Noncontiguous finished genome sequence and description of *Murdochiella massiliensis* strain SIT12 sp. nov.

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Abstract

Murdochiella massiliensis strain SIT12 (= CSUR P1987 = DSM 29078) is the type strain of *M. massiliensis* sp. nov. This bacterium was isolated from the stool of a healthy 2-year-old Senegalese boy. *M. massiliensis* is an anaerobic, Gram-positive coccus. The genome size of *M. massiliensis* strain SIT12 is 1 642 295 bp with 48.9% G+C content and assembled into two scaffolds.

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The human microbiome is a complex and dynamic mixture of microorganisms. It is composed of different microbial communities present in different parts of the human body, each of which interacts with its host and has an impact on human health and disease [1].

The human gastrointestinal tract contains an enormous variety of aerobic and anaerobic bacteria [2]. Gram-positive anaerobic cocci account for about 25 to 30% of all anaerobic isolates recovered from clinical samples [3]. *Murdochiella massiliensis* sp. nov. strain SIT12 (= CSUR P1987 = DSM 29078) is the type strain of *M. massiliensis* sp. nov., a new species within the genus *Murdochiella*. It is an anaerobic, Gram-stain-positive, coccus-shaped, nonmotile, non-spore-forming bacteria. *M. massiliensis* was isolated in Marseille from a stool sample of a healthy 2-year-old boy as part of the culturomics project, which was developed in 2012 to study human gut microbiota [1]. This work has significantly expanded our knowledge of the diversity of human gut bacterial species [4]. Currently, bacterial taxonomy is based on the combination of phenotypic and genotypic characteristics [5,6]. In the last few years, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as a promising technique for bacterial identification [7]. It offers for the first time the possibility of identifying clinical isolates with high accuracy and high speed for a low cost, in contrast to conventional procedures [8]. This polyphasic approach to describe new bacterial species, which includes genome sequences, MALDI-TOF MS spectra and phenotypic characteristics, was named taxonogenomics [9]. The first and only type species in the genus *Murdochiella* is *Murdochiella asaccharolytica*, isolated from human wound specimens [3].

The following is a summary classification and set of features for *Murdochiella massiliensis* sp. nov. strain SIT12 with a description of its genomic sequencing and annotation.

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Organism Information

Classification and features

A stool sample was obtained from a healthy 2-year-old boy living in Senegal. The stool was sent to and frozen in Marseille at −80°C until laboratory culture isolation. Strain SIT12 was isolated in May 2015 by cultivation on marine medium in anaerobic atmosphere after 21 days' incubation.

Growth of the strain was tested under anaerobic and microaerophilic conditions using GENbag anaer and GENbag microaer systems respectively (bioMérieux, Marcy l'Étoile, France), and in the presence of air, with or without 5% CO_2, but growth was achieved only under anaerobic conditions. *M. massiliensis* grew at mesophilic temperatures between 25 and 42°C after 48 hours' incubation on Columbia agar with 5% sheep's blood, chocolate agar and Müller-Hinton agar. Growth occurred at pH 6, 6.5, 7 and 8.5 and exhibited tolerance for NaCl until a concentration of 5 g/L.

Gram staining and electron microscopy of *M. massiliensis* were performed using a TechnaiG^2 Cryo (FEI Company, Limeil-Brevannes, France) at an operating voltage of 200 keV. Strain SIT12 was Gram positive (Fig. 1). The cells were of coccus morphology, 0.5 μm in diameter and occurred in pairs and short chains (Fig. 2). The sporulation test was performed using a thermic shock (80°C during 30 minutes), but no free spores were observed and no viable cells could be recovered from sporulating cultures. The motility test was negative. The strain did not show catalase and oxidase activity. API ZYM and API 50 CH (bioMérieux) gallery systems were used to perform biochemical assays.

Distinguishing results from biochemical tests between *M. massiliensis* SIT 12 and *Murdochiella asaccharolitica* are listed in Table 1.

