Draft genome and description of *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 in *Nocardioidaceae* in the phylum *Actinobacteria*. We describe here the main phenotypic characteristics, genome sequence and annotation of *Aeromicrobium phoceense* strain Marseille-Q0843^T, a new member of the *Aeromicrobium* genus, which we propose as the type strain.

Keywords: *Aeromicrobium*, culturomics, genome, sp. nov., taxonogenomics

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Corresponding author: B. La Scola, Méditerranée-Infection, Aix-Marseille Université, 19-21 Boulevard Jean Moulin, 13385, Marseille, cedex 05, France.

E-mail: bernard.la-scola@univ-amu.fr

Introduction

The genus *Aeromicrobium* comprises 22 species [1], most isolated from diverse environmental samples, such as air [2], soil and water [3–12], or associated with plants and birds [13,14]. Only one species has been isolated from a human: *Aeromicrobium massiliense* [15]. *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 [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 [19,20]. The genome of *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 *Aeromicrobium choanae* strain 9H-4 16S 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 *Aeromicrobium choanae* strain 9H-4. On the basis of these data, we propose *Aeromicrobium phoceense* strain Marseille-Q0843^T, a new member of the *Aeromicrobium* genus, as the type strain.

Materials and methods

Strain isolation and phenotypic tests

Aeromicrobium phoceense strain Marseille-Q0843^T was isolated from a swab sample taken from the skin of the back of the hand a 61-year-old healthy woman. Sampling was performed in the CosNat Provence laboratory (https://cosnat-loccitane.com, Marseille area, France). *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) [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).

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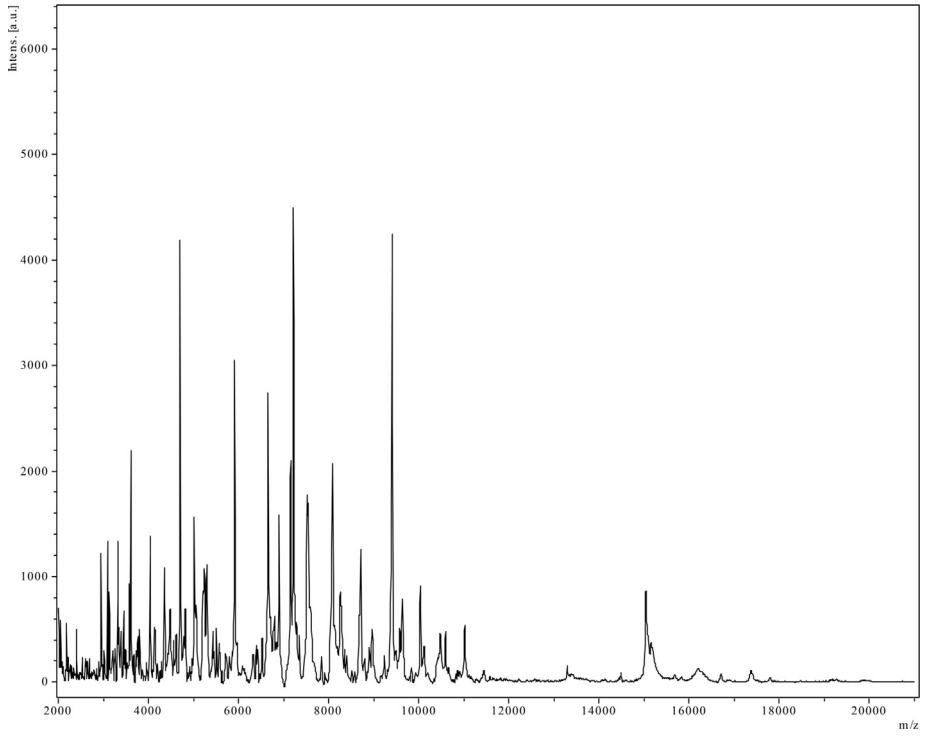

FIG. 1. Matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) reference mass spectrum. Spectra from 12 individual colonies of strain Marseille-Q0843^T were compared and reference spectrum generated.

