ORIGINAL ARTICLE

Dakarella massiliensis gen. nov., sp. nov., strain ND3^T: a new bacterial genus isolated from the female genital tract

N. Dione[1], J. Rathored[1], E. Tomei[1], J.-C. Lagier[1], S. Khelaifia[1], C. Robert[1], F. Bretelle[1,2], D. Raoult[1,3], P.-E. Fournier[1] and F. Fenollar[1]

1) *Aix-Marseille Université, URMITE, UM63, CNRS 7278, IRD 198, INSERM 1095, Institut Hospitalo-Universitaire Méditerranée-Infection, Faculté de médecine,* 2) *Department of Gynecology and Obstetrics, Gynépole, Hôpital Nord, Assistance Publique-Hôpitaux de Marseille, Aix-Marseille Université, Marseille, France and* 3) *Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia*

Abstract

Strain ND3^T was isolated from the genital tract of a 28-year-old woman with bacterial vaginosis. This strain exhibited a 16S rRNA gene sequence similarity of 92.4% with *Sutterella wadsworthensis,* the phylogenetically closest species with standing in nomenclature. Strain ND3^T was a strictly anaerobic Gram-negative rod and member of the family *Sutterellaceae.* It exhibited a genome of 2 476 884 bp containing 2175 protein-coding and 62 RNA genes. On the basis of these data, we propose the creation of *'Dakarella massiliensis'* sp. nov. with strain ND3^T (= CSUR P1938 = DSM 100447) as the type strain.

keywords: bacterial vaginosis, culturomics, taxono-genomics
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Corresponding author: F. Fenollar, Aix Marseille Université, URMITE, UM63, CNRS 7278, IRD 198, INSERM 1095, Institut Hospitalo-Universitaire Méditerranée-Infection, Faculté de médecine, 19-21 boulevard Jean Moulin, 13385 Marseille cedex 5, France
E-mail: florence.fenollar@univ-amu.fr

Introduction

Bacterial vaginosis is a serious worldwide public health problem that can affect women of childbearing age and may involved in premature birth [1,2]. Its prevalence has been estimated to be between 10% and 30% in developed countries and >50% in women in East Africa and Southern Africa [2]. It is also a risk factor for sexually transmitted diseases like herpes simplex virus type 2 and human immunodeficiency virus type 1 [2]. First-line recommended therapies are metronidazole or clindamycin for 5 to 7 days, but even with these treatments, failure rates are >50% within 6 to 12 months [2].

Bacterial vaginosis is characterized by a switch of the vaginal flora with the depletion of key *Lactobacillus* spp. for high bacterial species diversity with increased loads of anaerobes such as *Atopobium vaginae* or *Gardnerella vaginalis* compared to healthy controls [2]. The lack of extensive data on the vaginal microbiota diversity in cultured species is an impediment to understanding the aetiology and pathogenesis of bacterial vaginosis and searching for therapeutic strategies [3]. However, advances in molecular biology, particularly metagenomics, sequencing and phylogenetic analysis of the 16S rRNA gene, have enhanced the exploration of the human microbiome, and the vaginal microbiota in particular [4–6]. Today, with the advent of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and the culturomics approach [7,8], we have effective tools to explore the human microbiome diversity.

With the aim of exploring the microbial diversity of vaginal flora in patients with bacterial vaginosis, we cultivated a new bacterial strain named *'Dakarella massiliensis'* strain ND3^T (= CSUR P1938 = DSM 100447).

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Here we present a summary classification and a set of features for *'Dakarella massiliensis'* gen. nov., sp. nov., together with the description of the complete genome sequencing and annotation. These characteristics support the circumscription of the genus and species *'Dakarella massiliensis.'*

Organism Classification and Features

A vaginal specimen was collected from a 28-year-old French patient living in Marseille with bacterial vaginosis and diagnosed as previously reported [9]. After collection, the sample was transported directly to the laboratory. Part of the sample was grown directly in an anaerobic chamber. The remaining portion was stored at −80°C. The *'Dakarella massiliensis'* strain ND3^T was isolated in November 2013 by culture on Columbia agar (bioMérieux, Marcy l'Etoile, France) after 3 days of sample preincubation in a blood culture bottle (Becton Dickinson, Le Pont-de-Claix, France) with the addition of 5 mL of sheep rumen that was filter-sterilized through a 0.2 µm pore filter (Thermo Fisher Scientific, Villebon-sur-Yvette, France) in an anaerobic chamber.

MALDI-TOF MS (Microflex spectrometer; Bruker Daltonics, Bremen, Germany) was first performed to try to identify the bacterium [10]. In brief, 1.5 µL of matrix solution containing diluted α-cyano-4-hydroxycinnamic acid in 500 µL acetonitrile, 250 µL 10% trifluoroacetic acid and 250 µL HPLC water was deposited on each spot for ionization and crystallization. All protein spectra obtained were compared with those of the MALDI-TOF database. If the score was greater than or equal to 1.9, the strain was considered identified. Otherwise, the identification failed. When MALDI-TOF MS failed, bacterial identification was performed using 16S rRNA gene PCR amplification in combination with sequencing as previously described [11].

