Draft genome and description of \textit{Aeromicrobium phoceense} strain Marseille-Q0843$^T$ 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 sample from the back of the hand of a 61-year-old healthy woman and assessing it via the culturomics method, we isolated the new bacterial strain Marseille-Q0843$^T$ (= CSUR-Q0843). 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{Nocardioidaceae} in the phylum \textit{Actinobacteria}. We describe here the main phenotypic characteristics, genome sequence and annotation of \textit{Aeromicrobium phoceense} strain Marseille-Q0843$^T$, a new member of the \textit{Aeromicrobium} genus, which we propose as the type strain.

Introduction

The genus \textit{Aeromicrobium} comprises 22 species \cite{1}, most isolated from diverse environmental samples, such as air \cite{2}, soil and water \cite{3–12}, or associated with plants and birds \cite{13,14}. Only one species has been isolated from a human: \textit{Aeromicrobium massiliense} \cite{15}. \textit{Aeromicrobium phoceense} strain Marseille-Q0843$^T$ was isolated using the culturomics approach, which is based on using a large panel of culture conditions to describe the microbial composition of a sample by high-throughput culture \cite{16–18}. 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 \cite{19,20}. The genome of \textit{Aeromicrobium phoceense} strain Marseille-Q0843$^T$ is 3 270 508 bp long with 70.96% G + C content. This new bacterium is most closely related to \textit{Aeromicrobium choanae} strain 9H-4 with a 16S ribosomal RNA (rRNA) sequence similarity value of 99.46%. Genomic comparison using OrthoANI parameters provided a value of 93.67% and a digital DNA-DNA hybridization value of 21.8% (19.5–24.2) with \textit{Aeromicrobium choanae} strain 9H-4. On the basis of these data, we propose \textit{Aeromicrobium phoceense} strain Marseille-Q0843$^T$, a new member of the \textit{Aeromicrobium} genus, as the type strain.

Materials and methods

Strain isolation and phenotypic tests

\textit{Aeromicrobium phoceense} strain Marseille-Q0843$^T$ was isolated from a swab sample taken from the skin of the back of the hand of a 61-year-old healthy woman. Sampling was performed in the CosNat Provence laboratory (https://cosnat-loccitane.com, Marseille area, France). \textit{Aeromicrobium phoceense} strain Marseille-Q0843$^T$ was initially isolated by direct seeding of 50 μL of sample on Columbia agar with 5% sheep’s blood media (bioMérieux, Marcy l’Etoile, France) incubated in aerobiosis at 31°C. MALDI-TOF MS protein analysis was carried out with a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) \cite{8}. Spectra from strain Marseille-Q0843$^T$ were imported into MALDI BioTyper 3.0 software (Bruker) and analysed by standard pattern matching (with default parameter settings). The study was validated by the local ethics committee (ID-RCB: 2019-A01508-49).

© 2020 The Authors. Published by Elsevier Ltd.

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

https://doi.org/10.1016/j.nmni.2020.100809
Phenotypic characterization
Different growth temperatures (21, 28, 30, 37, 45 and 56°C), atmospheric conditions, anaerobic, aerobic and microaerophilic atmospheres (CampyGEN; Oxoid, Basingstoke, UK) and pH (5.5, 6.5, 7.5, 8.5) were tested. API ZYM, 20 NE and 50 CH strips (bioMérieux) were used according to the manufacturer’s instructions to evaluate the strain’s biochemical properties. 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.

**FIG. 1.** Matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) reference mass spectrum. Spectra from 12 individual colonies of strain Marseille-Q0843T were compared and reference spectrum generated.

**FIG. 2.** 16S ribosomal RNA–based phylogenetic tree highlighting position of Aeromicrobium phoceense sp. nov. strain Marseille-Q0843T (red) relative to other closely related bacterial taxa. Sequences were aligned using Muscle v3.8.31 with default parameters; phylogenetic relationship was inferred using maximum likelihood method, with 1000 bootstrap replicates, within MEGA 7 software.