The antibiotic susceptibility was studied using antibiotics discs (i2a, Montpellier, France). *Murdochiella massiliensis* SIT12 was resistant to fosfomycin, tobramycin, naxidic acid and colistin but was susceptible to gentamicin, ciprofloxacin, trimethoprim−sulfamethoxazole, teicoplanin, rifampicin, ceftazidime, erythromycin, imipenem, tazocillin and aztreonam.

Using 16S rRNA phylogeny analyses, we demonstrated that strain SIT12 exhibited a 97% 16S rRNA sequence identity with *Murdochiella asaccharolytica* (EU483153) and *Levyella massiliensis* (HM587324) species (Fig. 3). Investigation of the most closely related described species revealed that the novel species strain was member of the phylum *Firmicutes.* Its 16S rRNA sequence was deposited in GenBank under accession number LN866998.

Cellular fatty acid methyl ester analysis was performed by gas chromatography/mass spectrometry [10]. The major fatty acids for strain SIT12 are mainly composed of 16 or 18 carbons: 16:0 (34%), 18:1n9 (28%), 18:2n6 (19%) and 18:0 (12%). Moreover, several fatty acids are described with unusual longer chains such as 20:4n6, 20:5n3 and 22:6n3 (<1%) (Table 2).

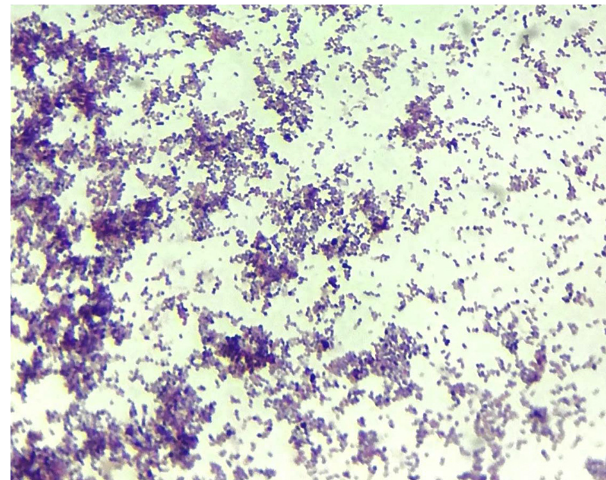

FIG. 1. Gram staining of *Murdochiella massiliensis* strain SIT12.

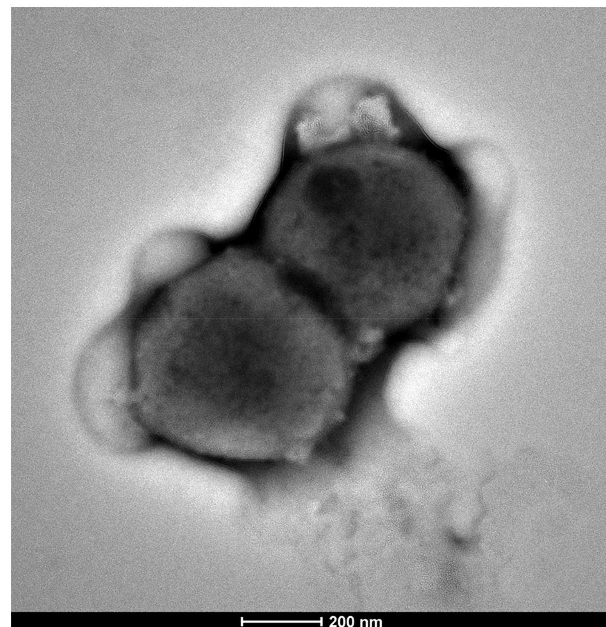

FIG. 2. Transmission electron microscopy of *Murdochiella massiliensis* strain SIT12 using TechnaiG^2 Cryo device (FEI Company) at an operating voltage of 200 keV. Scale bar = 200 nm.