Strain ND3^T exhibited 92.4% of 16S rRNA gene sequence similarity with *Sutterella wadsworthensis* strain SW4, which is the phylogenetically closest species with a validly published name [12]. As Stackebrant [13] suggested, if the 16S rRNA gene sequence similarity value was lower than 98.7% or 95%, the strain was defined as a new species or genus respectively, without performing DNA-DNA hybridization [14]. Phylogenetic analysis was performed by comparing the 16S rRNA gene sequences obtained from other *Sutterellaceae* family members. Sequences were aligned using CLUSTALW, and phylogenetic references were obtained using the maximum-likelihood method within the MEGA software (Fig. 1). The MALDI-TOF MS analysis of proteins was also performed, as previously described, to generate a reference spectrum. Spectra from 12 individual colonies of strain ND3^T were compared and a reference spectrum generated (Fig. 2).

Different growth temperatures (25, 30, 37 and 45°C) were tested. Growth was observed after 24 hours of inoculation

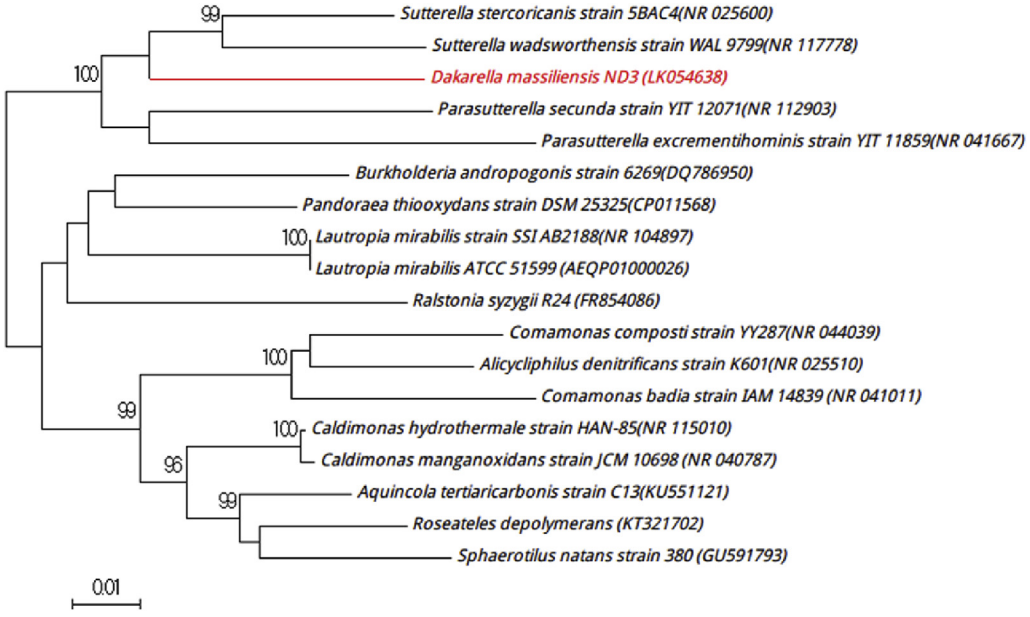

FIG. 1. Phylogenetic tree highlighting position of *'Dakarella massiliensis'* strain ND3^T relative to other type strains within *Sutterellaceae* family. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. GenBank accession numbers are indicated in tree. *Lautropia mirabilis* was used as outgroup. Scale bar represents 2% nucleotide sequence divergence.

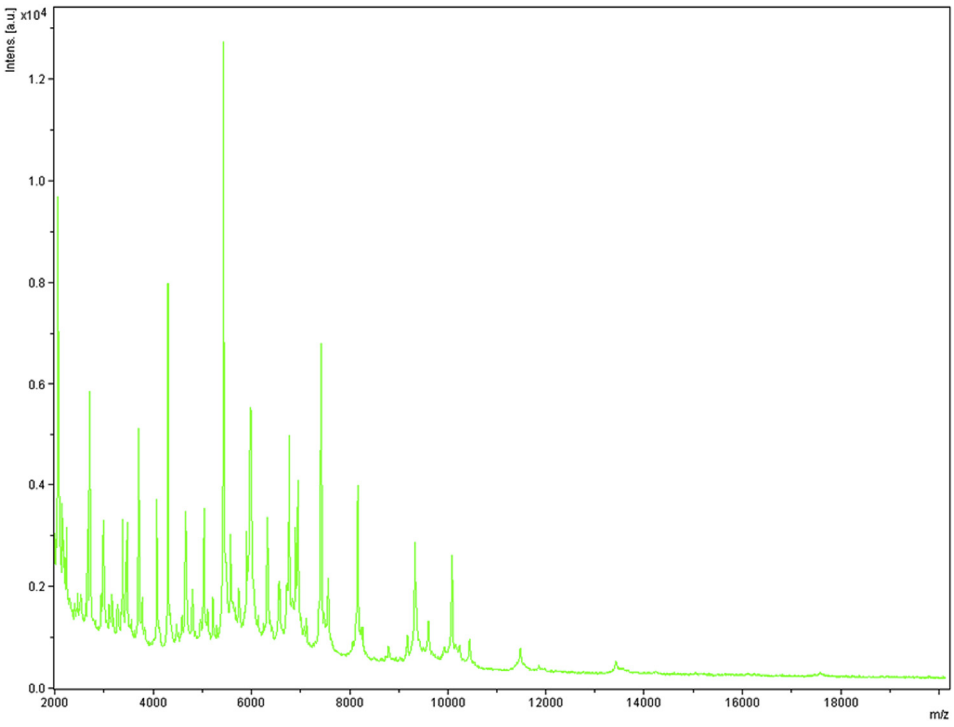

FIG. 2. Reference mass spectrum from *'Dakarella massiliensis'* strain ND3^T. Spectra from 12 individual colonies were compared and reference spectrum generated.

between 28 to 37°C, with the optimal growth temperature being 37°C. Colonies were dark grey and about 0.1 to 0.3 mm in diameter on 5% sheep's blood–enriched Columbia agar (bioMérieux). Gram staining performed using the Aerospray Gram series (ELITechGroup Biomedical Systems, Puteaux, France) showed rod-shaped Gram-negative bacilli (Fig. 3). These rods were not motile and were unable to form spores.

