Draft genome and description of *Microvirga mediterraneensis* strain Marseille-Q2068T sp. nov., a new bacterium isolated from human healthy skin

M. Boxberger[1,2], M. Ben Khedher[1,2], S. Magnien[1,2], N. Cassir[1,2] and B. La Scola[1,2]

1) *Aix-Marseille Université, IRD, AP-HM, MEPHI and* 2) *IHU-Méditerranée Infection, Marseille, France*

Abstract

In 2019, by culturing a skin swab from the forehead of a 70-year-old healthy woman via the culturomics method, we isolated the new bacterial strain Marseille-Q2068T (= CSUR-Q2068). Matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) failed to identify this isolate. Analysis of the 16S ribosomal RNA gene and genome-to-genome comparison suggested that this taxon belongs to a novel bacterial species within the family *Methylobacteriaceae* in the phylum Proteobacteria. We describe here its main phenotypic characteristics, genome sequence and annotation of *Microvirga mediterraneensis* strain Marseille-Q2068T, a new member of the *Microvirga* genus, which we propose as the type strain.

Keywords: Bacteria, culturomics, genome, *Microvirga mediterraneensis*, sp. nov., species, taxonogenomics
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Corresponding author: B. La Scola, Aix-Marseille Université, Méditerranée-Infection, 19-21 Boulevard Jean Moulin, 13385, Marseille cedex 05, France.
E-mail: bernard.la-scola@univ-amu.fr

Introduction

The genus *Microvirga* includes 22 species [1], most of which were isolated from diverse environmental samples, such as air [2], soils and springs [2–9], or in samples associated with plants [10–15]. Only one species was isolated from human, *Microvirga massiliensis* [16], which is the human commensal bacterium with the largest genome. *Microvirga mediterraneensis* strain Marseille-Q2068T was isolated using the culturomics approach, based on the use of a large panel of culture conditions, in order to describe the culturable microbial composition of a sample [17–19]. A taxonogenomics approach including matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF MS), phylogenetic analysis, main phenotypic description and genome sequencing was used to describe this species [20].

The genome of *Microvirga mediterraneensis* strain Marseille-Q2068T is 5,347,712 bp long with 63.83% G + C content. This new bacterium is most closely related to *Microvirga lotononidis* strain WSM3557 with a 16S ribosomal RNA (rRNA) sequence similarity value of 99.25%. Furthermore, digital DNA-DNA hybridization analysis between the novel organism and the *Microvirga lotononidis* strain WSM3557 type strain genome revealed an identity of only 39.7%, and genomic comparison using the OrthoANI parameter provided a value of 89.86%. On the basis of these data, we propose *Microvirga mediterraneensis* strain Marseille-Q2068T, a new member of the *Microvirga* genus, as the type strain.

Materials and methods

Strain isolation

The subject was registered at the L'Occitane Natural Cosmetic Assessment Center in Marseille (https://cosnat-loccitane.com; CosNat, Marseille France). The skin areas used for sampling were 10 cm^2. The samples were collected in a Z-stroked manner [21] using sterile swabs soaked in Culture Top transport medium (C-Top Ae-Ana, Eurobio, France). *Microvirga mediterraneensis* strain Marseille-Q2068T was initially isolated by direct seeding of 50 µL of sample on a homemade R2A incubated in aerobiosis at 31°C. MALDI-TOF MS protein analysis was carried out with a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [8]. Spectra from strain Marseille-Q2068T were imported into MALDI BioTyper software (Bruker) and analysed by standard pattern matching using

