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Khoudiadiopia massiliensis gen. nov., sp. nov. Strain Marseille-P2746^T, a New Gram-Stain-Positive Anaerobic Coccus Member of the Family *Peptoniphilaceae*, Isolated from the Human Vagina

Awa Diop[1], Florence Bretelle[1,2], Enora Tomei[1], Nicholas Armstrong[1], Didier Raoult[3,4], Florence Fenollar[1] and Pierre-Edouard Fournier[1]*

[1]UMR VITROME, Aix-Marseille Université, IRD, Service de Santé des Armées, Assistance Publique-Hôpitaux de Marseille, Institut Hospitalo-Universitaire Méditerranée-Infection, Marseille, France

[2]Department of Gynecology and Obstetrics, Gynépole, Marseille, Hôpital Nord, Assistance Publique-Hôpitaux de Marseille, Marseille, France

[3]UMR MEPHI, Aix-Marseille University, IRD, Assistance Publique-Hôpitaux de Marseille, Institut Hospitalo-Uuniversitaire Méditerranée Infection, Marseille, France

[4]Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

***Corresponding Author**: Pierre-Edouard Fournier, UMR VITROME, Aix-Marseille Université, IRD, Service de Santé des Armées, Assistance Publique-Hôpitaux de Marseille, Institut Hospitalo-Universitaire Méditerranée-Infection, Marseille, France.

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Abstract

A strictly anaerobic bacterium, strain Marseille-P2746^T, was isolated from the vaginal sample of a French woman suffering with bacterial vaginosis (BV) using the culturomics approach. It is a Gram-stain-positive, non-motile, non-spore-forming and mesophilic coccus. Cells were positive for oxidase and hydrolysis of aesculin, but negative for catalase, urease, nitrate reduction and indole production Strain Marseille-P2746^T exhibited 91.6% 16S rRNA gene sequence similarity with *Kallipyga massiliensis* strain ph2^T, the phylogenetically-closest species with standing in nomenclature. The major fatty acids were 12-methyl-tetradecanoic acid (28%), 9-Octadecenoic acid (14%), Octadecanoic acid (12%) and Hexadecanoic acid (11 %). The G+C content of the 1,533,2691-bp-long genome sequence of strain Marseille-P2746^T was 43.37 mol%. On the basis of its phenotypic, biochemical, phylogenetic and genomic features, we propose the creation of the genus *Khoudiadiopia* gen. nov., within the family *Peptoniphilaceae*, which contains strain Marseille-P2746^T (= CSUR P2746 = CECT9309) as type strain of the species *Khoudiadiopia massiliensis* gen. nov., sp. nov.

Keywords: *Khoudiadiopia massiliensis; Microbial Vaginosis; Culturomics; Taxono-Genomics; Anaerobic Bacteria, News Species*

Abbreviations

BV: Bacterial Vaginosis; CSUR: Stands for 'Collection de Souches de l'Unite´ des Rickettsies'; GPAC: Gram-Positive Anaerobic Cocci; dDDH: Digital DNA-DNA Hybridization; MALDI-TOF MS: Matrix-Assisted Laser-Desorption/Ionization Time-of-Flight Mass Spectrometry; GGDC: Genome-to-Genome Distance Calculator; DSMZ: Stands for 'Deutsche Sammlung von Mikroorganismen und Zellkulturen'; ML: Maximum Likelihood; MP: Maximum Parsimony; FAME: Cellular Fatty Acid Methyl Ester; GC/MS: Gas Chromatography/Mass Spectrometry; MICs: Minimal Inhibitory Concentrations; gDNA: Genomic DNA; AGIOS: Average Genomic Identity of Orthologous Gene Sequences; MAGI: Marseille Average Genomic Identity; AAI: Average Amino Acid Identity

Introduction

The vaginal flora composition, first described in 1892 by Doderleïn, is homogeneous and essentially composed of lactobacilli [1] that are Gram-positive, pleomorphic and asporogenic bacteria. Members of the *Lactobacillus* genus are the first line of defense against genital infections [2,3] and their imbalance causes a disease named bacterial vaginosis (BV). It is characterized by a decrease of *Lactobacillus* species inversely to an abnormal proliferation of Gram-negative anaerobic bacteria [4,5]. In order to investigate the vaginal microflora diversity of patients with bacterial vaginosis, we performed a "microbial culturomics" study [6,7] on a vaginal sample of a 26-year-old French woman suffering from BV. This strategy consists in optimizing culture conditions to explore in depth the human microbiota. Here, it enabled us to isolate a new and strictly anaerobic bacterial strain, Marseille-P2746^T, that was classified as belonging to the family *Peptoniphilaceae*.

First proposed by Johnson., *et al.* in 2014 [8], the family *Peptoniphilaceae* belongs to the order *Clostridiales* in the phylum *Firmicutes* and contains Gram-positive anaerobic cocci (GPAC) that are part of the human commensal flora and were often isolated from various clinical samples [9]. Currently, the family Peptoniphilaceae contains ten genera of GPAC that have standing in nomenclature (www.bacterio.net), including *Parvimonas, Finegoldia, Peptoniphilus, Anaerococcus* and *Gallicola* that once formed the genus Peptostreptococcus [10], and *Helcococcus, Anaerosphaera, Murdochiella, Ezakiella* and *Kallypiga* (www.bacterio.net).

Conventional parameters (such as 16S rRNA sequence identity and phylogeny, genomic (G + C content) diversity and DNA-DNA hybridization (DDH)) used in bacterial species delimitation, present some shortfalls, due to the variation of their cutoff values according to species and/or genus [11-14]. Thanks to the availability of genomic data of many bacteria, recently, a new approach was proposed for the classification and description of new bacterial taxa named taxono-genomics [15]. On the basis of the results of this polyphasic taxonomic study integrating phenotypic, phylogenetic, proteomic and genomic data [15-17], we described the new genus and species *Khoudiadiopia massiliensis* gen. nov., sp. nov., with strain Marseille-P2746^T (= CSUR P2746 = CECT9309) being the type strain of both the new genus and species.

Materials and Methods

Sample collection

In April 2016, a new member of the family *Peptoniphilaceae*, strain Marseille-P2746 was isolated from a vaginal sample of a 26 year-old French woman diagnosed with BV at the Nord hospital in Marseille, France. The sample was collected using a Sigma Transwab (Medical Wire, Corsham, United Kingdom) and then transported immediately to the microbiology laboratory of the Timone Hospital in Marseille. The patient was not treated with any antibiotic at the time of sampling. She gave her informed and signed consent and the study was authorized by the local ethics committee of the IFR48 (Marseille, France) under agreement 09-022.

