Draft genome and description of \textit{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 \textit{Methylobacteriaceae} in the phylum Proteobacteria. We describe here its main phenotypic characteristics, genome sequence and annotation of \textit{Microvirga mediterraneensis} strain Marseille-Q2068T, a new member of the \textit{Microvirga} genus, which we propose as the type strain.

© 2021 The Author(s). Published by Elsevier Ltd.

Keywords: Bacteria, culturomics, genome, \textit{Microvirga mediterraneensis}, sp. nov., species, taxonogenomics

Original Submission: 14 August 2020; Revised Submission: 9 November 2020; Accepted: 23 November 2020
Article published online: 15 January 2021

Introduction

The genus \textit{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, \textit{Microvirga massiliensis} [16], which is the human commensal bacterium with the largest genome. \textit{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 \textit{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 \textit{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 \textit{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 \textit{Microvirga mediterraneensis} strain Marseille-Q2068T, a new member of the \textit{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 cm². 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). \textit{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
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 1 ng 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/mm² 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
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/accesressources/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).

![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.](image-url)

**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.
### 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          | Fai-4           | TFB              |
| Call size (μm)                 | 0.9 × 1.7                           | 0.4–0.56 × 1.0–2.2  | NA                | 0.5–0.9 × 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                       | +                                   | −                   | NA                | NA               | −               | −                |
| Oxidase                        | −                                   | −                   | ++                | ++               | ++              | ++               |
| α-Glucosidase                  | −                                   | −                   | NA                | NA               | +               | +                |
| β-Galactosidase                | −                                   | −                   | NA                | NA               | −               | −                |
| Acid from:                     |                                     |                     |                   |                  |                 |                  |
| N-Acetylglucosamine            | −                                   | −                   | NA                | NA               | −               | −                |
| L-arabinose                    | −                                   | −                   | NA                | NA               | −               | −                |
| D-Ribose                       | −                                   | −                   | NA                | NA               | −               | −                |
| D-Mannose                      | −                                   | −                   | NA                | NA               | −               | −                |
| D-Manitol                      | −                                   | −                   | NA                | NA               | −               | −                |
| D-Glucose                      | −                                   | −                   | NA                | NA               | −               | −                |
| D-Fructose                     | −                                   | −                   | NA                | NA               | −               | −                |
| D-Maltose                      | −                                   | −                   | NA                | NA               | −               | −                |
| D-Lactose                      | −                                   | −                   | NA                | NA               | −               | −                |
| G + C content (mol%)           | 63.83                               | NA                  | 64.3              | NA               | 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.*

![Graphical circular map of genome from strain Marseille-Q2068T obtained by CGView tool](image)

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

---

**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 |
| [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              | 0           | 0            | Extracellular structures |
| [S]  | 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           | Molobiome: 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.

**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.
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 naphthol-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          | Mediterraneanis |
| Specific epithet     | Mediterraneanis |
| Species status       | sp. nov.        |
| Species etymology   | Microvirga mediterraneensis strain Marseille-Q2068T. Mi.cro.vir’ga Gr. adj. mikros, ‘small’; L. fem. n. virgo, ‘rood’; N.L. fem. n. Microvirga, ‘small rod’. Me.di.ter.ra.ne.en sis, L. masc. adj. mediterraneens, ‘of Mediterranean’, the Latin name of the Mediterranean Sea, by which Marseille is located and the bacteria isolated. | |
| Authors             | Manon Boxberger, Mariem Ben Khedher, Sévylle Magnien, Nadim Cassir, Bernard La Scala |
| Strain collection number | CSUR-Q2068 |
| 16S rRNA gene accession number | MT795959.1 |
| Genome accession number | GCA_013520865.1 |
| Genome size          | 5 347 712 bp |
| Genome status        | Draft |
| 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 stain           | 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 O₂    | Faculative |
| O₂ for strain testing | Anaerobiosis, microaerophilic, aerobicis |
| 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 Methylbacteriaceae found on forehead human skin. The main characteristics of this strain are summarized in Table 3. It was named Microvirga mediterraneensis strain Marseille-Q2068T, as follows: Mi.cro.vir’ga Gr. adj. mikros, ‘small’; L. fem. n. virgo, ‘rood’; N.L. fem. n. Microvirga, ‘a small rod’. Me.di.ter.ra.ne.en sis, L. masc. adj. mediterraneensis, ‘of Mediterranean’, 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