© 2020 The Authors. Published by Elsevier Ltd. NMNI, 38, 100809
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
with approximately 60 cm in height and 33 cm in width between the microscope detector and the slide to evaluate the bacterial structure using a TM4000Plus tabletop microscope (Hitachi High-Tech, Tokyo, Japan). Scale bar represents 5 μm.

Genome sequencing
Genomic DNA (gDNA) of Aeromicrobium phoceense strain Marseille-Q0843T was extracted in two steps: a mechanical treatment was first performed by glass beads acid washed (G4649-500g; Sigma-Aldrich, St Louis, MO, USA) using a FastPrep-24 5G Grinder (mpBio, Santa Ana, CA, USA) at maximum speed (setting 6.5) for 90 seconds. After 30 minutes’ lysozyme incubation at 37°C, DNA was extracted using the EZ1 biorobot (Qiagen, Germantown, MD, USA) with the EZ1 DNA tissue kit. The elution volume was 50 μL. gDNA of Aeromicrobium phoceense strain Marseille-Q0843 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 using MiSeq Technology (Illumina, San Diego, CA, USA) with the paired end strategy, and was barcoded in order to be mixed with 18 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

### TABLE 1. Differential characteristics of Aeromicrobium phoceense strain Marseille-Q0843T and other closely related species with standing in nomenclature

| Property                  | A. phoceense | A. alkaliterrae | A. flavum | A. tamlense | A. choanae | A. panaciterrae |
|---------------------------|--------------|----------------|-----------|-------------|------------|----------------|
| Strain                    | Marseille-Q0843 | KSL-107 | TYLN1 | SSW1-57 | 9H-4 | Gsoil 161T |
| Cell size                 | 0.3–0.5 × 1.0–1.4 μm | 0.3–0.56 × 0.8–1.4 μm | 0.2–0.4 × 0.3–1.2 μm | 0.4–0.66 × 0.8–1.2 μm | 0.56 × 3.3–4.8 μm | 1.5 μm |
| Oxygen requirement        | +            | +            | Facultative | Facultative | +          | +              |
| Gram stain                | +            | +            | +          | +          | +          | +              |
| Motility                  | —            | —            | NA         | —          | —          | —              |
| Endospore formation       | —            | —            | —          | —          | —          | —              |
| Optimum temperature for growth | 31°C | 25°C | 30°C | 30°C | 29–31°C | 15–30°C |
| Production of:            |              |              |            |            |            | NA            |
| Alkaline phosphatase      | +            | +            | +          | +          | NA         | NA            |
| Catalase                  | +            | +            | +          | +          | —          | —              |
| Oxidase                   | —            | —            | +          | +          | —          | —              |
| α-Glucosidase             | +            | +            | +          | +          | +          | NA            |
| β-Galactosidase           | +            | +            | —          | +          | NA         | NA            |
| Acid from:                |              |              |            |            |            | NA            |
| N-Acetylg lysine          | —            | —            | —          | —          | NA         | NA            |
| L-Arabinose               | —            | —            | —          | —          | —          | —              |
| d-Ribose                  | —            | —            | —          | —          | —          | —              |
| d-Mannose                 | —            | —            | —          | —          | —          | —              |
| d-Mannitol                | —            | —            | —          | —          | —          | —              |
| d-Glucose                 | —            | —            | —          | —          | —          | —              |
| d-Fructose                | —            | —            | —          | —          | —          | —              |
| d-Maltose                 | —            | —            | —          | —          | —          | —              |
| d-Lactose                 | —            | —            | —          | —          | —          | —              |
| Acid from:                |              |              |            |            |            | NA            |
| d-Galactose               | —            | —            | —          | —          | —          | —              |
| Glucose                   | —            | —            | —          | —          | —          | —              |
| d-Mannitol                | —            | —            | —          | —          | —          | —              |
| d-Glucose                 | —            | —            | —          | —          | —          | —              |
| d-Fructose                | —            | —            | —          | —          | —          | —              |
| d-Maltose                 | —            | —            | —          | —          | —          | —              |
| d-Lactose                 | —            | —            | —          | —          | —          | —              |
| C content (mol%)          | 70.96        | 71.5         | 73.3      | 72.7       | 70.8       | Soil          |
| Habitat                   | Human        | Various: soil, herbage, seawater | Air sample | Dried seaweed sample | Soil, air, plants, ascidians, human faeces, marine environment | Soil |