Strain isolation and identification by MALDI-TOF MS and 16S rRNA sequencing

The vaginal sample was initially inoculated in an anaerobic blood culture bottle (Bactec Lytic/10 Anaerobic/F Culture Vials, Becton-Dickinson, Le Pont de Claix, Isère, France) enriched with 4 mL filter-sterilized rumen fluid through a 0.2 µm pore filter (Thermo Fisher Scientific, Villebon-sur-Yvette, France) and 3 mL of sheep blood (bioMérieux, Marcy l'Etoile, France) and incubated at 37°C. After two days of incubation, 50 µL of the supernatant was inoculated on Schaedler agar and Trypticase soy agar (BD Diagnostics, Le pont de Claix, France) and incubated in anaerobic atmosphere at 37°C. After two days of incubation, isolated colonies were subcultured individually using the same conditions and each colony was deposited on a MTP 96 MALDI-TOF target plate (Bruker Daltonics, Leipzig, Germany) in duplicate for identification with a Microflex MALDI-TOF MS spectrometer (Bruker Daltonics, Leipzig, Germany), as described by Seng., *et al* [18]. The protein spectra obtained were compared with those of 8,687 reference spectra in the Bruker database constantly enriched with our own database [19]. If the MALDI-TOF MS score was greater than 1.9 and 2.3, the bacterium was identified at the genus and species levels, respectively. Conversely, if the score was lower than these thresholds, the identification was not considered as reliable and the 16S rRNA gene was sequenced using the GeneAmp PCR System 2720 thermal cycler (Applied Bio systems, Bedford, MA, USA) and an ABI Prism 3130-XL capillary sequencer (Applied Biosciences, Saint Aubin, France), respectively, as previously described [20]. The resulting sequence was corrected using the Chromas Pro 1.34 software (Technelysium Pty. Ltd., Tewantin, Australia) and then compared to the NCBI database using the BLASTn algorithm (https://blast.ncbi.nlm.nih.gov/) for taxonomic assignment.

Phylogenetic analysis

The 16S rRNA sequences of type strains from the species with a validly published name (http://www.bacterio.net/) exhibiting the closest phylogenetic relationship with strain Marseille-P2746 were downloaded from NCBI (ftp://ftp.ncbi.nih.gov/Genome/). Sequences were aligned using MUSCLE [21]. Then, the degree of pairwise 16S rRNA sequence similarity between strain Marseille-P2746 and other closely related species were calculated using the Meier-Kolthoff method [22] available at GGDC web server (http://ggdc.dsmz.de/) [23]. Phylogenetic trees were inferred by the GGDC web server [23] using the DSMZ phylogenomics pipeline [24] adapted to single genes. Maximum likelihood (ML, using the GTR+GAMMA model) and maximum parsimony (MP)-based trees were inferred from the alignment with RAxML [25] and TNT [26], respectively. For ML, rapid bootstrapping in conjunction with the autoMRE bootstopping criterion [27] and subsequent search for the best tree was used; for MP, 1000 bootstrapping replicates were used in conjunction with tree-bisection-and-reconnection branch swapping and ten random sequence addition replicates. The sequences were checked for a compositional bias using the X2 test as implemented in PAUP* [28]. If the 16S rRNA sequence similarity value was lower than 95% or 98.65% with the most closely related species with standing in nomenclature, as proposed by Stackebrandt and Ebers [11], the strain was proposed to belong to a new genus or species, respectively [29].

Growth conditions and morphological observation

To evaluate its ideal growth conditions, strain Marseille-P2746 was cultivated on 5% sheep blood-enriched Columbia agar (bioMérieux) at different temperatures (25, 28, 37, 45, and 56°C), in aerobic conditions with or without 5% CO_2, and in anaerobic and microaerophilic atmospheres using GENbag Anaer and GENbag Microaer systems (bioMérieux), respectively. The tolerance to various NaCl concentrations (5 - 100 g/l NaCl) and pH values (pH 5, 6, 6.5, 7, 8.5) was also tested. To observe the cell morphology, transmission electron microscopy was performed after negative staining as previously described [30]. Electron micrographs were acquired using a Tecnai G20 Cryo (FEI company, Limeil-Brevannes, France) transmission electron microscope operated at 200 keV. Gram-stain, motility, oxidase, catalase and sporulation tests were performed as previously described [31].

Biochemical and antimicrobial susceptibility tests

The biochemical properties of strain Marseille-P2746 were evaluated using API ZYM, API 20A, and API rapid ID 32A strips (bioMérieux) according to the manufacturer's instructions. The strips were incubated in anaerobic conditions for 4, 24, and 4 hours respectively. Catalase and oxidase activities were assessed in 3% hydrogen peroxide solution (bioMérieux) and using an oxidase reagent (Becton-Dickenson, Le Pont de Claix, and France), respectively.

Cellular fatty acid methyl ester (FAME) analysis was performed using Gas Chromatography/Mass Spectrometry (GC/MS). Strain Marseille-P2746 was grown on 5% sheep blood-enriched Columbia agar (bioMérieux). Two samples were then prepared with approximately 70 mg of bacterial biomass per tube harvested from several culture plates. FAMEs were prepared as described by Sasser [32]. GC/MS analyses were carried out as described before [33].

Minimal inhibitory concentrations (MICs) of amoxicillin, benzylpenicillin, ceftriaxone, metronidazole, rifampicin, erythromycin and kanamycin were evaluated for strain Marseille-P2746 using E-test gradient strips (bioMérieux) according to the EUCAST recommendations [34,35].

DNA extraction, Genome sequencing and assembly

After a pretreatment step by lysozyme incubation at 37°C for 2 hours, the Genomic DNA (gDNA) of strain Marseille-P2746 was extracted using an EZ1 biorobot and the EZ1 DNA Tissue kit (Qiagen, Hilden, Germany). The elution volume was 50 µL. The gDNA was quantified by a Qubit assay with the high sensitivity kit (Life technologies, Carlsbad, CA, USA) to 64.5 ng/µl. The gDNA of the strain Marseille-P2746 was sequenced on the MiSeq sequencer with the Mate-Pair strategy (Illumina Inc, San Diego, CA, USA) as previously described [30]. A total of sequencing output of 6.3 Gb was obtained from a 673K/mm^2 cluster density with a cluster passing quality control filters of 95.4% (12,453,000 clusters). A total of 1,146,113 reads were obtained, then trimmed and assembled using the Spades assembler program [36].

