Dakarella massiliensis gen. nov., sp. nov., strain ND3T: a new bacterial genus isolated from the female genital tract

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Abstract

Strain ND3T 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 ND3T was a strictly anaerobic Gram-negative rod and member of the family Sutterellaceae. It exhibited a genome of 2,476,884 bp containing 2,175 protein-coding and 62 RNA genes. On the basis of these data, we propose the creation of ‘Dakarella massiliensis’ sp. nov. with strain ND3T (= CSUR P1938 = DSM 100447) as the type strain.

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Introduction

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 ND3T (= CSUR P1938 = DSM 100447).
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 ND3T 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 ND3T 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 ND3T 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

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**FIG. 1.** Phylogenetic tree highlighting position of 'Dakarella massiliensis' strain ND3T 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.

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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. 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, Limel-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% CO₂. 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 ND3ᵀ was found to 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-
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 ND3T to antimicrobial agents was tested using the diffusion method with antibiotic disks (i2a, Montpellier, France) [16]. Strain ND3T 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 ND3T 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 ND3T 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. 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 ND3T was sequenced on the MiSeq sequencer (Illumina, San Diego, CA, USA) [17–21]. The gDNA was bar-coded in order to be mixed with 11 other projects with the Nextera Mate-Pair sample prep kit (Illumina), the Mate-Pair library was prepared with 1 μg of genomic DNA using the

### TABLE 1

| 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 |
| DNA G+C content (mol%) | 57 | 68 | 66 | 66 | 63.3 | 65.6 | 48.1 | 69.9 |
| Gram stain | Anaerobic | Anaerobic | Anaerobic | Anaerobic | Anaerobic | Anaerobic | Anaerobic | Anaerobic |
| Oxygen requirement | Anaerobic | Anaerobic | Anaerobic | Anaerobic | Anaerobic | Anaerobic | Anaerobic | Anaerobic |
| Motility | − | + | + | + | + | + | + | + |
| Endospore formation | − | NA | − | − | − | − | NA | NA |
| Oxidase | − | + | NA | + | + | + | + | + |
| Catalase | − | NA | + | + | + | + | + | + |
| Indole | + | NA | NA | NA | NA | − | NA |
| Nitrate reductase | − | NA | NA | NA | NA | − | NA |
| L-Arabinose | − | NA | − | − | − | − | − | − |
| Mannitol | − | NA | − | − | − | − | − | − |
| α-Maltose | − | NA | − | − | − | − | − | − |
| β-Lactose | − | NA | − | − | − | − | − | − |
| α-Glucosidase | − | NA | − | − | − | − | − | − |
| β-Glucosidase | − | NA | − | − | − | − | − | − |
| Urease | − | NA | − | − | − | − | − | − |
| Lipase | − | NA | − | − | − | − | − | − |
| Isolated from: Human gut and vagina | Sewage | Hot springs | Sludge | Food waste | Human oral cavity | Human gut | Freshwater |

*+, positive result; −, negative result; w, weakly positive result; NA, data not available.
Nextera Mate-Pair Illuma 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/mm² cluster density, with a cluster separation of 99.1% (22 579 000 clusters). The obtained reads were trimmed; assembly was then performed using the CLC genomics WB4 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 genome sequence alignment [31].

The mean level of nucleotide sequence similarity at the genome level between ‘Dakarella massiliensis’ strain ND3T 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 ORFans).

**Genome properties**

The genome of ‘Dakarella massiliensis’ strain ND3T 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 ND3T is smaller than those of Alcicycliphilus denitrificans strain K601, Comamonas composti strain YY287, Sphaerotilus natans strain DSM 6575, Comamonas bodia strain IAM 14839, Caldimonas manganoxidans strain JCM 10698, Lautropia mirabilis strain ATCC 51599 and Parasutterella excrementominis strain YIT

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

| Code | Value | % of total | 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     | 0.00       | Chromatin structure and dynamics |
| D    | 23    | 1.06       | Cell cycle control, mitosis and meiosis |
| Y    | 0     | 0.00       | 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     | 0.00       | Cytoplasm |
| W    | 6     | 0.28       | Extracellular structures |
| U    | 53    | 2.44       | Intracellular trafficking and secretion |
| O    | 87    | 4.00       | Posttranslational modification, protein turnover, chaperones |
| C    | 174   | 8.00       | 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 |
| P    | 113   | 5.20       | Lipid transport and metabolism |
| Q    | 27    | 1.24       | Inorganic ion transport and metabolism |
| R    | 260   | 11.96      | Secondary metabolites biosynthesis, transport and catabolism |
| S    | 118   | 5.43       | Function unknown |
| N    | 171   | 7.87       | Not in COGs |

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

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![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.](image)

### Table 3. Nucleotide content and gene count levels of genome

| Attribute                      | Genome (total) | % of total |
|--------------------------------|----------------|------------|
| Size (bp)                      | 2476884        | 100        |
| G+C content (%)                | 1411823        | 57.0       |
| Coding region (bp)             | 2126880        | 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       |

COGS, Clusters of Orthologous Groups database.