*+, positive result; −, negative result; NA, data not available.
on AMPure XP beads (Beckman Coulter, Fullerton, CA, USA), the libraries were normalized on specific beads according to the Nextera XT protocol (Illumina). Normalized libraries were pooled into a single library for sequencing on the MiSeq Technology device. 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 7.33 Gb was obtained from a 763K/mm² cluster density, with a cluster passing quality control filters of 77.80%. Within this run, the index representation for Aeromicrobium phoceense strain Marseille-Q0843T was determined to be 3.54%. The 14 825 474 paired end reads were filtered according to the read qualities.

**Genome annotation and genome comparison**

Genome assembly was performed on Spades v.3.10 software [22]. Genome annotation was obtained through the NCBI Prokaryotic Genome Annotation Pipeline [23]. A phylogenetic tree was obtained using the maximum likelihood method and Kimura 2-parameter model within MEGA 7 software on the annotated-genome-extracted 16S RNA gene sequence [11]. 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 (DDH) with digital DDH (dDDH) [15,16]. The degree of genomic similarity of Aeromicrobium phoceense strain Marseille-Q0843T with closely related species was estimated using OrthoANI software [24]. Antibiotic resistance genes as well as the presence of pathogenesis-related proteins and plasmid were investigated using the ABRicate [25] tools on the Online Galaxy platform by using the Resfinder, CARD, NCBI Bacterial Antimicrobial Resistance Reference Genes, PlasmidFinder and VFDB databases.
Aeromicrobium phoceense strain Marseille-Q0843T was isolated from a skin swab sample of the back of the hand of a 61-year-old healthy woman. Aeromicrobium phoceense strain Marseille-Q0843T failed to be identified by our systematic MALDI-TOF MS screening, suggesting that the corresponding species was not in the database (https://www.mediterranee-infection.com/acces-ressources/base-de-donnees/urms-data-base/) (Fig. 1).

Moreover, Aeromicrobium phoceense strain Marseille-Q0843T exhibited a 16S rRNA sequence similarity value of 99.46% with Aeromicrobium choanae strain 9H-4 (GenBank accession no. NR_156062.1), the phylogenetically closest bacterium.

### TABLE 3. Digital DNA-DNA hybridization (dDDH) values obtained by sequence comparison of all studied genomes using Genometo-Genome Distance Calculator (GGDC) server (formula 2a)

| Query strain | Subject strain | dDDH (%) | CI (%) | G + C content difference (%) |
|--------------|----------------|----------|--------|-----------------------------|
| PE1MN1 'spades' | Aeromicrobium choanae 9H-4 | 52.6 | 49.9–55.3 | 0.05 |
| Marseille-Q0843 | Aeromicrobium tamiae DSM 19087 | 31.9 | 29.5–34.4 | 0.29 |
| Marseille-Q0843 | Aeromicrobium flaccumfaciens DSM 50003 | 29.2 | 26.8–31.7 | 0.18 |
| Marseille-Q0843 | Aeromicrobium eutrochum ATCC 51598 | 20.4 | 18.2–22.9 | 1.17 |
| Marseille-Q0843 | Aeromicrobium zozeae JG/14 | 20.2 | 18.0–22.7 | 1.69 |
| Marseille-Q0843 | Aeromicrobium camelliae YS17 | 20.2 | 17.9–22.6 | 1.59 |
| Marseille-Q0843 | Aeromicrobium endophyticum 9W16Y-2 | 20.1 | 17.9–22.5 | 2.06 |
| Marseille-Q0843 | Aeromicrobium ginsengisoli JCM14732 | 19.8 | 17.6–22.2 | 1.12 |
| Marseille-Q0843 | Aeromicrobium marinum DSM 15272 | 19.7 | 17.5–22.2 | 0.15 |
| Marseille-Q0843 | Nocardioides marinisabuli DSM 18965 | 19.5 | 17.4–21.9 | 2.21 |
| Marseille-Q0843 | Nocardioides jensenii NBRC 14765 | 19.3 | 17.2–21.7 | 2.04 |

CI, Confidence Interval.