Genome annotation and comparison

Prodigal was used for Open Reading Frame (ORF) prediction [37] with default parameters. Predicted ORFs spanning a sequencing gap region were excluded. The bacterial proteome was predicted using BLASTP (E-value 1e^{-03} coverage 0.7 and identity percent 30%) against the Clusters of Orthologous Groups (COG) database. If no hit was found, a search against the nr database [38] was performed using BLASTP with a E-value of 1e^{-03}, a coverage of 70% and an identity of 30%. If sequence lengths were smaller than 80 amino acids, we used an E-value of 1e^{-05}. Pfam conserved domains (PFAM-A an PFAM-B domains) were searched on each protein with the hhmscan tools analysis [39]. RNAmmer [40] and tRNAScanSE [41] were used to identify ribosomal RNAs and tRNAs, respectively. We predicted lipoprotein signal peptides and the number of transmembrane helices using Phobius [42]. ORFans were identified if the BLASTP search was negative (E-value smaller than 1e^{-03} for ORFs with a sequence size larger than 80 aas or E-value smaller than 1e^{-05} for ORFs with sequence length smaller than 80 aas). Artemis [43] and DNA Plotter [44] were used for data management and for visualization of genomic features, respectively. Genomes from members of the *Peptoniphilaceae* family and closely related to our strain were used for the calculation of AGIOS values. Genomic informations from strain Marseille-P2746 and comparatively closest related species are presented in table 6. Annotation and comparison processes were performed using the multi-agent software system DAGOBAH [45], which includes Figenix [46] libraries that provide pipeline analysis. We estimated the degrees of genomic sequence similarity among compared genomes using the following tools. First, the average genomic identity of orthologous gene sequences (AGIOS) among compared genomes [15] was calculated using the MAGI home-made software as previously described [47]. Then, the average amino acid identity (AAI) was calculated, based on the overall similarity between datasets of proteins of genome pairs belonging to the same family of *Peptoniphilaceae* [48] available at (http://enve-omics.ce.gatech.edu/aai/index). Finally, digital DNA-DNA hybridization (dDDH) analysis was performed using the GGDC web server, as previously reported [23].

Results and Discussion

Identification and phylogenetic analysis of the new genus

Strain Marseille-P2746 was first cultivated after 48 hours of incubation in anaerobic atmosphere at 37°C on Schaedler and Trypticase soy agars (BD Diagnostics, Le pont de Claix, France), after 4 days of pre-incubation in a blood culture bottle enriched with rumen and sheep blood. A score of 1.3 was obtained with MALDI-TOF-MS identification for strain Marseille-P2746 against our database (Bruker database) suggesting that this isolate was not referenced in the database. Its MALDI-TOF MS spectrum was added to our database to improve its content and the 16S rRNA of the strain was sequenced.

The 16S rDNA-based similarity analysis of strain Marseille-P2746 (deposited in EMBL-EBI under accession number LT223702) against GenBank yielded a highest nucleotide sequence similarity of 91.6% with *Kallipyga massiliensis* strain ph2^T (GenBank accession number JN837487), the phylogenetically closest species with a validly published name. As this value was lower than the 95% 16S rRNA sequence identity threshold proposed to define a new genus [11,49], strain Marseille-P2746 was considered as a representative of a potentially new genus within the family *Peptoniphilaceae*. The resulting combined ML/MP tree highlighting the position of strain Marseille-P2746 relative to other closely related species with a validly published name is shown in figure 1.

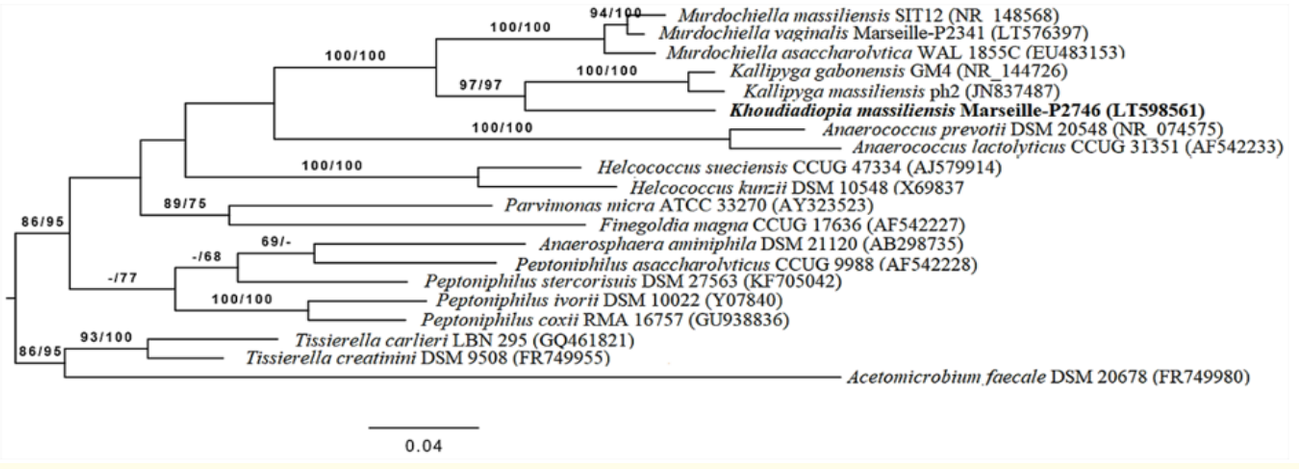

Figure 1: *16S rRNA-based Maximum likelihood phylogenetic tree of Khoudiadiopia massiliensis gen. nov., sp. nov., Marseille-P2746T inferred under the GTR+GAMMA model. The branches are scaled in terms of the expected numbers of substitutions per site. GenBank accession numbers of each 16S rRNA are noted in parentheses. The numbers above the branches are support values when larger than 60% from ML (left) and MP (right) bootstrapping. Acetomicrobium faecale was used as outgroup. The scale bar represents a 4% nucleotide sequence divergence.*

For the phylogenetic inferences, the input nucleotide matrix comprised 20 operational taxonomic units and 1,573 characters, 553 of which were variable and 436 of which were parsimony-informative. The base-frequency check indicated no compositional bias ($p = 0.29$, $\alpha = 0.05$). ML analysis under the GTR+GAMMA model yielded a highest log likelihood of -10000.07, whereas the estimated alpha parameter was 0.22. The ML bootstrapping did not converge, hence 1000 replicates were conducted; the average support was 79.53%. MP analysis yielded a best score of 1807 (consistency index 0.50, retention index 0.56) and a single best tree. The MP bootstrapping average support was 82.06%.