*Total is based on either size of genome in base pairs or total number of protein-coding genes in annotated genome.*
Pairwise comparison of Dakarella massiliensis with species of this family (Table 6).  

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

| Microorganism | INSDC          | Size (Mb) | G+C (%) | Protein-coding genes | Total genes |
|---------------|----------------|-----------|---------|----------------------|-------------|
| Dakarella massiliensis strain ND3 | CVTT00000000.1 | 2.47      | 57      | 2174                 | 2236        |
| Alicycliphilus denitrificans strain K601 | CP002557.1 | 5.0       | 68      | 4573                 | 4705        |
| Caldimonas manganoxidans strain JCM 10698 | ARLH00000000.1 | 3.53     | 66      | 3187                 | 3385        |
| Comamonas badia strain IAM 14829 | AKVP00000000.1 | 3.68     | 66      | 3388                 | 3499        |
| Comamonas compositi strain YT287 | AQQ90000000.1 | 4.63     | 63.3    | 3893                 | 4078        |
| Lautropia mirabilis strain ATCC 51599 | AEOQ00000000.1 | 3.15     | 65.6    | 2413                 | 2569        |
| Parasutterella excrementihominis strain YIT 11859 | ARFB00000000.1 | 2.83     | 48.1    | 2470                 | 2570        |
| Sphaerotilus natans strain DSM 6375 | ACPA00000000.1 | 4.59     | 69.9    | 3898                 | 4143        |

**TABLE 5. Numbers of orthologous protein shared between genomes (upper right)**

| Microorganism | Alicycliphilus denitrificans | Caldimonas manganoxidans | Comamonas badia | Comamonas compositi | 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. compositi     | 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                   |

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

| Microorganism | Alicycliphilus denitrificans | Caldimonas manganoxidans | Comamonas badia | Comamonas compositi | 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             | 22.4% ± 2.53    | 24.7% ± 2.53        | 18.6% ± 2.58           | 32.1% ± 2.52         | 33.7% ± 2.52                  | 20.1% ± 2.69        |
| C. badia        | 100% ± 00                    | 20.8% ± 2.71             | 26.7% ± 2.53    | 17.9% ± 2.56        | 26.8% ± 2.53           | 19.1% ± 2.65         | 22.1% ± 2.53                  | 18.7% ± 2.57        |
| C. compositi    | 100% ± 00                    | 26.9% ± 2.52             | 23.2% ± 2.53    | 22.8% ± 2.53        | 26.8% ± 2.53           | 22.1% ± 2.53         | 21.5% ± 2.52                  | 21.5% ± 2.52        |
| L. mirabilis    | 100% ± 00                    | 19.2% ± 2.55             | 32.7% ± 2.52    | 19.8% ± 2.60        | 35.0% ± 2.52           | 18.7% ± 2.57         | 33.5% ± 2.52                  | 33.5% ± 2.52        |
| P. excrementihominis | 100% ± 00                | 100% ± 00                 | 100% ± 00       | 100% ± 00            | 100% ± 00              | 100% ± 00             | 100% ± 00                     | 100% ± 00           |

DDH, DNA-DNA hybridization; GGDC, Genome-to-Genome Distance Calculator; HSP, high-scoring segment pairs.  
*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 ND3T 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, trypsin, α-chymotrypsin, phosphatase acid, naphth-phosphohydrolase, α-galactosidase, α-galactosidase, β-glucosidase, α-mannosidase, β-galactose, β-glucose, esculin, salicin, α-cellobiose, β-maltose, β-lactose, D-saccharose, D-trehalose, D-raffinose, amidon, glycogen, D-turanose, D-tagatose, glycero and arginine arylamidase. Habitat: human vaginal flora. Type species: ‘Dakarella massiliensis’.
Description of ‘Dakarella massiliensis’ gen. nov., sp. nov.

‘Dakarella massiliensis’ (mas.ill’en’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 nonsporulating. 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 (bio-Mérieux). 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, trypsin, α-chymotrypsin, phosphatase acid, naphthol phosphoehydrolase, α-galactosidase, α-galactosidase, β-glucosidase, α-mannosidase, β-galactosidase, glucose, galactose, esculin, salicin, D-celllobiose, D-maltose, D-lactose, D-saccharose, D-trehalose, D-raffinose, amidon, glycogen, D-turanose, D-taraganose, glycerol and arginine arylamidase. Strain ND3\(^T\) is susceptible to penicillin, amoxicillin, amoxicillin–clavulanate, ceftriaxone, ampicillin, 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 CVTT00000000.1 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.

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