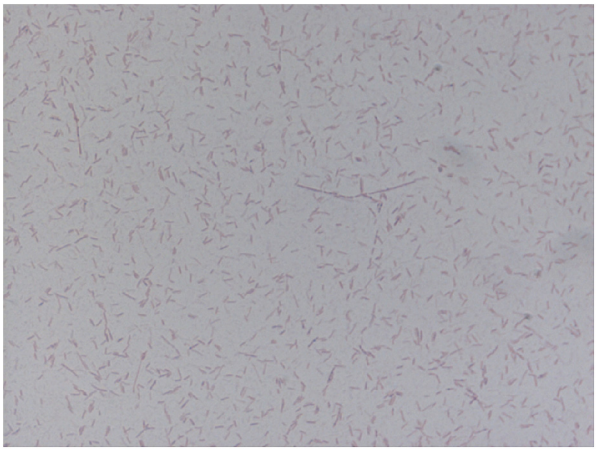

FIG. 3. Transmission electron microscopy of *'Dakarella massiliensis'* strain ND3^T using Tecnai G20(Fei) at operating voltage of 60 kV. Scale bar = 500 nm.

For electronic microscopy, detection coated grids were deposited on a 40 µL bacterial suspension drop and incubated for 30 minutes at 37°C. The grids were incubated for 1 second on ammonium molybdate 1%, dried on blotting paper and then observed with a Tecnai G20 transmission electron microscope (FEI Company, Limeil-Brevannes, France) at an operating voltage of 60 kV. Using electron microscopy, cells had a mean length of 2.1 µm (range, 1.7–2.6 µm) and width of 0.9 µm (range, 0.60–1.2 µm) (Fig. 4).

Growth of the strain was tested under anaerobic and microaerophilic conditions using GENbag anaer and GENbag microaer systems respectively (bioMérieux), and under aerobic conditions, with and without 5% CO_2. Growth was only observed in anaerobic conditions. Salinity was tested on agar plates at different concentrations of salt (0, 15, 50 and 100 g/L), but no growth was observed with salt. Strain ND3^T did not exhibit oxidase or catalase activities.

Using API ZYM strips (bioMérieux), positive reactions were observed for phosphatase alkaline, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, phosphatase acid, naphtol phosphohydrolase, α-galactosidase, α-galactosidase, β-glucosidase and α-mannosidase. Using an API 50CH strip (bioMérieux), positive reactions were observed for D-galactose, D-glucose, esculin, salicin, D-cellobiose, D-maltose, D-lactose, D-

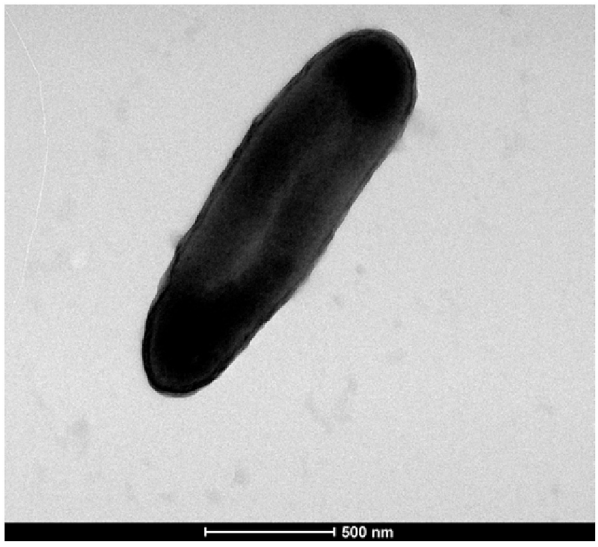

FIG. 4. Gram staining of *'Dakarella massiliensis'* strain ND3^T.

saccharose, D-trehalose, D-raffinose, amidon, glycogen, D-turanose, D-tagatose and glycerol. Using API Rapid ID 32A strip (bioMérieux), a positive reaction was only observed for arginine arylamidase. Overall, these biochemical results are consistent with those of *Sutterella parvirubra* [15].

The *in vitro* susceptibility of strain ND3^T to antimicrobial agents was tested using the diffusion method with antibiotic disks (i2a, Montpellier, France) [16]. Strain ND3^T was susceptible to penicillin, amoxicillin, amoxicillin–clavulanate, ceftriaxone, imipenem, ciprofloxacin, clindamycin, doxycycline, erythromycin, gentamicin, metronidazole and rifampicin but resistant to trimethoprim–sulfamethoxazole and vancomycin.

Phenotypic comparison between strain ND3^T and *Sutterella wadsworthensis* as well as other representative species from validly published members of the family *Sutterellaceae* are summarized in Table 1.