The MB PhD grant is supported by the collaboration between M&L Laboratories and Aix-Marseille Université (reference PVM:2018-200). Supported by the French state, managed by the National Research Agency under the ‘Investissements d’avenir’ (Investments for the Future) programme under reference ANR-10-IAHU-03 (Méditerranée Infection) and by the Région Provence-Alpes-Côte-d’Azur and European funding from FEDER PRIMI. The authors are indebted to L. Brechard for sequencing the genome and helping with electron microscopy at IHU-Méditerranée Infection.

References

[1] Parte AC. Lpsn — list of prokaryotic names with standing in nomenclature (bacterio.net), 20 years on. Int J Syst Evol Microbiol 2018;68: 1825–9. https://doi.org/10.1099/ijsem.0.002786.
[2] Weon HY, Kwon SW, Son JA, Jo EH, Kim SJ, Kim YS, et al. Description of Microvirga aerophila sp. nov. and Microvirga ornata sp. nov., isolated from air, reclassification of Balneimonas fluctuans Takeda et al. 2004 as Microvirga fluctuans comb. nov. and emended description of the genus Microvirga. Int J Syst Evol Microbiol 2010;60:2596–600. https://doi.org/10.1099/ijsem.0.018770-0.
[3] Liu ZT, Xian WD, Li MM, Liu L, Ming YZ, Yao JY, et al. Microvirga makkahensis sp. nov., an arsenate reduction bacterium isolated from sandy arid soil. Antonie Van Leeuwenhoek 2016;109:287–96. https://doi.org/10.1007/s10482-015-0631-z.
[4] Zhang X, Zhang J, Yao Q, Feng G, Zhu HH. Microvirga flavescens sp. nov., a novel bacterium isolated from forest soil and emended description of the genus Microvirga. Int J Syst Evol Microbiol 2019;69:667–71. https://doi.org/10.1099/ijsem.0.003189.
[5] Veysioglu A, Tatar D, Saygin H, Inan K, Cetin D, Guven K, et al. Microvirga makkahensis sp. nov., and Microvirga ornata sp. nov., isolated from sandy arid soil. Antonie Van Leeuwenhoek 2016;109:287–96. https://doi.org/10.1007/s10482-015-0631-z.
[6] Amin A, Ahmed I, Habib N, Abbas S, Hasan F, Xiao M, et al. Microvirga pakistanensis sp. nov., a novel bacterium isolated from desert soil of Cholistan, Pakistan. Arch Microbiol 2016;198:933–9. https://doi.org/10.1007/s00203-016-1251-3.
[7] Huq MdA. Microvirga rosea sp. nov.: a nanoparticle producing bacterium isolated from soil of rose garden. Arch Microbiol 2018;200:1439–45. https://doi.org/10.1007/s00203-018-1558-3.
[8] Dahal RH, Kim J. Microvirga soli sp. nov., an alphaproteobacterium isolated from soil. Int J Syst Evol Microbiol 2017;67:127–32. https://doi.org/10.1099/ijsem.0.001582.

© 2021 The Author(s). Published by Elsevier Ltd. NMNI, 40, 100839
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0).
[9] Kanso S, Patel BKC, Microvirga subterranea gen. nov., sp. nov., a moderate thermophile from a deep subsurface Australian thermal aquifer. Int J Syst Evol Microbiol 2003;53:401-6. https://doi.org/10.1099/ijs.0.02348-0.

[10] Jiménez-Gómez A, Sasti-Santamaría Z, Igual JM, Rivas R, Mateos PF, García-Fraile P. Genome insights into the novel species Microvirga brassicacearum, a rapped endophyte with biotechnological potential. Microorganisms 2019;7:354. https://doi.org/10.3390/microorganisms7090354.