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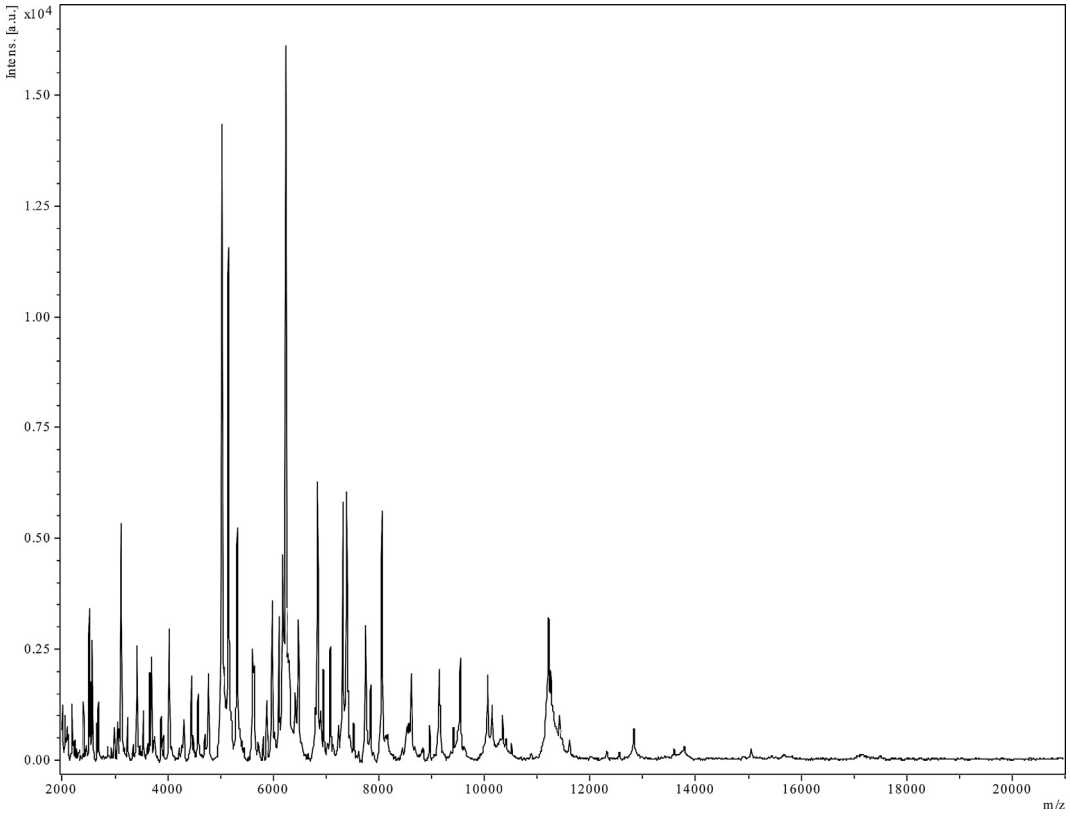

FIG. 1. Matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) reference mass spectrum. Spectra from 12 individual colonies of *Microvirga mediterraneensis* strain Marseille-Q2068T were compared and reference spectrum generated.

default parameter settings (Fig. 1). The study was validated by the local ethics committee (ID-RCB 2019-A01508-49).

Phenotypic characterization

Different growth temperatures (20, 30, 37, 45 and 56°C), atmospheric conditions (anaerobic, aerobic and microaerophilic using CampyGEN, Oxoid, Basingstoke, UK) and pH (5, 6.5, 7.2 and 8.5) were tested. API ZYM, API NE and API 50CH strips (bioMérieux, Marcy l'Etoile, France) were used to evaluate the biochemical properties of the strain according to the manufacturer's instructions. For scanning electronic microscopy, a colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. The slide was gently washed in water, air dried and examined with approximately 60 cm in height and 33 cm in width to evaluate bacterial structure on a TM4000 microscope (Hitachi High-Technologies, Tokyo, Japan). Motility test was performed using the semisolid TCC media as described by Tittsler and Sandholzer [22].