Extended feature descriptions

MALDI-TOF MS protein analysis was carried out as previously described [11]. The SIT12 spectra were imported into MALDI BioTyper 3.0 software (Bruker Daltonics, Leipzig, Germany) and analysed by standard pattern matching (with default parameter settings) against 7765 spectra of bacteria. The

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TABLE 1. Differential characteristics of *Murdochiella massiliensis* SIT12 and *Murdochiella asaccharolitica* WAL 1855C^T

Property	*M. massiliensis*	*M. asaccharolitica*
Cell diameter (μm)	0.5	0.5–0.6
Oxygen requirement	Strictly anaerobic	Strictly anaerobic
Gram stain	Positive	Positive
Motility	Nonmotile	Nonmotile
Endospore training	−	−
Production of:		
Catalase	−	NA
Oxidase	−	NA
Esterase	−	+
Esterase lipase	+	−
Leucine arylamidase	+	+
Phosphatase acid	+	−
Naphtol phosphohydrolase	+	−
β-Galactosidase	+	−
β-Glucuronosidase	+	−
N-Acetyl-β-glucosamidase	+	−
A-Mannosidase	+	−
Acid from:		
Glycerol	+	−
D-Ribose	+	−
D-Galactose	+	−
D-Glucose	+	−
D-Fructose	+	−
D-Mannose	+	−
D-Mannitol	+	−
N-acetylglucosamine	+	−
Potassium 5-ketogluconate	+	−
G+C satisfied (%)	48.9	NA
Habitat	Human gut	Human gut

NA, data not available.

method of identification included m/z from 3000 to 15 000 Da. A maximum of 100 peaks were compared with spectra in the database for every spectrum. The resulting score enabled the identification (or not) of tested species: a score of ≥2 with a validly published species enabled identification at the species level, a score of ≥1.7 but <2 enabled identification at the genus level and a score of <1.7 did not enable any identification. No significant MALDI-TOF MS score was obtained for strain SIT12 against the Bruker database, suggesting that our isolate was not a member of a known species. We added the spectrum from strain SIT12 to our database.

Genome description

Genome sequencing and assembly

Genomic DNA of *M. massiliensis* was sequenced on the MiSeq Technology (Illumina, San Diego, CA, USA) with the mate-pair strategy. Automated cluster generation and sequencing run were performed in a single 39-hour run at a 2 × 151 bp read length. Total information of 2.9 Gb was obtained from a 297K/mm^2 cluster density, with a cluster passing quality control filters of 97% (5 808 000 passing filter paired reads). Within this run, the index representation for *M. massiliensis* was determined to 7.58%. The 440 495 paired reads were trimmed, then assembled in two scaffolds using the SPAdes assembler.

Genome annotation and properties

Open reading frames (ORFs) were predicted using Prodigal [12] with default parameters, but the predicted ORFs were excluded if they spanned a sequencing gap region. The tRNAScanSE tool [13] was used to find tRNA genes, whereas ribosomal RNAs were found by using RNAmmer [14].

The genome is 1 642 295 bp long with 48.9% GC content. It is composed of two scaffolds comprising two contigs. Of the