[11] Wang F, Yang L, Deng J, Liu X, Lu Y, Chen W, et al. Microvirga colopogoni sp. nov., a novel alphaproteobacterium isolated from a root nodule of Calopogonium mucanoides in Southwest China. Antonie Van Leeuwenhoek 2019;112:1593–602. https://doi.org/10.1007/s10482-019-01285-5.

[12] Ardley JK, Parker MA, De Meyer SE, Trengove RD, O’Hara GW, Reeve WG, et al. Microvirga lupini sp. nov., Microvirga lotonisidis sp. nov. and Microvirga zamhensi sp. nov. are alphaproteobacterial root-nodule bacteria that specifically nodulate and fix nitrogen with geographically and taxonomically separate legume hosts. Int J Syst Evol Microbiol 2012;62:2579–88. https://doi.org/10.1099/ijs.0.035097-0.

[13] Safronova VI, Kuznetsova IG, Sazanova AL, Belinov AA, Andronov EE, Chirak ER, et al. Microvirga assetco sp. nov., a species of rhizobia isolated from root nodules of the legume species Vicia alpestris Steven. Int J Syst Evol Microbiol 2017;67:94–100. https://doi.org/10.1099/ijs.0.001577.

[14] Msaddak A, Rejili M, Durán D, Mars M, Palacios JM, Ruiz-Argüeso T, et al. Microvirga turitani sp. nov., a root nodule symbiotic bacterium isolated from Lupinus microcarthus and L. luteus grown in Northern Tunisia. Syst Appl Microbiol 2019;42:126015. https://doi.org/10.1016/j.syapm.2019.126015.

[15] Radl V, Simões-Araújo JL, Leite J, Passos SR, Martins LMV, Xavier GR, et al. Microvirga vignae sp. nov., a root nodule symbiotic bacterium isolated from cowpea grown in semi-arid Brazil. Int J Syst Evol Microbiol 2014;64:725–30. https://doi.org/10.1099/ijs.0.053082-0.

[16] Caputo A, Lagier JC, Azza S, Robert C, Mouelhi D, Fournier PE, et al. Microvirga massiliensis sp. nov., the human commensal with the largest genome. Microbiologyopen 2016;5:307–22. https://doi.org/10.1002/mbo3.329.

[17] Lagier JC, Dubourg G, Million M, Cadoret F, Bilen M, Fenollar F, et al. Culture of the human microbiota and culturomics. Nat Rev Microbiol 2018;16:540–50. https://doi.org/10.1038/s41579-018-0041-0.

[18] 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. https://doi.org/10.1111/j.1469-0691.2012.

[19] Lagier JC, Khelifa S, Alou MT, Ndongo S, Dione N, Hugon P, et al. Culture of previously uncultured members of the human gut microbiota by culturomics. Nat Microbiol 2016;1:16203. https://doi.org/10.1038/nmcr.2016.203.

[20] Ramasamy D, Mishra AK, Lagier JC, Padmanabhan R, Rossi M, Sentaux A, 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. https://doi.org/10.1099/ijs.0.057091-0.

[21] Rushing J. Obtaining a wound culture specimen. Nursing 2007;37:18. https://doi.org/10.1097/01.NURSE.0000298181.53662.e6.

[22] Tittsler RP, Sandholzer LA. The use of semi-solid agar for the detection of bacterial motility. J Bacteriol 1936;31:575–80.

[23] Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, et al. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 2016;44:6614–24. https://doi.org/10.1093/nar/gkw569.

[24] Lee I, Ouk Kim Y, Park SC, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–3. https://doi.org/10.1099/ijs.0.00760.

[25] McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, et al. The comprehensive antibiotic resistance database. Antimicrob Agents Chemother 2013;57:3348–57. https://doi.org/10.1128/AAC.00419-13.

[26] Chen L. VFDB: a reference database for bacterial virulence factors. Nucleic Acids Res 2004;33:D325–8. https://doi.org/10.1093/nar/gku008.

[27] Grant JR, Stothard P. The CGView Server: a comparative genomics tool for circular genomes. Nucleic Acids Res 2008;36:W181–4. https://doi.org/10.1093/nar/gkn179.