Genome Sequencing Information

Growth conditions and genomic DNA preparation

Strain ND3^T was grown anaerobically on 5% sheep's blood–enriched Columbia agar (bioMérieux) at 37°C. Colonies from five petri dishes were collected and resuspended in 4 × 100 µL of Tris-EDTA buffer (TE) buffer. Then 200 µL of this suspension was diluted in 1 mL of TE buffer for lysis treatment including a 30-minute incubation with 2.5 µg/µL lysozyme at 37°C, followed by overnight incubation with 20 µg/µL proteinase K at 37°C. Extracted DNA was then purified using 3 successive phenol–chloroform extractions and ethanol precipitations at −20°C overnight. After centrifugation, the DNA was resuspended in 160 µL of TE buffer.

Genome sequencing and assembly

Using the mate-pair strategy, genomic DNA of *'Dakarella massiliensis'* strain ND3^T was sequenced on the MiSeq sequencer (Illumina, San Diego, CA, USA) [17–21]. The gDNA was barcoded 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

TABLE 1. Phenotypic features that distinguish *Dakarella massiliensis* strain ND3^T from the closely related type strains for *Alicycliphilus denitrificans* strain K601, *Caldimonas manganoxidans* strain JCM 10698, *Comamonas badia* strain IAM 14839, *Comamonas composti* strain YY287, *Lautropia mirabilis* strain ATCC 51599, *Parasutterella excrementihominis* strain YIT 11859 and *Sphaerotilus natans* strain DSM 6575

Characteristic	*D. massiliensis*	*A. denitrificans*	*C. manganoxidans*	*C. badia*	*C. composti*	*L. mirabilis*	*P. excrementihominis*	*S. natans*
Cell diameter (µm)	0.70	0.5–1.0	0.5–0.7	0.8–0.9	0.5	1.5–2	0.4–1.1	1.2–2.06
Oxygen requirement	Anaerobic	Anaerobic	Aerobic	Anaerobic	Anaerobic	Facultative anaerobic	Anaerobic	Anaerobic
Gram stain	−	−	−	−	−	−	−	−
Motility	−	+	+	+	+	+	−	+
Endospore formation	−	NA	−	−	−	−	−	NA
DNA G+C content (mol%)	57	68	66	66	63.3	65.6	48.1	69.9
Biochemical								
Oxidase	−	+	NA	+	+	+	−	+
Catalase	−	NA	+	+	+	+	−	+
Indole	+	NA	−	NA	−	NA	−	−
Nitrate reductase		NA	NA	NA	+	+	−	+
L-Arabinose	−	−	NA	NA	−	−	−	+
Mannitol	−	NA	+	−	−	+	NA	−
D-Maltose	−	+	−	NA	+	+	NA	+
D-Lactose	−	+	NA	NA	−	−	NA	+
α-Glucosidase	−	NA	+	NA	−	NA	−	NA
β-Glucosidase	+	NA	−	NA	−	NA	−	NA
Urease	−	NA	−	−	−	+	−	NA
Lipase	+	NA	−	+	+	NA	w	NA
Isolated from:	Human gut and vagina	Sewage	Hot spring	Sludge	Food waste	Human oral cavity	Human gut	Freshwater

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

Nextera Mate-Pair Illumina guide, and the gDNA sample was simultaneously fragmented and tagged with a Mate-Pair junction adapter. The pattern of the fragmentation was validated on an Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) with a DNA 7500 labchip. The DNA fragments ranged in size from 1 to 10 kb, with an optimal size at 4.08 kb. No size selection was performed, and only 464 ng of tagged fragments were circularized [17–21]. The circularized DNA was mechanically sheared to small fragments with an optimal size of 569 bp in microtubes on the Covaris S2 device (Covaris, Woburn, MA, USA). The library profile was visualized on a High Sensitivity Bioanalyzer LabChip (Agilent Technologies), and the final library concentration was measured at 24.4 nmol/L. The libraries were normalized at 2 nM and pooled. After a denaturation step and dilution at 15 pM, the pool of libraries was loaded onto the reagent cartridge and then onto the instrument along with the flow cell. Automated cluster generation and sequencing run were performed in a single 39-hour run in a 2 × 251 bp read length. Total information of 10.1 Gb was obtained from a 1189K/mm^2 cluster density, with a cluster passing quality control filters of 99.1% (22 579 000 clusters). The obtained reads were trimmed; assembly was then performed using the CLC genomicsWB4 software [17–21].

Genome annotation

Open reading frames (ORFs) were predicted using Prodigal [22] with default parameters. However, the predicted ORFs were excluded if they spanned a sequencing gap region. The predicted bacterial protein sequences were searched against the GenBank [23] and Clusters of Orthologous Groups (COGs) databases using BLASTP. The tRNAs and rRNAs were predicted using tRNAScan-SE [24] and RNAmmer [25] tools respectively. Signal peptides and numbers of transmembrane helices were predicted using SignalP [26] and TMHMM [27] respectively. Mobile genetic elements were predicted using PHAST [27] and RAST [28]. ORFans were identified if their BLASTP *E* value was lower than 1e-03 for alignment length greater than 80 amino acids. If alignment length was smaller than 80 amino acids, we used an *E* value of 1e-05. Such parameter thresholds have already been used in previous works to define ORFans. Artemis [29] and DNA Plotter [30] were used for data management and visualization of genomic features respectively. The Mauve alignment tool (version 2.3.1) was used for multiple genomic sequence alignment [31].