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

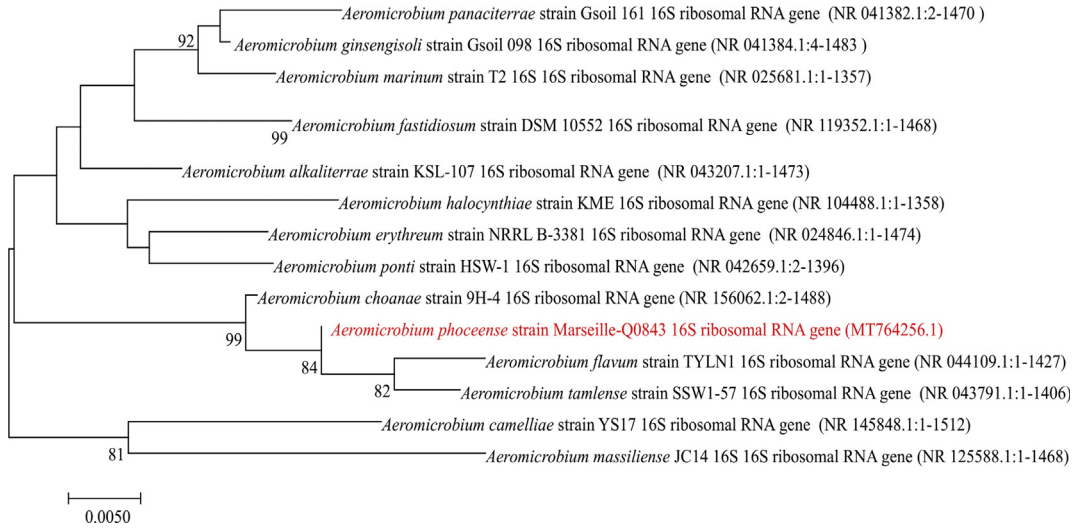

FIG. 2. 16S ribosomal RNA–based phylogenetic tree highlighting position of *Aeromicrobium phoceense* sp. nov. strain Marseille-Q0843^T (red) relative to other closely related bacterial taxa. Sequences were aligned using Muscle v3.8.31 with default parameters; phylogenetic relationship was inferred using maximum likelihood method, with 1000 bootstrap replicates, within MEGA 7 software.

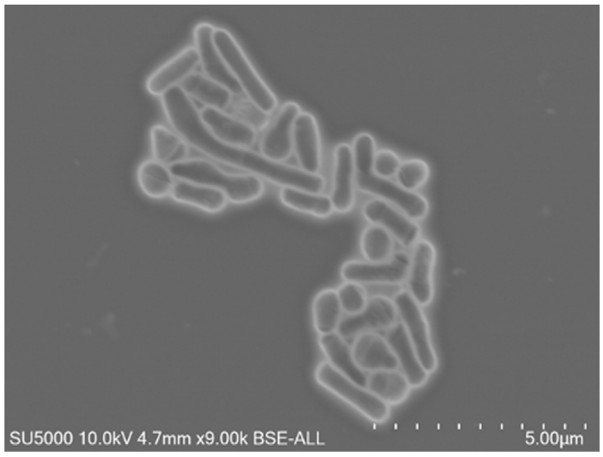

FIG. 3. Scanning electron microscopy of *Aeromicrobium phoceense* sp. nov. strain Marseille-Q0843^T using tabletop microscope TM4000Plus tabletop microscope (Hitachi High-Tech, Tokyo, Japan). Scale bar represents 5 µm.

with approximately 60 cm in height and 33 cm in width between the microsocpe detector and the slide to evaluate the bacterial structure using a TM4000Plus tabletop microscope (Hitachi High-Tech, Tokyo, Japan). A sporulation test was performed by applying a heat shock to the bacteria for 20 minutes at 80°C. Motility test was performed using the semi solid 2,3,5- triphenyltetrazolium chloride (TCC) media as described by Tittsler and Sandholzer [21].

Genome sequencing

Genomic DNA (gDNA) of *Aeromicrobium phoceense* strain Marseille-Q0843^T 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-Q0843^T 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 to 0.56 × 3.8–4.8 µm	1.5 µm	0.2–0.4 mm
Oxygen requirement	+	+	Facultative	Facultative	+	+
Gram strain	+	+	+	+	+	+
Motility	—	—	—	—	—	—
Endospore formation	—	—	NA	—	—	—
Optimum temperature for growth	31°C	25°C	30°C	30°C	29–31°C	15–30°C
Production of:						
Alkaline phosphatase	+	—	—	+	+	NA
Catalase	+	+	+	+	+	—
Oxidase	—	—	+	—	—	—
α-Glucosidase	+	+	+	+	—	NA
β-Galactosidase	+	—	—	—	+	NA
Acid from:						
N-Acetylglucosamine	—	—	—	—	—	NA
L-Arabinose	—	+	+	—	NA	—
D-Ribose	—	NA	+	NA	NA	NA
D-Mannose	—	—	—	+	+	+
D-Mannitol	—	NA	NA	+	+	—
D-Glucose	—	+	+	+	NA	+
D-Fructose	+	—	+	+	NA	—
D-Maltose	—	+	+	+	NA	+
D-Lactose	—	NA	+		NA	NA
G + C content (mol%)	70.96	71.5	73.3	72.7	70.8	65.5
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.