Physiological and biochemical characteristics

After 2 days of growth at 37°C in anaerobic atmosphere on Schaedler agar and Trypticase soy agars (BD Diagnostics, Le pont de Claix, France), colonies from strain Marseille-P2746 were bright grey, circular with a mean diameter of 0.2 µm. Cells were Gram-stain-positive (Figure 2A), non-motile and non-spore-forming cocci with a mean diameter of 0.55 µm (Figure 2B). Strain Marseille-P2746 was strictly anaerobic and exhibited positive oxidase activity but no catalase activity. Growth was obtained at temperatures ranging from 28 to 45°C, with optimal growth observed at 37°C in anaerobic atmosphere. No growth was obtained in neither aerobic nor microaerophilic atmospheres. Growth of strain Marseille-P2746 was obtained with NaCl concentrations below 5 g/L and at a pH ranging from 6.5 to 7.0.

Using an API ZYM strip (bioMérieux), positive results were obtained for esterase (C4), esterase lipase (C8), alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucuronidase and α-glucosidase. No reaction was observed for lipase (14), trypsin, α-chymotrypsin, α-galactosidase, β-glucosidase,

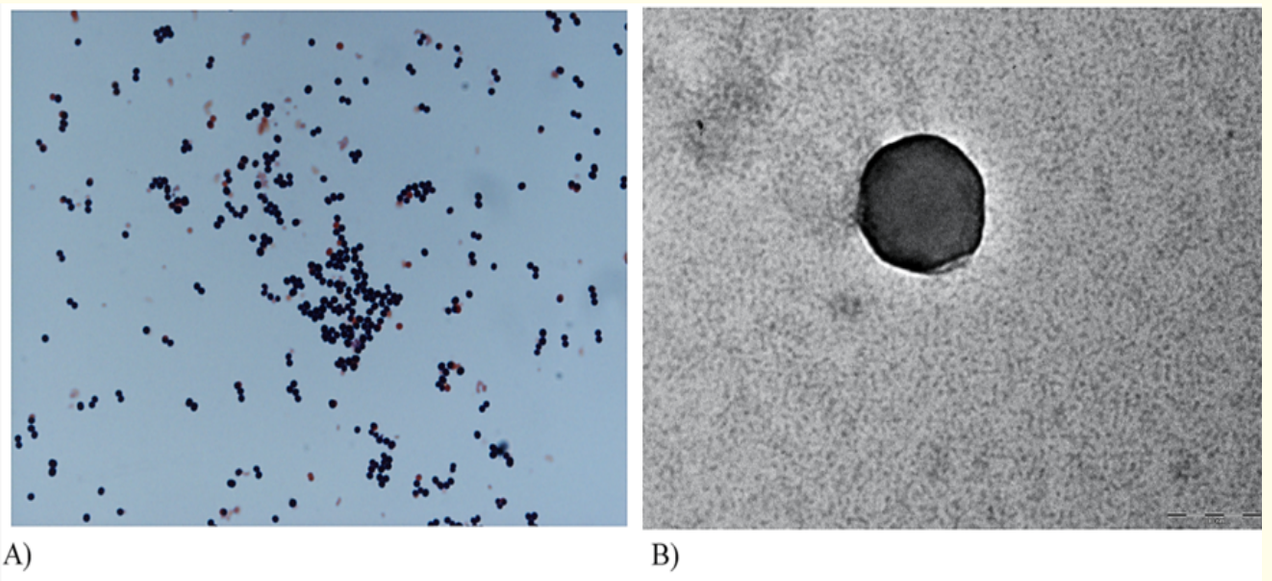

Figure 2: *A: Gram-staining of Khoudiadiopia massiliensis gen. nov., sp. nov., strain Marseille-P2746^T. B: Transmission electron microscopy of Khoudiadiopia massiliensis strain Marseille-P2746^T using a Tecnai G20 transmission electron microscope (FEI Company). The scale bar represents 100 nm.*

N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Using a Rapid ID32A strip (bioMérieux), positive reactions were obtained for arginine dihydrolase, β-glucuronidase, mannose fermentation, raffinose fermentation, alkaline phosphatase, arginine arylamidase, leucine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase. Cells showed no nitrate reduction, indole production, urease, α-galactosidase, β-galactosidase, 6-phospho-β-galactosidase, α-glucosidase, β-glucosidase, α-arabinosidase, N-Acetyl-ß-glucosaminidase, glutamic acid decarboxylase, α-fucosidase, proline arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase and glutamyl-glutamic acid arylamidase activities. Using an API 20A strip (bioMérieux), strain Marseille-P2746 produced acid from D-glucose and gelatin but not from D-mannitol, D-lactose, D-saccharose, D-maltose, salicin, D-xylose, L-arabinose, glycerol, D-cellobiose, D-mannose, D-melezitose, D-raffinose, sorbitol, D-rhamnose and D-trehalose. Esculin ferric citrate was hydrolyzed. Strain Marseille-P2746 differed from other members of the Peptoniphilaceae family [9,10,50-54] in ß-galactosidase activity (Table 1). The classification and general features of strain Marseille-P2746 are summarized in table 2. The most abundant cellular fatty acids found for strain Marseille-P2746 were 12-methyl-tetradecanoic acid (28%), 9-Octadecenoic acid (14%), Octadecanoic acid (12%) and Hexadecanoic acid (11%). Many branched fatty acids (iso and anteiso) were described. Unusual C19 and C20 fatty acids were also detected with significant abundances (Table 3). Cells were susceptible to benzylpenicillin (MIC 0.016 µg/mL), amoxicillin (0.023 µg/mL), ceftriaxone (0.25 µg/mL), metronidazole (0.032 µg/mL), rifampicin (0.004 µg/mL) and erythromycin (2 µg/mL), but resistant to kanamycin (48 µg/mL).