Genome sequencing

Genomic DNA (gDNA) of *Microvirga mediterraneensis* strain Marseille-Q2068T was quantified by a Qubit assay with the high sensitivity kit (Life Technologies, Carlsbad, CA, USA) to 0.2 ng/µL. Genomic DNA was next sequenced on the MiSeq Technology (Illumina, San Diego, CA, USA) with the paired end strategy and was barcoded in order to be mixed respectively with 23 other genomic projects prepared with the Nextera XT DNA sample prep kit (Illumina). To prepare the paired end library, dilution was performed to require 1ng of each genome as input to prepare the paired end library. The tagmentation step fragmented and tagged the DNA. Then limited-cycle PCR amplification (12 cycles) completed the tag adapters and introduced dual-index barcodes. After purification on AMPure XP beads (Beckman Coulter, Fullerton, CA, USA), the libraries were then normalized on specific beads according to the Nextera XT protocol (Illumina). Normalized libraries were pooled into a single library for sequencing on the MiSeq. The pooled single strand library was loaded onto the reagent cartridge and then onto the instrument along with the flow cell. Automated cluster generation and paired end sequencing with dual index reads were performed in a single 39-hour run at a 2 × 250 bp read length. Total information of 9.5 Gb was obtained from a 1063K/mm^2 cluster density, with a cluster passing quality control filters of 89.2%. Within this run, the index representation for *Microvirga mediterraneensis* was determined to index 3.4%. The 20 050 916 paired end reads were filtered according to the read qualities. 16S RNA gene sequence was extracted, and a

phylogenetic tree was obtained using the maximum likelihood method and Kimura two-parameter within MEGA 7 software [11].

Genome annotation and genome comparison

Genome annotation was obtained through the NCBI Prokaryotic Genome Annotation Pipeline [23]. BlastP was used to predict the bacterial proteome (*E* value of 1e03, coverage of 0.7 and percent identity of 30) according to the Clusters of Orthologous Groups (COGs) database. The Genome-to-Genome Distance Calculator (GGDC) web server (http://ggdc.dsmz.de) was used to estimate the overall similarity among compared genomes and to replace the wet-lab DNA-DNA hybridization by a digital version. The degree of genomic similarity of *Microvirga mediterraneensis* strain Marseille-Q2068 with closely related species was estimated using the OrthoANI software [24]. Antibiotic resistance genes (ARG) were searched using the Comprehensive Antibiotic Resistance Database (CARD) [25]. Assembled sequences were searched against the CARD database under moderately stringent conditions (e-value of 10−5) for the *in silico* ARG prediction. The presence of pathogenesis-related proteins was investigated using the virulence factor database (VFDB) [26].

Results

Strain identification and classification

Microvirga mediterraneensis strain Marseille-Q2068T was isolated from the forehead skin swab of a 70-year-old healthy woman. *Microvirga mediterraneensis* strain Marseille-Q2068T failed to be identified by our systematic MALDI-TOF MS screening, suggesting that the corresponding species was not in our database (https://www.mediterranee-infection.com/acces-ressources/base-de-donnees/urms-data-base/) (Fig. 1). Moreover, *Microvirga mediterraneensis* strain Marseille-Q2068T exhibited a 99.25% 16S rRNA sequence similarity to the *Microvirga lotononidis* strain WSM3557 type strain (GenBank accession no. NR_117846.1), the phylogenetically closest bacterium with standing in nomenclature (Fig. 2).

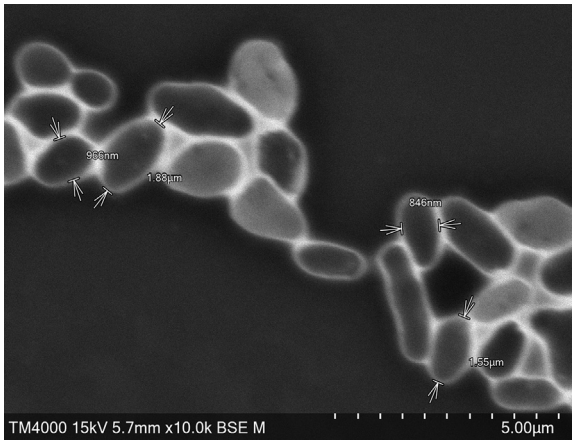

FIG. 3. Scanning electron microscopy of Microvirga *mediterraneensis* sp. nov., strain Marseille-Q2068T, using TM 4000plus Tabletop microscope (Hitachi, Tokyo, Japan). Scale bar represents 5 µm.