The mean level of nucleotide sequence similarity at the genome level between *'Dakarella massiliensis'* strain ND3^T and other bacteria was estimated using the average genomic identity of orthologous gene sequences (AGIOS) homemade software. This software can combine with others: Proteinortho (to detect orthologous proteins between genomes compared two by two, and then retrieve the corresponding genes) and the Needleman-Wunsch global alignment algorithm (to determine the mean percentage of nucleotide sequence identity among orthologous ORFs).

TABLE 2. Number of genes associated with 25 general COGs functional categories

Code	Value	% of total[a]	Description
J	153	7.04	Translation
A	2	0.09	RNA processing and modification
K	141	6.49	Transcription
L	132	6.07	Replication, recombination and repair
B	0	0.0	Chromatin structure and dynamics
D	23	1.06	Cell cycle control, mitosis and meiosis
Y	0	0.0	Nuclear structure
V	26	1.20	Defense mechanisms
T	80	3.68	Signal transduction mechanisms
M	145	6.67	Cell wall/membrane biogenesis
N	1	0.05	Cell motility
Z	0	0.0	Cytoskeleton
W	6	0.28	Extracellular structures
U	53	2.44	Intracellular trafficking and secretion
O	87	4.0	Posttranslational modification, protein turnover, chaperones
C	174	8.0	Energy production and conversion
G	74	3.40	Carbohydrate transport and metabolism
E	198	9.11	Amino acid transport and metabolism
F	57	2.62	Nucleotide transport and metabolism
H	77	3.54	Coenzyme transport and metabolism
I	58	2.67	Lipid transport and metabolism
P	113	5.20	Inorganic ion transport and metabolism
Q	27	1.24	Secondary metabolites biosynthesis, transport and catabolism
R	260	11.96	General function prediction only
S	118	5.43	Function unknown
—	171	7.87	Not in COGs

COGs, Clusters of Orthologous Groups database.
[a]Total is based on total number of protein-coding genes in annotated genome.

Genome properties

The genome of *'Dakarella massiliensis'* strain ND3^T is 2 476 884 bp long with a 56.98% G+C content (Table 2, Fig. 5). It is composed of seven scaffolds (composed of seven contigs). Of the 2236 predicted genes, 2175 were protein-coding genes and 62 were RNA genes (five 5S rRNA, two 16S rRNA, five 23S rRNA and 50 tRNA genes). A total of 1780 genes (79.6%) were assigned a putative function. A total of 59 genes (2.63%) were identified as ORFans. The remaining genes were annotated as hypothetical proteins. The properties and statistics of the genome are summarized in Table 3, while the distribution of genes into COGs functional categories is presented in Table 2.

Insights From the Genome Sequence

The genome size of *'Dakarella massiliensis'* strain ND3^T is smaller than those of *Alicycliphilus denitrificans* strain K601, *Comamonas composti* strain YY287, *Sphaerotilus natans* strain DSM 6575, *Comamonas badia* strain IAM 14839, *Caldimonas manganoxidans* strain JCM 10698, *Lautropia mirabilis* strain ATCC 51599 and *Parasutterella excrementihominis* strain YIT