TABLE 2. Description of *Aeromicrobium phoceense* strain Marseille-Q0843^T

Type of description	New description
Species name	*phoceense*
Genus name	*Aeromicrobium*
Specific epithet	*Aeromicrobium*
Species status	sp. nov.
Species etymology	*Aeromicrobium phoceense* strain Marseille-Q0843^T. Aer.o.mi.cro'bi.um, Gr. masc. n. *aer* (gen. *aeros*), 'air'; N.L. neut. n. *microbium*, 'microbe'; N.L. neut. n. *Aeromicrobium*, 'aerobic microbe'. 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 Q0843
Strain collection number	CSUR-Q0843
16S rRNA gene accession number	MT764256.1
Genome accession number	JACEOG000000000.1
Genome status	Draft
Genome size	3.270.508
GC%	70.96
Country of origin	Marseille, France
Date of isolation	2019
Source of isolation	Human skin
Growth medium, incubation	Columbia agar with 5% sheep's blood media (bioMérieux, France) at 31°C
Gram strain	Positive
Cell shape	Irregular rods
Cell size	0.3–0.5 × 1.0–1.4 µm
Motility	Nonmotile
Sporulation	Nonsporulating
Colony morphology	
Temperature range	31–45°C
Temperature optimum	31°C
Relationship to O_2	Necessary
O_2 requirement	Strict aerobe
Oxidase	Negative
Catalase	Positive

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/mm^2 cluster density, with a cluster passing quality control filters of 77.80%. Within this run, the index representation for *Aeromicrobium phoceense* strain Marseille-Q0843^T 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 obtain 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-Q0843^T 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.

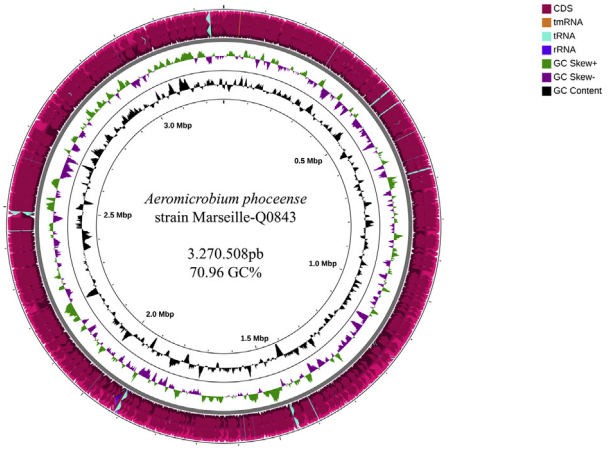

FIG. 4. Graphical circular map of genome from strain *Aeromicrobium phoceense* sp. nov. strain Marseille-Q0843^T obtained by CGView tool [26].

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

Query strain	Subject strain	dDDH (%)	CI (%)	G + C content difference (%)
Q0843 PE1MN1 spades'	*Aeromicrobium choanae* 9H-4	52.6	49.9–55.3	0.05
Aeromicrobium phoceense strain Marseille-Q0843	*Aeromicrobium tamlense* DSM 19087	31.9	29.5–34.4	0.29
Aeromicrobium phoceense strain Marseille-Q0843	*Aeromicrobium flavum* NBRC 107625	29.2	26.8–31.7	0.18
Aeromicrobium phoceense strain Marseille-Q0843	*Aeromicrobium erythreum* ATCC 51598	20.4	18.2–22.9	1.17
Aeromicrobium phoceense strain Marseille-Q0843	*Aeromicrobium massiliense* JC14	20.2	18.0–22.7	1.69
Aeromicrobium phoceense strain Marseille-Q0843	*Aeromicrobium camelliae* YS17	20.2	17.9–22.6	1.59
Aeromicrobium phoceense strain Marseille-Q0843	*Aeromicrobium endophyticum* 9W16Y-2	20.1	17.9–22.5	2.06
Aeromicrobium phoceense strain Marseille-Q0843	*Aeromicrobium ginsengisoli* JCM14732	19.8	17.6–22.2	1.12
Aeromicrobium phoceense strain Marseille-Q0843	*Aeromicrobium marinum* DSM 15272	19.7	17.5–22.2	0.15
Aeromicrobium phoceense strain Marseille-Q0843	*Nocardioides marinisabuli* DSM 18965	19.5	17.4–21.9	2.21
Aeromicrobium phoceense strain Marseille-Q0843	*Nocardioides jensenii* NBRC 14755	19.3	17.2–21.7	2.04