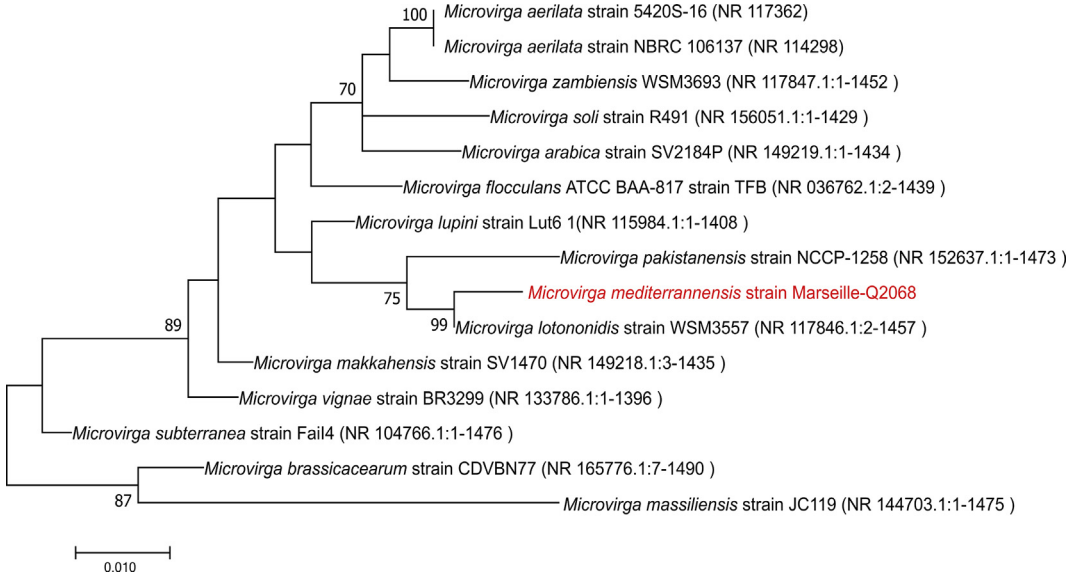

FIG. 2. 16S rRNA–based phylogenetic tree highlighting position of *Microvirga mediterraneensis* sp. nov., strain Marseille-Q2068T (red), relative to other closely related bacterial taxa. Sequences were aligned using Muscle 3.8.31 with default parameters, and phylogenetic relationship was inferred using maximum likelihood method with 1000 bootstrap replicates within MEGA 7.0 software.

TABLE 1. Differential characteristics of *Microvirga mediterraneensis* strain Marseille-Q2068T and its most closely related species with standing in nomenclature

Characteristic	*M. mediterranensis*	*M. lotononidis*	*M. pakistanensis*	*M. calopogonii*	*M. subterranea*	*M. flocculans*
Property	Marseille-Q2068	WSM3557	NCCP-1258	SSW1-57	Fail-4	TFB
Cell size (µm)	0.9 × 1.7	0.4–0.56 × 1.0–2.2	NA	0.5–0.9 × 9 1.3–2.0	1 × 1.5–4	0.8 × 1.1
Oxygen requirement	Facultative	+	+	Facultative	+	+
Gram Strain	—	—	—	—	—	—
Motility	—	—	—	+	+	—
Endospore formation	—	+	—	—	—	—
Optimum temperature for growth (°C)	30–56	41	40	20–45	41	10–35
Production of:						
Alkaline phosphatase	+	—	+	NA	NA	—
Catalase	+	+	?	+	+	+
Oxidase	—	—	+	+	—	—
α-Glucosidase	—	+	—	NA	+	—
β-Galactosidase	—	—	—	NA	—	—
Acid from:						
N-Acetylglucosamine	—	—	—	NA	NA	—
L-arabinose	—	+	—	?	+	NA
D-Ribose	—	NA	—	NA	NA	NA
D-Mannose	—	NA	+	+	NA	—
D-Mannitol	—	+	—	NA	NA	NA
D-Glucose	—	+	+	NA	+	—
D-Fructose	—	+	+	+	—	NA
D-Maltose	—	+	—	NA	—	NA
D-Lactose	—	NA	—	NA	NA	NA
G + C content (mol%)	63.83	62.8–63	64.3	64.48	63.5	62.2
Habitat	Human healthy skin	Various: soils, herbage, seawater	Desert soil of Cholistan, Pakistan	Root nodule in Southwest China	Deep surface Australian thermal aquifer	Air samples