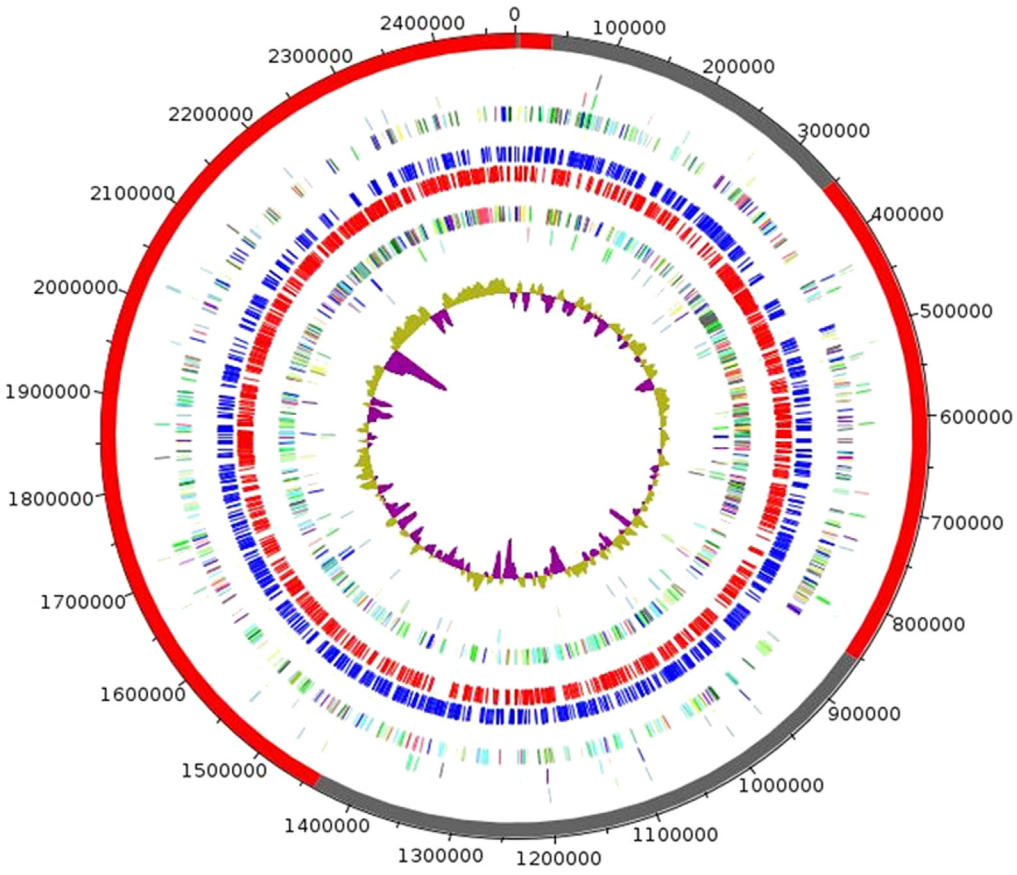

FIG. 5. Graphical circular map of chromosome. From outside to centre: genes on forward strand coloured by COGs categories (only genes assigned to COGs), genes on reverse strand coloured by COGs categories (only genes assigned to COGs), RNA genes (tRNAs green, rRNAs red), GC content and GC skew. COGS, Clusters of Orthologous Groups database.

TABLE 3. Nucleotide content and gene count levels of genome

	Genome (total)	
Attribute	**Value**	**% of total**[a]
Size (bp)	2 476 884	100
G+C content (%)	1 411 823	57.0
Coding region (bp)	2 126 880	85.86
Total genes	2236	100
RNA genes	62	2.77
Protein-coding genes	2174	97.22
Genes with function prediction	1780	79.60
Genes assigned to COGs	1609	71.95
Genes with peptide signals	420	18.78
No. of pseudogenes	76	3.39
Genes with transmembrane helices	428	19.14
CRISPR repeats	01	0.04
No. of genes with Pfam-A domains	2001	89.49
ORFan genes	59	2.63

COG2, Clusters of Orthologous Groups database.
[a]Total is based on either size of genome in base pairs or total number of protein-coding genes in annotated genome.

11859 (5.0, 4.63, 4.59, 3.68, 3.53, 3.15 and 2.83 Mb respectively). The G+C content of *'Dakarella massiliensis'* is lower than those of *Sphaerotilus natans, Alicycliphilus denitrificans, Caldimonas manganoxidans, Comamonas badia* and *Comamonas composti* (69.9, 68, 66, 66, 65.6 and 63.3% respectively) but higher than that of *Parasutterella excrementihominis* (48.1%). The protein-coding genes of *Dakarella massiliensis* (2175) are smaller than those of *Alicycliphilus denitrificans, Sphaerotilus natans, Comamonas composti, Comamonas badia, Caldimonas manganoxidans, Parasutterella excrementihominis* and *Lautropia mirabilis* (4573, 3898, 3893, 3388, 3187, 2470 and 2413 respectively). The gene content of *'Dakarella massiliensis'* (2236) is smaller than that of *Alicycliphilus denitrificans, Sphaerotilus natans, Comamonas composti, Comamonas badia, Caldimonas manganoxidans, Parasutterella excrementihominis* and *Lautropia mirabilis* (4705, 4143, 4705, 4078, 3499, 3385, 2570 and 2569) (Table 4). In addition, the comparison according the numbers of orthologous protein shared between genomes is summarized in Table 5.

Among species with standing in nomenclature, AGIOS values ranged from 79.04 between *Parasutterella excrementihominis* and

TABLE 4. Genome comparison of closely related species to *Dakarella massiliensis* strain ND3^T

Microorganism	INSDC	Size (Mb)	G+C (%)	Protein- coding genes	Total genes
Dakarella massiliensis strain ND3	CVTY00000000.1	2.47	57	2174	2236
Alicycliphilus denitrificans strain K601	CP002657.1	5.0	68	4573	4705
Caldimonas manganoxidans strain JCM 10698	ARLH00000000.1	3.53	66	3187	3385
Comamonas badia strain IAM 14839	AXVM00000000.1	3.68	66	3388	3499
Comamonas composti strain YY287	AUCQ00000000.1	4.63	63.3	3893	4078
Lautropia mirabilis strain ATCC 51599	AEQP00000000.1	3.15	65.6	2413	2569
Parasutterella excrementihominis strain YIT 11859	AFBP00000000.1	2.83	48.1	2470	2570
Sphaerotilus natans strain DSM 6575	AZRA00000000.1	4.59	69.9	3898	4143

INSDC, International Nucleotide Sequence Database Collaboration.

TABLE 5. Numbers of orthologous protein shared between genomes (upper right)[a]

	Alicycliphilus denitrificans	*Caldimonas manganoxidans*	*Comamonas badia*	*Comamonas composti*	*Lautropia mirabilis*	*Parasutterella excrementihominis*	*Sphaerotilus natans*	*Dakarella massiliensis*
A. denitrificans	**4706**	1597	1710	1871	1089	786	1564	771
C. manganoxidans	72.74	**3369**	1399	1530	1020	756	1453	729
C. badia	79.04	71.25	**3479**	1679	1011	739	1360	724
C. composti	77.53	70.89	74.56	**4058**	1063	776	1523	755
L. mirabilis	69.18	67.93	67.73	67.53	**2541**	701	999	680
P. excrementihominis	60.00	60.65	60.17	60.59	60.54	**2552**	744	757
S. natans	74.24	74.69	72.47	71.46	69.40	60.13	**4085**	726
D. massiliensis	63.15	62.83	62.95	62.66	63.25	63.91	63.84	**2174**

[a]Average percentage similarity of nucleotides corresponding to orthologous protein shared between genomes (lower left) and numbers of proteins per genome (bold).