Genome properties

The draft genome of strain Marseille-P2746 (Table 4 and Figure 3) measures 1,533,2691 bp long with a 43.37 mol% G+C content. It is composed of ten scaffolds and ten contigs. Of the 1,425 predicted genes; 1,368 were protein-coding genes and 57 were RNAs (three 5S rRNAs, three 16S rRNAs, one 23S rRNA, 47 tRNAs and three ncRNAs). A total of 1,097 genes (80.19%) were assigned a putative function (by BLAST against the COGS or NR databases). The genome also exhibited 1 clustered regularly interspaced short palindromic repeat region. The distribution of the genes into COGs functional categories is presented in table 5.

Characteristic	1	2	3	4	5	6	7	8
Cell diameter (µm)	0.55	0.67	0.5 - 0.6	na	na	0.3 - 0.7	0.8 - 1.6	na
Oxygen requirement	Anaerobic	Anaerobic	Anaerobic	Facultative	Facultative	Anaerobic	Anaerobic	Anaerobic
Gram stain	+	+	+	+	+	+	+	+
DNA G+C content (mol%)	43.4	51.4	na	29.5	30	28.65	32.3	33
Spore-forming	-	-	-	-	-	-	-	na
Motility	-	-	-	-	-	-	-	-
Production of Alkaline phosphatase	+	-	-	+	-	+	Variable	-
Indole	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	Variable	Variable	+
Nitrate reductase	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	+/-
β-galactosidase	+	-	-	-	-	-	-	-
Acid form								
Mannose	-	-	-	-	-	-	-	+
Glucose	+	+	-	+	-	-	-	+/-
Lactose	-	na	-	+	+	-	-	-
Raffinose	-	-	-	-	-	-	-	+
Habitat	Vaginal discharges	Human gut	Human wound	Human wound	Human specimen	Human specimen	Human specimen	Human specimen

Table 1: *Differential phenotypic characteristics of Khoudiadiopia massiliensis gen. nov., sp. nov., strain Marseille-P2746^T and related species of the family Peptoniphilaceae. 1: Khoudiadiopia massiliensis strain Marseille-P2746^T; 2: Kallipyga massiliensis strain ph2^T; 3: Murdochiella asaccharolytica strain WAL 1855C^T; 4: Helcococcus sueciensis strain CCUG 47334^T; 5: Helcococcus kunzii strain NCFB 2900^T; 6: Parvimonas micra strain CCUG 46357^T; 7: Finegoldia magna strain CCUG 17636^T; 8: Anaerococcus prevotii strain ATCC 9321^T. +: Positive Reaction; -: Negative Reaction; na: No Available Data.*

Fatty acids	Name	Mean relative % (a)
15:0 anteiso	12-methyl-tetradecanoic acid	27.9 ± 2.9
18:1ω9	9-Octadecenoic acid	13.7 ± 2.0
18:00	Octadecanoic acid	12.2 ± 0.4
16:00	Hexadecanoic acid	11.0 ± 2.5
18:2ω6	9,12-Octadecadienoic acid	5.3 ± 0.7
15:0 iso	13-methyl-tetradecanoic acid	5.2 ± 1.0
20:00	Eicosanoic acid	3.3 ± 0.2
17:0 iso	15-methyl-Hexadecanoic acid	3.2 ± 1.2
17:0 anteiso	14-methyl-Hexadecanoic acid	2.8 ± 0.4
20:1ω11	9-Eicosenoic acid	2.3 ± 0.1
19:0 iso	17-methyl-Octadecanoic acid	2.1 ± 0.5
14:00	Tetradecanoic acid	1.8 ± 0.7
14:0 iso	12-methyl-Tridecanoic acid	1.6 ± 0.1
19:0 anteiso	16-methyl-Octadecanoic acid	1.1 ± 0.1
13:0 iso	11-methyl-Dodecanoic acid	1.0 ± 0.1
18:1ω7	11-Octadecenoic acid	TR
19:00	Nonadecanoic acid	TR
20:2ω6	11,14-Eicosadienoic acid	TR

15:00	Pentadecanoic acid	TR
18:1ω5	13-Octadecenoic acid	TR
5:0 iso	3-methyl-Butanoic acid	TR
17:00	Heptadecanoic acid	TR
16:0 iso	14-methyl-Pentadecanoic acid	TR
16:1ω7	9-Hexadecenoic acid	TR
13:0 anteiso	10-methyl-Dodecanoic acid	TR
13:00	Tridecanoic acid	TR
10:00	Decanoic acid	TR
12:00	Dodecanoic acid	TR

Table 2: *Cellular fatty acid composition (%) of Khoudiadiopia massiliensis gen. nov., sp. nov., strain Marseille-P2746^T.*

[a]: Mean peak area percentage; TR: Trace Amounts < 1 %.

Properties	Terms
Taxonomy	Kingdom: *Bacteria*
	Phylum: *Firmicutes*
	Class: *Tissierellia*
	Order: *Tissierellales*
	Family: *Peptoniphilaceae*
	Genus: *Khoudiadiopia*
	Species: *Khoudiadiopia massiliensis*
Type strain	Marseille P2746^T
Isolation site	Human vagina
Isolation country	France
Gram stain	Positive
Cell shape	Cocci
Motility	No
Oxygen requirements	Anaerobic
Optimal temperature	37°C
Temperature range	Mesophilic

Table 3: *Classification and general features of Khoudiadiopia massiliensis gen. nov., sp. nov., strain Marseille-P2746^T.*

Attribute	Value	% of total[a]
Size (bp)	1,533,261	100
G+C content (bp)	664,941	43.37
Coding region (bp)	1,399,110	91.25
Total genes	1,425	100
RNA genes	57	4.00
Total protein-coding genes	1,368	96.00
Genes with function prediction	1097	80.19
Genes assigned to COGs	1,111	81.21
Genes with peptide signals	84	6.14
Genes with transmembrane helices	379	27.70

Table 4: *Nucleotide content and gene count levels of the genome from Khoudiadiopia massiliensis gen. nov., sp. nov., strain Marseille-P2746^T.*

[a]: The total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome.