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

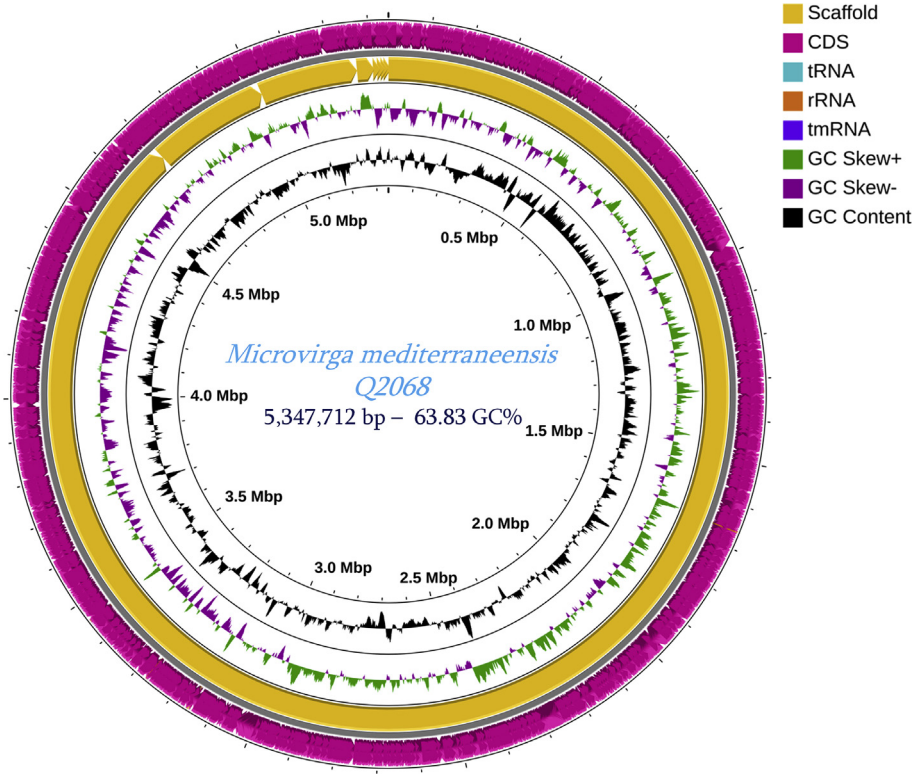

FIG. 4. Graphical circular map of genome from strain Marseille-Q2068T obtained by CGView tool [27].

TABLE 2. **Number of genes associated with 25 general COGs functional categories of *Microvirga mediterraneensis* strain Marseille-Q2068 and closely related species *Microvirga lupini*, *Microvirga lotononidis*, *Microvirga ossetica* and *Microvirga flocculans***

Code	*M. mediterraneensis*	*M. lupini*	*M. lotononidis*	*M. ossetica*	*M. flocculans*	Description
[J]	208	219	229	231	211	Translation, ribosomal structure and biogenesis
[A]	0	0	0	0	0	RNA processing and modification
[K]	203	384	312	370	158	Transcription
[L]	203	163	152	220	100	Replication, recombination and repair
[B]	128	4	3	2	2	Chromatin structure and dynamics
[D]	3	31	34	40	24	Cell cycle control, cell division, chromosome partitioning
[Y]	28	0	0	0	0	Nuclear structure
[V]	0	91	84	99	50	Defense mechanisms
[T]	75	404	416	526	189	Signal transduction mechanisms
[M]	321	234	215	264	160	Cell wall/membrane/envelope biogenesis
[N]	193	57	71	67	41	Cell motility
[Z]	54	0	0	0	0	Cytoskeleton
[W]	0	0	0	1	0	Extracellular structures
[U]	0	34	31	34	21	Intracellular trafficking, secretion and vesicular transport
[O]	24	193	173	210	145	Posttranslational modification, protein turnover, chaperones
[X]	175	178	197	568	10	Mobilome: prophages, transposons
[C]	76	319	284	320	189	Energy production and conversion
[G]	227	475	366	524	176	Carbohydrate transport and metabolism
[E]	276	638	505	567	315	Amino acid transport and metabolism
[F]	410	84	92	98	84	Nucleotide transport and metabolism
[H]	89	208	184	190	129	Coenzyme transport and metabolism
[I]	129	256	190	281	147	Lipid transport and metabolism
[P]	167	217	227	241	117	Inorganic ion transport and metabolism
[Q]	168	140	110	156	103	Secondary metabolites biosynthesis, transport and catabolism
[R]	91	314	275	369	150	General function prediction only
[S]	177	174	146	183	112	Function unknown