TABLE 6. Pairwise comparison of *Dakarella massiliensis* strain ND3 with other species using GGDC, formula 2 (DDH estimates based on identities/HSP length)[a] (upper right)

	Alicycliphilus denitrificans	*Caldimonas manganoxidans*	*Comamonas badia*	*Comamonas composti*	*Dakarella massiliensis*	*Lautropia mirabilis*	*Parasutterella excrementihominis*	*Sphaerotilus natans*
A. denitrificans	100% ± 00	18.8% ± 2.65	18.6% ± 2.60	19.6% ± 2.58	22.4% ± 2.53	19.2% ± 2.55	34.4% ± 2.52	20.1% ± 2.67
C. manganoxidans		100% ± 00	22.8% ± 2.93	22.3% ± 2.82	24.7% ± 2.53	18.6% ± 2.58	32.1% ± 2.52	20.1% ± 2.69
C. badia			100% ± 00	20.8% ± 2.71	26.7% ± 2.53	17.9% ± 2.56	33.7% ± 2.52	19.1% ± 2.65
C. composti				100% ± 00	26.9% ± 2.52	23.2% ± 2.53	26.8% ± 2.53	22.1% ± 2.53
D. massiliensis					100% ± 00	19.2% ± 2.55	32.7% ± 2.52	19.8% ± 2.60
L. mirabilis						100% ± 00	35.0% ± 2.52	18.7% ± 2.57
P. excrementihominis							100% ± 00	31.5% ± 2.52
S. natans								100% ± 00

DDH, DNA-DNA hybridization; GGDC, Genome-to-Genome Distance Calculator; HSP, high-scoring segment pairs.
[a]Confidence intervals indicate the inherent uncertainty in estimating DDH values from intergenomic distances based on models derived from empirical test data sets (which are always limited in size) These results are in accordance with the 16S rRNA (Fig. 1) and phylogenomic analyses as well as the GGDC results.

Alicycliphilus denitrificans to 60.00 between *Comamonas badia* and *Alicycliphilus denitrificans*. The genomic similarity of strain ND3T with species of *Comamonadaceae* family was also evaluated by two parameters: DNA-DNA hybridization (DDH) and AGIOS [32–34]. The values found in DDH and AGIOS of *'Dakarella massiliensis'* are in the range of those observed in the other genera of this family (Table 6).

Conclusion

Having analysed the phenotypic, phylogenetic and genomic results, we formally propose a new genus *'Dakarella'* with *'Dakarella massiliensis'* as the type strain. Strain ND3^T was isolated among the vaginal flora of a 28-year-old woman with bacterial vaginosis.

Description of *'Dakarella'* gen. nov.

'Dakarella' (Da.ka.rel'la, M.L. dim. suffix, usel'la; M.L. fem. n.) was chosen to honor Dakar, the capital of Senegal. Gram-negative rods. Strictly anaerobic. Mesophilic. Nonmotile. Does not exhibit catalase, oxidase. Positive for phosphatase alkaline, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, trypsine, α-chymotrypsin, phosphatase acid, naphtol phosphohydrolase, α-galactosidase, α-galactosidase, β-glucosidase, α-mannosidase, D-galactose, D-glucose, esculin, salicin, D-cellobiose, D-maltose, D-lactose, D-saccharose, D-trehalose, D-raffinose, amidon, glycogen, D-turanose, D-tagatose, glycerol and arginine arylamidase. Habitat: human vaginal flora. Type species: *'Dakarella massiliensis.'*

Description of *'Dakarella massiliensis'* gen. nov., sp. nov.

'Dakarella massiliensis' (mas.il'ien'sis, L. gen. fem. n., *massiliensis,* 'of Massilia,' the Latin name of Marseille, where strain ND3^T was isolated).

Gram-negative rods. Strictly anaerobic. Mesophilic. Nonmotile. Optimal growth at 37°C. Nonmotile and non-sporulating. Strain ND3^T exhibited neither catalase nor oxidase activities. Colonies are dark grey with a diameter of 0.1 to 0.3 mm on 5% sheep's blood–enriched Columbia agar (bioMérieux). Cells are rods with a mean length of 2.1 µm and width of 0.9 µm. Positive for phosphatase alkaline, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, trypsine, α-chymotrypsin, phosphatase acid, naphtol phosphohydrolase, α-galactosidase, α-galactosidase, β-glucosidase, α-mannosidase, D-galactose, D-glucose, esculin, salicin, D-cellobiose, D-maltose, D-lactose, D-saccharose, D-trehalose, D-raffinose, amidon, glycogen, D-turanose, D-tagatose, glycerol and arginine arylamidase. Strain ND3^T is susceptible to penicillin, amoxicillin, amoxicillin–clavulanate, ceftriaxone, imipenem, ciprofloxacin, clindamycin, doxycycline, erythromycin, gentamicin, metronidazole and rifampicin but resistant to trimethoprim–sulfamethoxazole and vancomycin.

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LK054638 and CVTY00000000.1 respectively. The genome is 2 476 884 bp long, with a G+C content of 56.98%. The type strain ND3^T (= CSUR P1938 = DSM 100447) was isolated from the vaginal flora of a 28-year-old woman with bacterial vaginosis.

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Conflict of Interest

None declared.