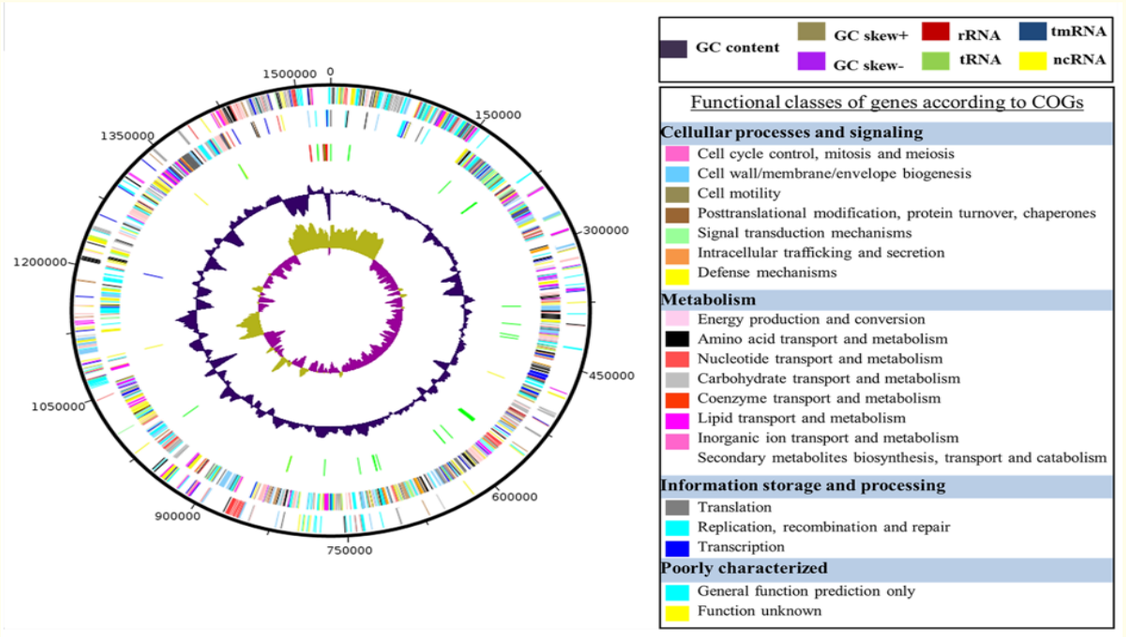

Figure 3: *Graphical circular map of the genome from Khoudiadiopia massiliensis gen. nov., sp. nov., strain Marseille-P2746T. From outside to the center: Contigs (red/gray), COG category of genes on the forward strand (three circles), genes on forward strand (blue circle), genes on the reverse strand (red circle), COG category on the reverse strand (three circles), GC content.*

Code	Value	% of total	Description
[J]	131	9.58	Translation
[A]	0	0	RNA processing and modification
[K]	66	4.82	Transcription
[L]	85	6.21	Replication, recombination and repair
[B]	0	0	Chromatin structure and dynamics
[D]	15	1.10	Cell cycle control, mitosis and meiosis
[Y]	0	0	Nuclear structure
[V]	48	3.51	Defense mechanisms
[T]	26	1.90	Signal transduction mechanisms
[M]	73	5.33	Cell wall/membrane biogenesis
[N]	0	0	Cell motility
[Z]	0	0	Cytoskeleton
[W]	0	0	Extracellular structures
[U]	12	188	Intracellular trafficking and secretion
[O]	50	3.65	Post-translational modification, protein turnover, chaperones
[X]	0	0	Mobilome: prophages, transposons
[C]	61	4.46	Energy production and conversion
[G]	91	6.65	Carbohydrate transport and metabolism
[E]	84	6.14	Amino acid transport and metabolism
[F]	57	4.17	Nucleotide transport and metabolism
[H]	30	2.19	Coenzyme transport and metabolism
[I]	30	2.19	Lipid transport and metabolism
[P]	53	3.87	Inorganic ion transport and metabolism
[Q]	3	0.22	Secondary metabolites biosynthesis, transport and catabolism
[R]	111	8.11	General function prediction only
[S]	85	6.21	Function unknown
-	257	18.79	Not in COGs

Table 5: *Number of genes from Khoudiadiopia massiliensis gen. nov., sp. nov., strain Marseille-P2746^T associated with the 25 general COG functional categories.*

Genomic comparison

Comparison with the closest related species (Table 6) revealed that the genome sequence of strain Marseille-P2746 (1.53 Mbp) was smaller than those of all compared genomes. The G+C content of strain Marseille-P2746 (43.4 mol%) was greater than those of *Helcococcus sueciensis, Parvimonas micra, Helcococcus kunzii, Finegoldia magna, Peptoniphilus asaccharolyticus, Anaerococcus prevotii* and *Anaerococcus lactolyticus*, (28.4, 28.6, 29.3, 32.3, 32.3, 36.1 and 39.4%, respectively). but smaller than those of *Murdochiella massiliensis* and *Kallipyga massiliensis* (49.0 and 51.4%, respectively).The gene content of strain Marseille-P2746 (1,425) was smaller than those of all compared genomes (Table 6). However, in all compared genomes, the distribution of genes into COG categories was similar (Figure 4). Moreover, strain Marseille-P2746 shared 672 to 680 orthologous genes with the two most closely related species (*Kallipyga massiliensis* and *Murdochiella massiliensis*, respectively) and AGIOS values ranged from 59.6 to 66.0% with *Parvimonas micra* and *Kallipyga massiliensis,* respectively (Table 7). In addition, strain Marseille-P2746 exhibited dDDH values ranging from 24.6% with *Finegoldia magna* to 43.4% with *Peptoniphilus asaccharolyticus* (Table 7). Moreover, we observed AAI values ranging from 44.35% with *Parvimonas micra* to 58.57% with *Kallipyga massiliensis* (Table 7).

Species	INSDC identifier[a]	Number of Contigs	N50	Size (Mb)	G+C (mol%)	Gene Content
Khoudiadiopia massiliensis Marseille-P2746^T	OKQS00000000	10	800,826	1.53	43.4	1425
Kallipyga massiliensis ph2^T	CAHC00000000	22	177,233	1.77	51.4	1645
Murdochiella massiliensis SIT12^T	FIZW00000000	2	1,293,679	1.64	49.0	1492
Anaerococcus lactolyticus ATCC 51172^T	ABYO00000000	298	83,276	2.2	39.4	2241
Anaerococcus prevotii DSM 20548^T	CP001708	Complete genome	90,722	1.88	36.1	1782
Helcococcus kunzii ATCC51366	AGEI00000000	39	98,606	2.1	29.3	1,886
Helcococcus sueciensis DSM 17243^T	AUHK00000000	45	75,015	1.57	28.4	1,445
Peptoniphilus asaccharolyticus DSM 20463^T	FWWR00000000	17	1,358,172	2.23	32.3	2,268
Finegoldia magna ATCC 29328^T	AP008971	Complete genome	1,797,577	1.8	32.3	1715
Parvimonas micra ATCC 33270^T	CP009761	Complete genome	1,627,009	1.63	28.6	1531

Table 6: *Genome comparison of Khoudiadiopia massiliensis gen. nov., sp. nov., strain Marseille-P2746^T and closely related species.*

[a]: INSDC: International Nucleotide Sequence Database Collaboration.