COGs, Clusters of Orthologous Groups database.

Phenotypic characteristics

Growth of *Microvirga mediterraneensis* strain Marseille-Q2068T was initially isolated by direct seeding of 50 µL of sample on a homemade R2A (Reasoner 2A agar) incubated in aerobiosis at 31°C. Colonies from strain Marseille-Q2068T showed a pink pigmentation and no haemolysis. They were circular, with a diameter of 0.5 to 1.5 mm. Bacterial cells were Gram-negative, nonmotile rods with a length of about 1.70 µm and a width of

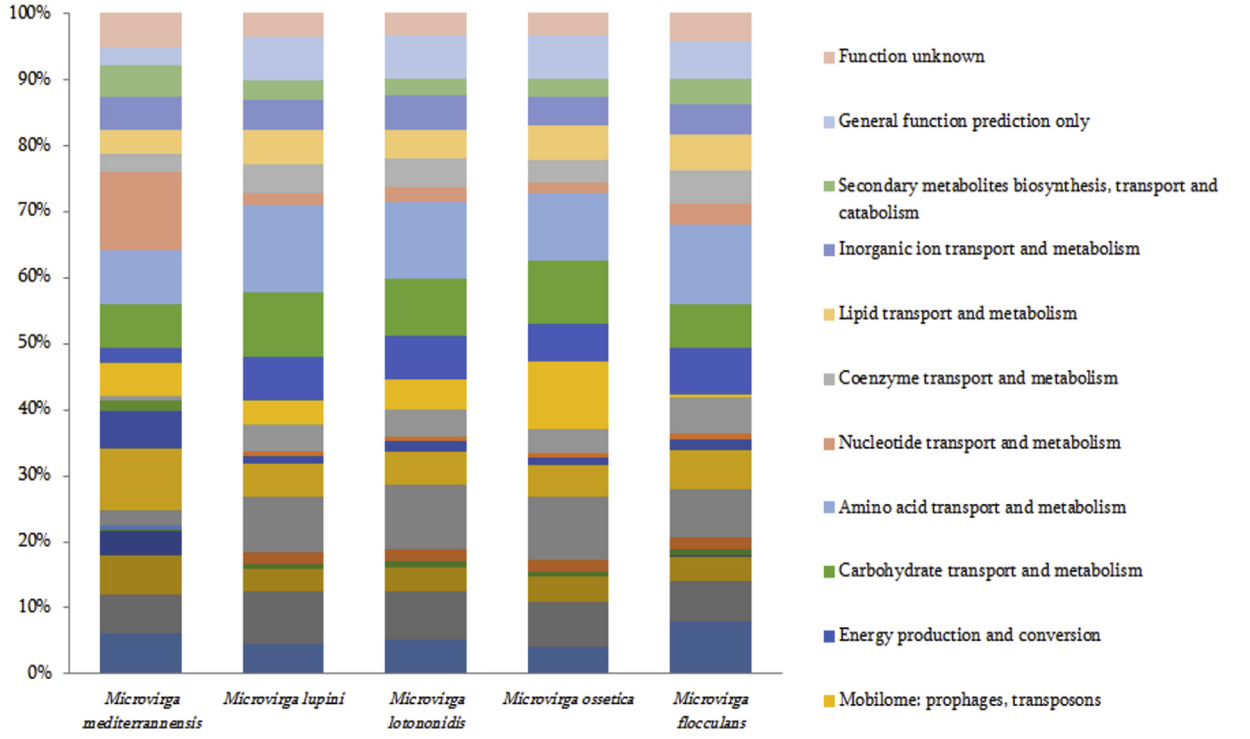

FIG. 5. Distribution of functional classes of predicted genes in *Microvirga mediterraneensis, Microvirga lotononidis*, *Microvirga lupini*, *Microvirga ossetica* and *Microvirga flocculans* according to Clusters of Orthologous Groups (COGs) database groups of proteins.