References

[1] Madhivanan P, Krupp K, Chandrasekaran V, Karat C, Arun A, Cohen C, et al. Prevalence and correlates of bacterial vaginosis among young women of reproductive age in Mysore, India. Indian J Med Microbiol 2008;26:132–7.

[2] Bradshaw CS, Brotman RM. Making inroads into improving treatment of bacterial vaginosis—striving for long-term cure. BMC Infect Dis 2015;15:292.

[3] Bradshaw CS, Morton AN, Hocking J, Garland SM, Morris MB, Moss LM, et al. High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. J Infect Dis 2006;193:1478–86.

[4] Fredricks DN, Fiedler TL, Marrazzo JM. Molecular identification of bacteria associated with bacterial vaginosis. N Engl J Med 2005;353:1899–911.

[5] Fredricks DN, Fiedler TL, Thomas KK, Oakley BB, Marrazzo JM. Targeted PCR for detection of vaginal bacteria associated with bacterial vaginosis. J Clin Microbiol 2007;45:3270–6.

[6] Fredricks DN, Fiedler TL, Thomas KK, Mitchell CM, Marrazzo JM. Changes in vaginal bacterial concentrations with intravaginal metronidazole therapy for bacterial vaginosis as assessed by quantitative PCR. J Clin Microbiol 2009;47:721–6.

[7] Lagier JC, 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.

[8] Pfleiderer A, Lagier JC, Armougom F, Robert C, Vialettes B, Raoult D. Culturomics identified 11 new bacterial species from a single anorexia nervosa stool sample. Eur J Clin Microbiol Infect Dis 2013;32:1471–81.

[9] Menard JP, Fenollar F, Henry M, Bretelle F, Raoult D. Molecular quantification of *Gardnerella vaginalis* and *Atopobium vaginae* loads to predict bacterial vaginosis. Clin Infect Dis 2008;47:33–43.

[10] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, 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.

[11] Rossi-Tamisier M, Benamar S, Raoult D, Fournier PE. Cautionary tale of using 16S rRNA gene sequence similarity values in identification of human-associated bacterial species. Int J Syst Evol Microbiol 2015;65:1929–34.

[12] Mukhopadhya I, Hansen R, Nicholl CE, Alhaidan YA, Thomson JM, Berry SH, et al. A comprehensive evaluation of colonic mucosal isolates of *Sutterella wadsworthensis* from inflammatory bowel disease. PLoS One 2011;6:e27076.

[13] Stackebrant E. Molecular taxonomic parameters. Microbiol Aust 2011;32:59–61.

[14] Rosselló-Mora R. DNA-DNA reassociation methods applied to microbial taxonomy and their critical evaluation. In: Stackebrandt PDE, editor. Molecular identification, systematics, and population structure of prokaryotes. Berlin: Springer; 2006. p. 23–50.

[15] Sakon H, Nagai F, Morotomi M, Tanaka R. *Sutterella parvirubra* sp. nov. and *Megamonas funiformis* sp. nov., isolated from human faeces. Int J Syst Evol Microbiol 2008;58:970–5.

[16] Jorgensen JH, Turnidge JD. Susceptibility test methods: dilution and disk diffusion methods. In: Pfaller MA, Richter SS, Funke G, Jorgensen JH, Landry ML, Carroll KC, et al., editors. Manual of clinical microbiology. 11th ed. Washington, DC: American Society for Microbiology; 2015. p. 1253–73.

[17] Lagier JC, El Karkouri K, Nguyen TT, Armougom F, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of *Anaerococcus senegalensis* sp. nov. Stand Genomic Sci 2012;6:116–25.

[18] Lagier JC, Armougom F, Mishra AK, Nguyen TT, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of *Alistipes timonensis* sp. nov. Stand Genomic Sci 2012;6:315–24.

[19] Mishra AK, Lagier JC, Robert C, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of *Clostridium senegalense* sp. nov. Stand Genomic Sci 2012;6:386–95.

[20] Mishra AK, Lagier JC, Robert C, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of *Peptoniphilus timonensis* sp. nov. Stand Genomic Sci 2012;7:1–11.
[21] Mishra AK, Lagier JC, Nguyen TT, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of *Peptoniphilus senegalensis* sp. nov. Stand Genomic Sci 2013;7:370–81.
[22] Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 2010;11:119.
[23] Benson DA, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic Acids Res 2015;43:D30–5.
[24] Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 1997;25:955–64.
[25] Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 2007;35:3100–8.
[26] Bendtsen JD, Nielsen H, von Heijne G, Brunak S. Improved prediction of signal peptides: SignalP 3.0. J Mol Biol 2004;340:783–95.
[27] Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. PHAST: a fast phage search tool. Nucleic Acids Res 2011;39:W347–52.
[28] Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 2008;9:75.
[29] Rutherford K, Parkhill J, Crook J, Horsnell T, Rice P, Rajandream MA, et al. Artemis: sequence visualization and annotation. Bioinforma Oxf Engl 2000;16:944–5.
[30] Carver T, Thomson N, Bleasby A, Berriman M, Parkhill J. DNAPlotter: circular and linear interactive genome visualization. Bioinforma Oxf Engl 2009;25:119–20.
[31] Darling ACE, Mau B, Blattner FR, Perna NT. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res 2004;14:1394–403.
[32] Auch AF, von Jan M, Klenk HP, Göker M. Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. Stand Genomic Sci 2010;2:117–34.
[33] Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence–based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 2013;14:60.
[34] Ramasamy D, Mishra AK, Lagier JC, 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.