Strains	1	2	3	4	5	6	7	8	9	10
AGIOS										
1. Khoudiadiopia massiliensis	**1,348**	672	680	659	645	615	574	551	536	530
2. Kallipyga massiliensis	66.0	**1,567**	724	674	631	626	596	558	544	521
3. Murdochiella massiliensis	49.9	49.7	**1,489**	631	612	606	583	537	526	524
4. Anaerococcus lactolyticus	60.9	57.6	49.0	**2,099**	852	749	698	651	614	625
5. Anaerococcus prevotii	61.4	57.0	49.1	72.7	**1,725**	731	679	655	619	609
6. Finegoldia magna	59.7	55.1	48.8	65.2	64.1	**1,656**	681	604	572	654
7. Peptoniphilus asaccharolyticus	60.0	55.8	48.9	64.8	63.5	65.6	**2,291**	532	521	594
8. Helcococcus kunzii	60.3	54.6	48.3	64.5	64.0	65.8	64.7	**1,878**	687	549
9. Helcococcus sueciensis	60.1	54.1	47.9	64.7	64.5	66.3	65.4	74.8	**1,420**	523
10. Parvimonas micra	59.6	53.8	48.2	64.0	63.6	67.2	66.0	66.5	66.9	**1,490**
dDDH										
1. Khoudiadiopia massiliensis	100	27.7 ± 2.4	31.1 ± 2.5	43.1 ± 2.3	30.8 ± 2.5	24.6 ± 2.4	43.4 ± 2.4	30.6 ± 2.5	27.9 ± 2.5	25.1 ± 2.4
2. Kallipyga massiliensis		100	38.4 ± 2.3	30.9 ± 2.5	21.6 ± 2.4	20.3 ± 2.4	31.0 ± 2.5	24.7 ± 2.4	24.4 ± 2.4	24.3 ± 2.4
3. Murdochiella massiliensis			100	30.7 ± 2.5	25.3 ± 2.4	23.9 ± 2.4	28.4. ± 2.5	25.6 ± 2.4	26.5 ± 2.4	24.4 ± 2.4

4. Anaerococcus lactolyticus				100	19.3 ± 2.3	37.3 ± 2.3	43.4 ± 2.3	31.7 ± 2.5	32.5 ± 2.5	23.5 ± 2.4
5. Anaerococcus prevotii					100	25.1 ± 2.4	19.5 ± 2.3	26.7 ± 2.4	28.7 ± 2.4	24.8 ± 2.4
6. Finegoldia magna						100	26.0 ± 2.4	22.6 ± 2.4	21.1 ± 2.4	21.8 ± 2.4
7. Peptoniphilus asaccharolyticus							100	30.6 ± 2.5	26.8 ± 2.5	24.1 ± 2.4
8. Helcococcus kunzii								100	19.6 ± 2.3	22.5 ± 2.4
9. Helcococcus sueciensis									100	21.9 ± 2.4
10. Parvimonas micra										100
AAI										
1. Khoudiadiopia massiliensis	100	58.57	53.20	46.94	46.77	45.40	44.6	45.29	45.95	44.35
2. Kallipyga massiliensis		100	55.39	46.06	44.84	45.05	46.15	44.30	44.98	43.98
3. Murdochiella massiliensis			100	45.26	44.23	44.59	44.79	44.09	44.58	44.19
4. Anaerococcus lactolyticus				100	65.14	49.89	49.11	46.85	46.93	46.46
5. Anaerococcus prevotii					100	48.10	46.19	46.70	47.20	45.75
6. Finegoldia magna						100	48.33	46.71	47.23	50.52
7. Peptoniphilus asaccharolyticus							100	43.59	44.74	46.83
8. Helcococcus kunzii								100	63.07	45.67
9. Helcococcus sueciensis									100	46.35
10. Parvimonas micra										100

Table 7: *Genomic indices (AGIOS, dDDH and OrthoANI) (%) among the genomes of Khoudiadiopia massiliensis Marseille-P2746^T and other members of the family peptoniphilaceae. AGIOS analysis: numbers of orthologous proteins shared between genomes (upper right) and AGIOS values obtained (lower left). The numbers of proteins per genome are indicated in bold.*

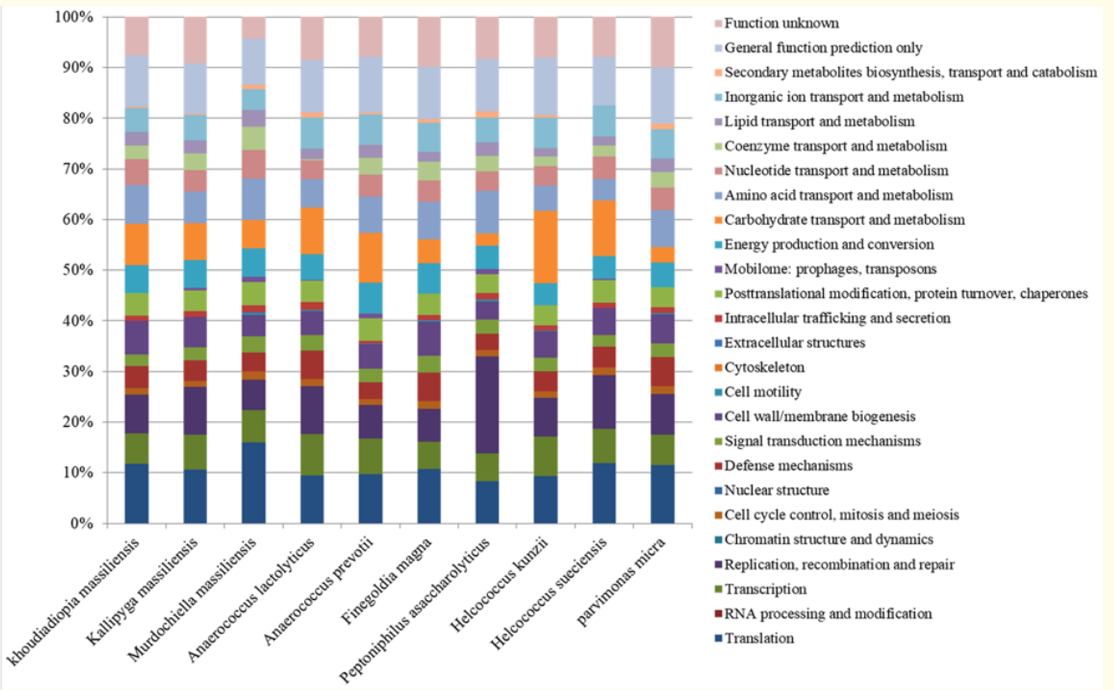

Figure 4: *Distribution of functional classes of predicted genes according to the clusters of orthologous groups of proteins of Khoudiadiopia massiliensis gen. nov., sp. nov., strain Marseille-P2746T among other member of the family Peptoniphilaceae.*