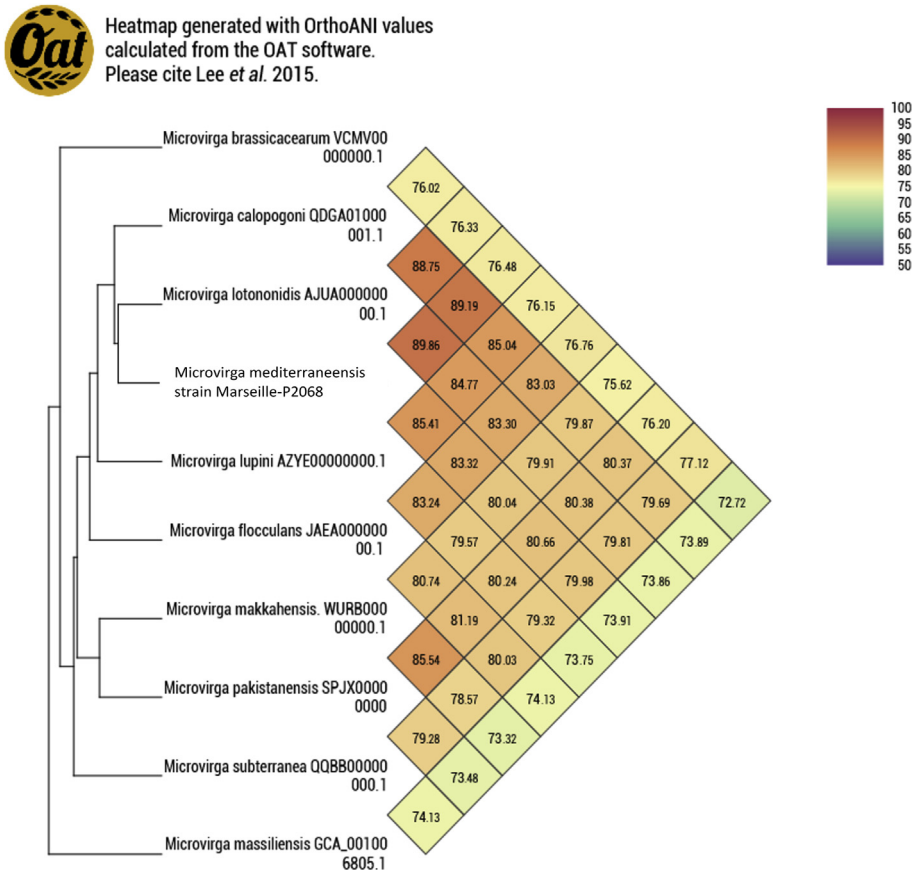

FIG. 6. Heat map generated with OrthoANI values calculated using OAT software between *Microvirga mediterraneensis* sp. nov., strain Marseille-Q2068T, and other closely related species with standing in nomenclature.

about 0.9 µm, as determined by scanning electron microscopy (Fig. 3). Strain Marseille-Q2068T is a facultative aerobe. The sporulation test (20 minutes at 80°C) was negative. Using an API strip, positive reactions were shown for esculin, gelatin, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase and naphtol-AS-BI-phosphohydrolase. All other reactions tested were negative. In addition, this bacterium shows catalase positivity and oxidase negativity. The results are summarized in Table 1.