CI, Confidence Interval.

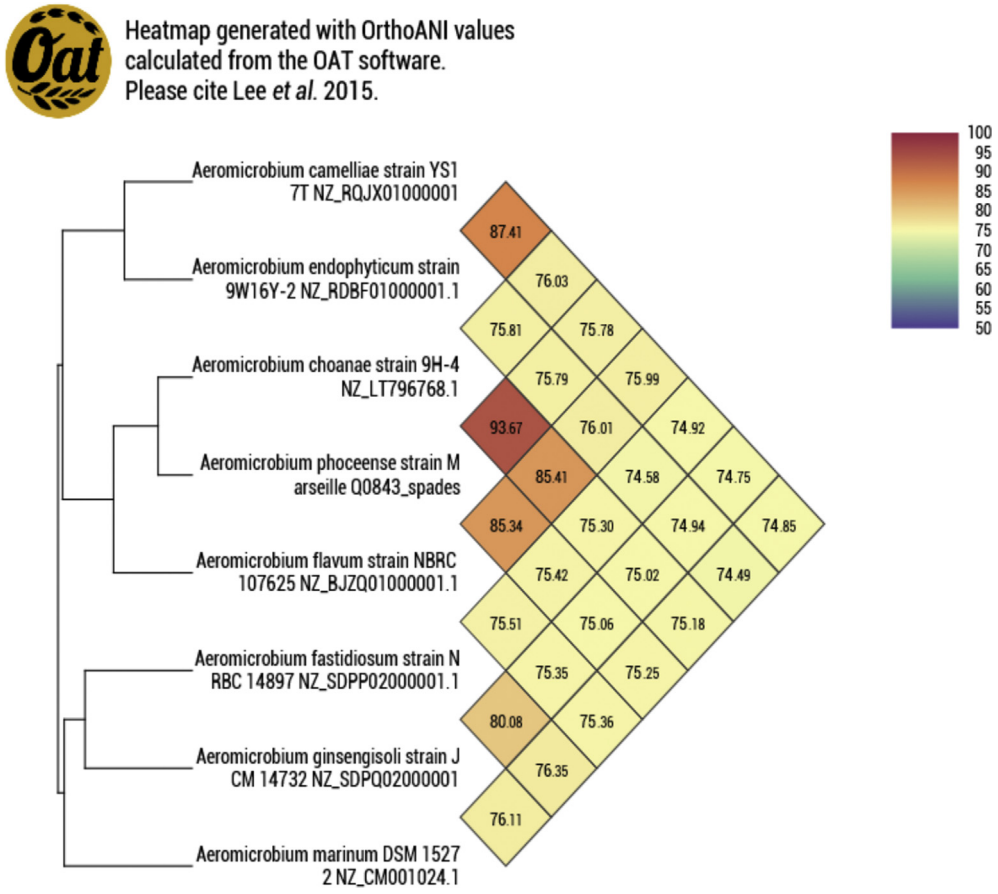

FIG. 5. Heat map generated with OrthoANI values calculated using OAT software between *Aeromicrobium phoceense* sp. nov. strain Marseille-Q0843^T and other closely related species with standing in nomenclature.

Results

Strain identification and classification

Aeromicrobium phoceense strain Marseille-Q0843^T was isolated from a skin swab sample of the back of the hand of a 61-year-old healthy woman. *Aeromicrobium phoceense* strain Marseille-Q0843^T 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-Q0843^T 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-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, D-fructose and D-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, 3161were 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* resistome 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 *Nocardioidaceae* 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. *aeros*), 'air'; N.L. neut. n. *microbium*, 'microbe'; N.L. neut. n. *Aeromicrobium*, 'aerobic microbe'. 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.

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

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