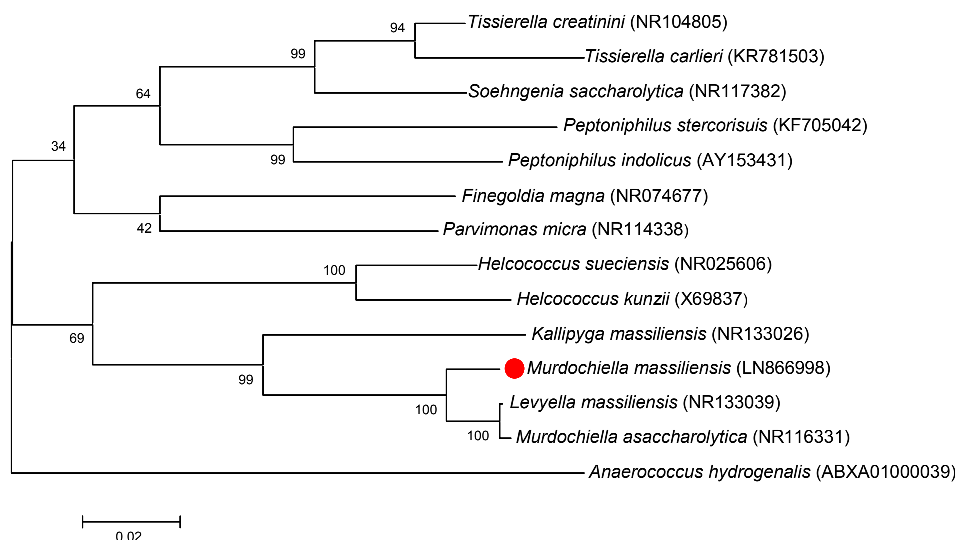

FIG. 3. Phylogenetic tree showing relationship of *Murdochiella massiliensis* strain SIT12 to most closely related species and other representative member of genus *Murdochiella*. Tree was constructed using maximum composite likelihood method based on Kimura two-parameter model using bootstrap method of 1000 replication.

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TABLE 2. Cellular fatty acid composition (%)

Fatty acid	Name	Mean relative %[a]
16:0	Hexadecanoic acid	32.9 ± 1.0
18:1n9	9-Octadecenoic	27.9 ± 0.4
18:2n6	9,12-Octadecadienoic acid	18.4 ± 0.2
18:0	Octadecanoic acid	11.4 ± 0.7
18:1n7	11-Octadecenoic acid	2.5 ± 0.1
18:1n6	12-Octadecenoic acid	1.7 ± 0.2
16:1n7	9-Hexadecenoic acid	1.1 ± 0.2
14:0	Tetradecanoic acid	1.0 ± 0.2
17:0 anteiso	14-Methyl-hexadecanoic acid	TR
20:4n6	5,8,11,14-Eicosatetraenoic acid	TR
17:0	Heptadecanoic acid	TR
15:0	Pentadecanoic acid	TR
17:0 iso	15-Methyl-hexadecanoic acid	TR
17:1n7	10-Heptadecanoic acid	TR
20:5n3	5,8,11,14,17-Eicosapentanoic acid	TR
15:0 iso	13-Methyl-tetradecanoic acid	TR
12:0	Dodecanoic acid	TR
22:6n3	4,7,10,13,16,19-Docosahexaenoic acid	TR
15:0 anteiso	12-Methyl-tetradecanoic acid	TR
5:0 anteiso	2-Methyl-butanoic acid	TR
10:0	Decanoic acid	TR

TR, trace amounts <1%.
[a]Mean peak area percentage.

1478 predicted genes, 1426 were protein-coding genes and 52 were RNAs (two genes were 5S rRNA, two 16S rRNA, one 23S rRNA and 47 tRNA). A total of 1002 genes (70.27%) were assigned as putative function (by cogs or by NR blast). Ninety-seven genes were identified as ORFans (6.8%). The remaining 284 genes were annotated as hypothetical proteins (19.92%).

Genome analyses

The resistome was analysed with the ARGANNOT (Antibiotic Resistance Gene-ANNOTation) database [15]. The exhaustive bacteriocin database available in our laboratories (Bacteriocins from the URMITE database) was performed by collecting all currently available sequences from the databases and from the National Center for Biotechnology Information. Protein sequences from this database allowed putative bacteriocins from human gut microbiota to be identified using BLASTp methodology [16]. Analysis of presence of polyketide synthases–nonribosomal peptide synthetases (PKS/NRPS) was performed by discriminating the gene with large size using a database realized in our laboratory; predicted proteins were compared against nonredundant GenBank database using BLASTP and then examined using antiSMASH [17]. *Murdochiella massiliensis* did not contain bacteriocins or NRPS, and the analysis of the resistome showed no resistance genes. PHAST (PHAge search tool) was used to identify phage sequences [18]. The results indicated the presence of an incomplete phage with 48.2% G+C content and a complete phage with 49.5% G+C content (Fig. 4).