Genome properties

The genome size of strain Marseille-Q2068 was 5 347 712 bp long with a 63.83% G + C content. The genome assembly of this strain was achieved on eight contigs. Of the 5099 predicted genes, 4933 were protein-coding genes and 86 were RNAs (five 16S rRNA, five additional 5S rRNAs, five additional 23S rRNAs and 67 transfer RNAs and four noncoding RNAs). A total of 4106 genes (83.2%) were assigned a putative function, and 824 genes (16.7%) were annotated as hypothetical proteins (Fig. 4). The genome properties and distribution of genes into COGs functional categories are detailed in Table 2. The *in silico* resistome of the strain Marseille-Q2068 obtained by searching the CARD database and the search for virulence factors via the VFDataBase of this strain showed no genes with high identity percentage. Genes with putative function (by COGs analysis) were 3425 (67%). Analysis of the COGs categories showed that the nucleotide transport and metabolism category, the replication, recombination and repair category, and the chromatin structure and dynamics category of the *Microvirga mediterraneensis* genome appear to be more numerous than those of the genomes of the *Microvirga* genus (categories F, L and B respectively) (Fig. 5). Finally, a digital DNA-DNA hybridization analysis between the novel organism and the *Microvirga lotononidis* strain *WSM3557* type strain revealed an identity of only 39.7%. Furthermore, genomic comparison using the OrthoANI parameter provided a value of 89.86% with the *Microvirga lotononidis* strain WSM3557 type strain (Fig. 6).

TABLE 3. Description of *Microvirga mediterraneensis* sp. nov. strain Marseille-Q2068T

Type of description	New description
Species name	*Microvirga*
Genus name	*Mediterraneesis*
Specific epithet	*Mediterraneesis*
Species status	sp. nov.
Species etymology	*Microvirga mediterraneensis* strain Marseille-Q2068T. Mi.cro.vir'ga, Gr. adj. *mikros*, 'small'; L. fem. n. *virga*, 'rod'; N.L. fem. n. *Microvirga*, 'small rod'. Me.di.ter.ra.ne.en'sis, L. masc. adj., *mediterraneensis*, 'of Mediterraneum', the Latin name of the Mediterranean Sea, by which Marseille is located and the bacteria isolated.
Authors	Manon Boxberger, Mariem Ben Khedher, Sibylle Magnien, Nadim Cassir, Bernard La Scola
Designation of the type strain	Marseille Q2068
Strain collection number	CSUR-Q2068
16S rRNA gene accession number	MT795959.1
Genome accession number	GCA_013520865.1
Genome status	Draft
Genome size	5 347 712 bp
GC%	63.83
Country of origin	Marseille, France
Date of isolation	2019
Source of isolation	Human healthy skin
Growth medium, incubation	Routinely COS, 31°C
Gram strain	Negative
Cell shape	Rods
Cell size	1.7µm
Motility	—
Sporulation	—
Colony morphology	Pink, smooth
Temperature range	31–56°C
Temperature optimum	31°C
Relationship to O_2	Facultative
O_2 for strain testing	Anaerobiosis, microaerophilic, aerobiosis
Oxidase	Negative
Catalase	Positive

Discussion and conclusion

In the past 8 years, the use of the culturomics approach has resulted in the discovery of more than 500 bacterial species [17]. Using the taxonogenomics concept – the combination of the genomic and phenotypic properties of a putative new taxon [27] – we have characterized a new bacterial species within the family *Methylobacteriaceae* found on forehead human skin. The main characteristics of this strain are summarize in Table 3. It was named *Microvirga mediterraneensis* strain Marseille-Q2068T, as follows: Mi.cro.vir'ga Gr. adj. *mikros*, 'small'; L. fem. n. *virga*, 'rod'; N.L. fem. n. *Microvirga*, 'a small rod'. Me.di.ter.ra.ne.en'sis, L. masc. adj., mediterraneensis, 'of Mediterraneum', the Latin name of the Mediterranean Sea, by which Marseille is located and the bacteria isolated (Table 3).

Deposit in culture collections and sequences database

Microvirga mediterraneensis strain Marseille-Q2068T was deposited in CSUR collection under accession number CSUR-Q2068. The 16S rRNA and genome sequences and annotation are available in GenBank under accession numbers MT795959.1 and GCA_013520865.1 respectively.

Conflict of interest

None declared.

Acknowledgements

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