**FIG. 5.** Heat map generated with OrthoANI values calculated using OAT software between Aeromicrobium phoceense sp. nov. strain Marseille-Q0843T and other closely related species with standing in nomenclature.

### Results

#### Strain identification and classification

Aeromicrobium phoceense strain Marseille-Q0843T was isolated from a skin swab sample of the back of the hand of a 61-year-old healthy woman. Aeromicrobium phoceense strain Marseille-Q0843T failed to be identified by our systematic MALDI-TOF MS screening, suggesting that the corresponding species was not in the database (https://www.mediterranee-infection.com/acces-ressources/base-de-donnees/urms-data-base/) (Fig. 1). Moreover, Aeromicrobium phoceense strain Marseille-Q0843T exhibited a 16S rRNA sequence similarity value of 99.46% with Aeromicrobium choanae strain 9H-4 (GenBank accession no. NR_156062.1), the phylogenetically closest bacterium.
with standing in nomenclature (Fig. 2). A dDDH analysis between the novel organism and the Aeromicrobium choanae strain 9H-4 type strain revealed an identity of 52.6% (49.9–55.3%), and OrthoANI software parameter provided a value of 93.67%.

**Phenotypic characteristics**
Growth of Aeromicrobium phoceense strain Marseille-Q0843\(^T\) was initially isolated by direct seeding of 50 μL of sample on Columbia agar with 5% sheep’s blood media (bioMérieux) incubated in aerobiosis at 31°C (known to approach the mean physiologic skin temperature of humans). Colonies from strain Marseille-Q0843\(^T\) showed a yellow pigmentation and no haemolysis. Bacterial cells were Gram-positive, nonmotile, irregular rods with a size of 0.3–0.5 × 1.0–1.4 μm determined by electronic scanning microscopy (Fig. 3). Strain Marseille-Q0843\(^T\) is strictly aerobic. Optimum pH of this bacteria is 7.5, and the optimal temperature growth range 31 to 45°C. The sporulation test (20 minutes at 80°C) was negative. Using API strips, positive reactions were shown for potassium nitrate, esculin ferric citrate, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-Bl-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, α-fructose and α-saccharose. All other reactions tested were negative. In addition, this bacterium shows catalase positivity and oxidase negativity. The results are summarized in Table 1. Table 2 compares the characteristics of Aeromicrobium phoceense strain Marseille-Q0843\(^T\) with other bacterial species.

**Genome properties**
The genome size of strain Marseille-Q0843\(^T\) was 3 270 508 bp long with a 70.96% G + C content. The genome assembly of this strain was achieved on three contigs. Of 3235 predicted genes, 3161 were protein-coding genes and 51 were RNAs (21 6S rRNA, two additional 5S rRNAs, two additional 23S rRNAs, 45 tRNAs, three noncoding RNAs) (Fig. 4). The in silico resis-tome of the strain Marseille-Q0843 showed no genes with high identity percentage; neither virulence gene nor plasmid were found. Finally, dDDH and OrthoANI analysis among closely related species showed a similarity index of 21.8% and 93.67% respectively (Table 3 and Fig. 5).

**Discussion and conclusion**
In the past 8 years, a culturomics approach has resulted in the discovery of more than 500 bacterial species [1,16]. Using the taxonogenomics concept – i.e. the combination of the genomic and phenotypic properties of a putative new taxon [16] – we have characterized a new bacterial species representing a new species within the family Nocardioidaeae found on human skin. It was named as Aeromicrobium phoceense strain Marseille-Q0843\(^T\): Aer.o.mi.cro.bi.um, Gr. masc. n. aer (gen. aero), ‘air’; N.L. neut. n. microbiöm, ‘microbe’; N.L. neut. n. Aeromicrobium, ‘aerobic microbe’. Medi.ter.rra.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.