The obtained dDDH and AGIOS values confirmed that the genomic similarity between strain Marseille-P2746 and its closest relatives are in accordance with the proposal of creating a new genus within the *Peptoniphilaceae* family. Moreover strain Marseille-P2746 exhibited genomic G+C content differences ranging from -15.0% when compared with *Helcococcus sueciensis* to +8.0% with *Kallipyga massiliensis.* In addition, strain Marseille-P2746 shared only 49.1% proteins (672/1368) with K. massiliensis to 49.7% (680/1368) with *M. massiliensis*. As Qin., *et al*. demonstrated that a strain from a new genus has less than 50% of pairwise percentage of conserved proteins with its closest phylogenetic neighbours [55], the percentages observed for strain Marseille-P2746 supported its new genus status (Table 7). Furthermore, the 91.6% 16S rRNA nucleotide sequence similarity exhibited by strain Marseille-P2746 with *Kallipyga massiliensis* strain ph2^T, its phylogenetically-closest neighbor, was lower than the value described by Yarza., *et al.* [56] to distinguish two genera, further confirming the proposal of a novel genus. Finally, the AAI analysis showing values lower than 65% also confirmed that strain Marseille-P2746 belonged to a new genus [48,57].

Conclusion

The strictly anaerobic strain Marseille-P2746 was isolated from a vaginal sample of a 26-year-old French woman patient suffering with BV as part of study of the bacterial community of the vaginal microbiota in healthy individuals and patients suffering from bacterial vaginosis. By taking into consideration its phenotypic, phylogenetic and genomic characteristics, strain Marseille-P2746 was proposed to belong to a new genus within this family, for which we propose the name *Khoudiadiopia* gen. nov., with *Khoudiadiopia massiliensis* gen. nov., sp. nov. being the type species.

Description of *Khoudiadiopia* gen. nov.

Khoudiadiopia (khou.di.a.di.o'pi.a, N.L. fem. n. *Khoudiadiopia*, in honor of Dr Khoudia Diop, a Senegalese microbiologits, for her contribution to the description of new species from the human microbiota).

Cells are Gram-stain-positive and non-motile cocci exhibiting a mean diameter of 0.55 µm. *Khoudiadiopia* bacteria are strictly anaerobic and optimal growth is observed at 37°C. Cells do not produce catalase but exhibited oxidase activity. In contrast with other members from the *Peptoniphilaceae* family, *Khoudiadiopia* bacteria exhibit a ß-galactosidase activity. *Khoudiadiopia massiliensis* gen. nov., sp. nov. is the type species of the genus. The type strain of the genus, Marseille-P2746^T (= CSUR P2746 = CECT9309) was isolated from the vaginal sample of a French woman suffering from bacterial vaginosis.

Description of *Khoudiadiopia massiliensis* gen. nov., sp. nov.

Khoudiadiopia massiliensis (mas.il'ien'sis. L. gen. fem. n. *massiliensis*, of Massilia, the Latin name of Marseille where was cultivated strain Marseille-P2746^T).

Khoudiadiopia massiliensis is a strictly anaerobic bacterium that grows from 28 to 45°C, with NaCl concentrations below 5 g/L and at a pH ranging from 6.5 to 7.0. Cells are Gram-stain-positive, not motile, non-spore-forming and mesophilic cocci with a mean diameter of 0.55 µm. Colonies grown on Schaedler and Trypticase soy agars in anaerobic atmosphere are bright grey and circular, with a diameter of 0.2 µm. Esculin ferric citrate is hydrolyzed but nitrate reduction, indole formation, catalase and urease activities are not detected. Using an API ZYM strip (bioMérieux), esterase (C4), esterase lipase (C8), alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucuronidase and α-glucosidase activities are observed. No activity is observed for lipase (14), trypsin, α-chymotrypsin, α-galactosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Using a Rapid ID32A strip (bioMérieux), positive reactions are observed for arginine dihydrolase, β-glucuronidase, mannose fermentation, raffinose fermentation, alkaline phosphatase, arginine arylamidase, leucine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase. Cells show no α-galactosidase, β-galactosidase, 6-phospho-β-galactosidase, α-glucosidase, β-glucosidase, α-arabinosidase, N-Acetyl-ß-glucosaminidase, glutamic acid decarboxylase, α-fucosidase, proline arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase and glutamyl-glutamic acid arylamidase activities. Using an API 20A strip (bioMérieux), strain Marseille-P2746^T produces acid from D-glucose and gelatin but not from D-mannitol, D-lactose, D-saccharose, D-maltose, salicin, D-xylose, L-arabinose, glycerol, D-cellobiose, D-mannose, D-melezitose, D-raffinose, sorbitol, D-rhamnose and D-trehalose. The most abundant fatty acids are 12-methyl-tetradecanoic acid, 9-Octadecenoic acid, Octadecanoic acid and Hexadecanoic acid. Strain Marseille-P2746^T is susceptible to benzylpenicillin, amoxicillin, ceftriaxone, metronidazole, rifampicin and erythromycin but resistant to kanamycin.

The type strain Marseille-P2746^T (= CSUR P2746 = CECT9309) was isolated from the vaginal sample of a French woman suffering from bacterial vaginosis. The genome of the type strain is 1,533,2691 bp long and exhibits a G+C content of 43.37 mol%. The 16S rRNA and whole-genome sequences are deposited in EMBL-EBI under accession numbers LT223702 and OKQS00000000, respectively.

Conflict of Interest

The authors declare no competing interest in relation to this research

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