Genome comparison

The draft gene sequence of *M. massiliensis* is smaller than those of *Anaerococcus hydrogenalis, Peptoniphilus indolicus, Parvimonas micra* and *Helcococcus kunzii* (1642, 1889, 2238,

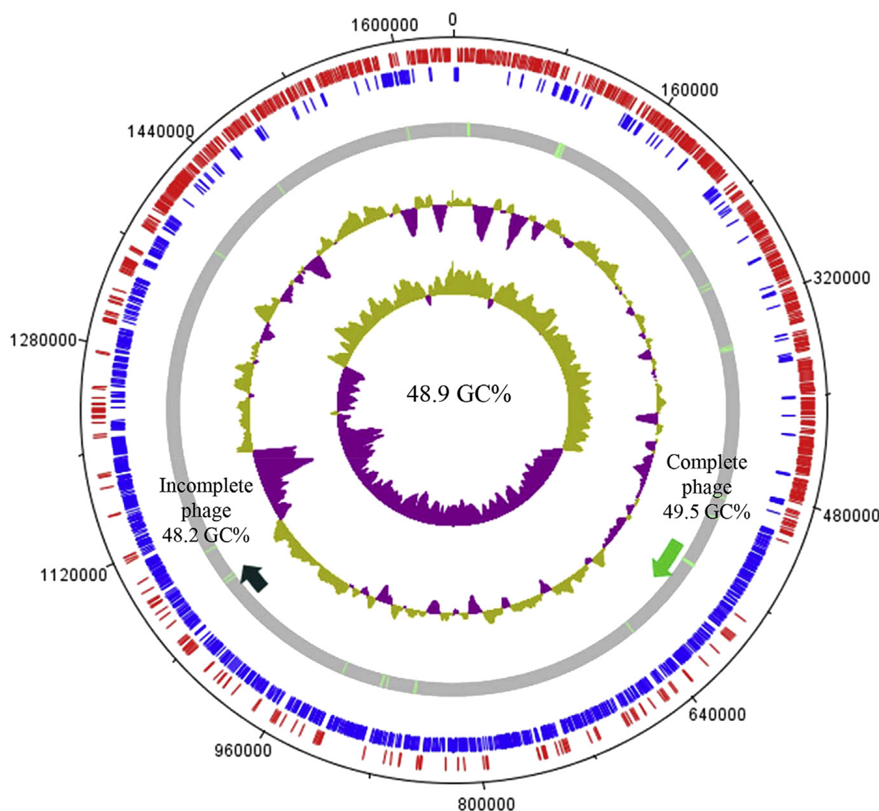

FIG. 4. Graphical circular map of chromosome. From outside to centre: genes on forward (red).

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1704 and 2096 Mb respectively), but larger than those of *Helcococcus sueciensis* (1574 Mb). The G+C content of *M. massiliensis* is larger than those of *A. hydrogenalis, P. indolicus, H. sueciensis, P. micra and H. kunzii* (48.97, 29.64, 31.69, 28.4, 28.66 and 29.35% respectively). The gene content of *M. massiliensis* is smaller than those of *A. hydrogenalis, H. sueciensis, P. micra* and *H. kunzii* (1.426, 2.069, 2.269, 1.445, 1.678 and 1.882 respectively).

Conclusion

On the basis of taxonogenomic analyses, we formally propose the creation of *Murdochiella massiliensis* sp. nov. that contains the strain SIT12.

Description of *Murdochiella massiliensis* sp. nov.

The *Murdochiella massiliensis* name come from Massilia, the ancient Roman name for Marseille, France, where the type strain was isolated. The strain was anaerobic, Gram positive, non−endospore forming, nonmotile and coccus shaped. Growth was achieved anaerobically between 25 and 42°C after 48 hours' incubation. Growth occurred in the presence of 5 g/L NaCl. Catalase and oxidase were negative. The genome is 1 642 295 bp long, and G+C content is 48.9%. The 16S rRNA gene sequence and whole-genome shotgun sequence of *M. massiliensis* strain SIT12 are deposited in GenBank under accession numbers LN866998 and FIZW00000000.1 respectively. The type strain SIT12 (= CSUR P1987 = DSM 29078) was isolated from the stool of a healthy 2-year-old Senegalese boy.

Acknowledgement

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Conflict of Interest

None declared.

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