**Deposit in culture Collections and sequence databases**
Aeromicrobium phoceense strain Marseille-Q0843\(^T\) was deposited in Collection de Souches de l’Unité des Rickettsies (CSUR) under accession number CSUR-Q0843. The 16S rRNA and genome sequences are available in GenBank under accession numbers MT764256.1 and JACEOG000000000.1 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] Tang Y, Zhou G, Zhang L, Mao J, Luo X, Wang M, et al. Aeromicrobium flavum sp. nov., isolated from air. Int J Syst Evol Microbiol 2008;58:1860–3. https://doi.org/10.1099/ijsem.0.065443-0.
[3] Yoon JH, Lee CH, Oh TK. Aeromicrobium alkaliterrae sp. nov., isolated from an alkaline soil, and emended description of the genus Aeromicrobium. Int J Syst Evol Microbiol 2005;55:2171–5. https://doi.org/10.1099/ijsem.0.03582-0.
KIM MK, Park MJ, Im WT, Yang DC. Aeromicrobium ginsengisoli sp. nov., isolated from a ginseng field. Int J Syst Evol Microbiol 2008;58:2025–30. https://doi.org/10.1099/ijs.0.64871-0.

Kim SH, Yang HO, Sohn YC, Kwon HC. Aeromicrobium halocynthiae sp. nov., a taurocholic acid-producing bacterium isolated from the marine ascidian Halocynthia roretzi. Int J Syst Evol Microbiol 2010;60:2793–8. https://doi.org/10.1099/ijs.0.016618-0.

Sun Y, Liu WH, Ai MJ, Su J, Zhang YQ. Aeromicrobium lacus sp. nov., a novel actinobacterium isolated from a drinking-water reservoir. Int J Syst Evol Microbiol 2019;69:460–4. https://doi.org/10.1099/ijsem.0.03181.

Bruns A. Aeromicrobium marinum sp. nov., an abundant pelagic bacterium isolated from the German Wadden Sea. Int J Syst Evol Microbiol 2003;53:1917–23. https://doi.org/10.1099/ijs.0.02735-0.

Cui YS, Im WT, Yin CR, Lee JS, Lee KC, Lee ST. Aeromicrobium panacierrae sp. nov., isolated from soil of a ginseng field in South Korea. Int J Syst Evol Microbiol 2007;57:687–91. https://doi.org/10.1099/ijs.0.64697-0.

Lee SD, Kim SJ. Aeromicrobium tamlense sp. nov., isolated from dried seaweed. Int J Syst Evol Microbiol 2008;58:987–91. https://doi.org/10.1099/ijs.0.65575-0.

Lee SD, Kim SJ. Aeromicrobium endophyticum sp. nov., an endophytic actinobacterium isolated from the choana of a garden warbler. Int J Syst Evol Microbiol 2017;67:357–61. https://doi.org/10.1099/ijsem.0.001632.

Li FN, Liao SL, Liu SW, Jin T, Sun CH. Aeromicrobium endophyticum sp. nov., an endophytic actinobacterium isolated from reed (Phragmites australis). J Microbiol 2019;57:725–31. https://doi.org/10.1007/s12275-019-8705-7.

Ramasamy D, Kokcha S, Lagier JC, Nguyen TT, Raoult D, Fournier PE. Genome sequence and description of Aeromicrobium massiliense sp. nov. Stand Genomic Sci 2012;7:246–57. https://doi.org/10.4056/sigs.3306717.

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

Afgan E, Baker D, van den Beek M, Blankenberg D, Bouvier D, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19:455–77. https://doi.org/10.1089/cmb.2012.0021.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19:455–77. https://doi.org/10.1111/j.1365-2958.2010.01790.x.