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# Draft genome and description of *Aeromicrobium phoceense strain Marseille-Q0843*$^{T}$ sp. nov., a new bacterium isolated from human healthy skin

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**Abstract**

In 2019, by culturing a skin swab from the back of the hand of a 61-year-old healthy woman through the culturomic 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 rRNA gene and Genome-to-Genome comparison suggested that this taxon belongs to a novel bacterial species within the family in *Nocardioidaceae* the phylum *Actinobacteria*. We described here its main phenotypic characteristics, genome sequence and annotation of *Aeromicrobium phoceense* strain Marseille-Q0843$^T$, a new member of the *Aeromicrobium* genus, that we propose as type strain.

**Keywords: *Aeromicrobium*; culturomics; genome; sp. nov.; taxono-genomics;**

## Introduction

The genus *Aeromicrobium* counts 22 species [1], most of them were isolated from diverse samples of the environment such as air [2], soils and water [3–12] or associated with plants and birds [13,14]. Only one species were isolated from human, *Aeromicrobium massiliense* [15]. *Aeromicrobium phoceense* strain Marseille-Q0843$^T$ was isolated using the culturomics approach, based on the use of a large panel of culture conditions in order to describe the microbial composition of a sample by high-throughput culture [16–19] A taxono-genomics 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 [20,21]. 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 rRNA sequence similarity value of 99.46%. Genomic comparison using the OrthoANI parameter provided a value of 93.67 % and a dDDH value of 21.8% [19.5 - 24.2] with *Aeromicrobium choanae* strain 9H-4. Based on these data, we propose *Aeromicrobium phoceense* strain Marseille-Q0843$^T$, a new member of the *Aeromicrobium* genus, as type strain.

## Materials and methods

### Strain isolation and phenotypic tests

*Aeromicrobium phoceense* strain Marseille-Q0843$^T$ was isolated from the skin of the back of the hand swab of a 61-year-old healthy woman. Sampling was performed in CosNat Provence laboratory (https://cosnat-loccitane.com, Marseille area). *Aeromicrobium phoceense* strain Marseille-Q0843$^T$ was initially isolated by direct seeding of 50µL of sample on Columbia Agar with 5% Sheep Blood media (BioMérieux, France) incubated in aerobiosis at 31°C, MALDI-TOF mass spectrometry (MS) protein analysis was carried out using a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [8]. Spectra from strain Marseille-Q0843$^T$ were imported into the MALDI BioTyper software (version 3.0, Bruker) and analyzed by standard pattern matching (with default parameter settings). The study was validated by the ethics committee ID-RCB: 2019-A01508-49.

### Phenotypic characterization

Different growth temperatures (21, 28, 30, 37, 45 and 56°C), atmosphere conditions, anaerobic, aerobic and microaerophilic (CampyGEN, Oxoid, USA) and pH (5,5; 6.5; 7.5; 8.5) were tested. API ZYM, API 20NE and API 50 CH 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 centimeters in height and 33 cm in width to evaluate bacterial structure on a TM4000 microscope. (Hitachi High-Technologies Corp., Tokyo, Japan). Sporulation test was performed by applying a heat choc to the bacteria 20 minutes at 80°C. Motility test was performed using the semi solid TCC media as described by Tittsler *et al.*[22]

### 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) using a FastPrep-24™ 5G Grinder (mpBio) at maximum speed (6.5) for 90s. Then after 30 minutes lysozyme incubation at 37°C, DNA was extracted on the EZ1 biorobot (Qiagen) with EZ1 DNA tissues kit. The elution volume is 50µL. 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

on the MiSeq Technology (Illumina Inc, San Diego, CA, USA) with the paired end strategy and was barcoded in order to be mixed respectively 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 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 Inc, 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-hours run in 2x250-bp. Total information of 7.33 Gb was obtained from a 763 K/mm2 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 index 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 software (v.3.10). [23] Genome annotation was obtain through the NCBI Prokaryotic Genome Annotation Pipeline [24]. A phylogenetic tree was obtained using the Maximum Likelihood method and Kimura 2-parameter within the 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) by a digital DDH (dDDH) [15, 16]. The degree of genomic similarity of *Aeromicrobium phoceense* strain Marseille-Q0843$^T$ with closely related species was estimated using the OrthoANI software [25]. Antibiotic resistance genes, presence of pathogenesis-related proteins and plasmid was investigated using the ABRicate [26] tools on Online Galaxy platform by using Resfinder, CARD , NCBI Bacterial Antimicrobial Resistance Reference Genes, PlasmidFinder and VFDB databases.

96 **Results**

97 **Strain identification and classification**

98 *Aeromicrobium phoceense* strain Marseille-Q0843$^T$ was isolated from the skin of the back of
99 the hand swab of a 61-year-old healthy woman. *Aeromicrobium phoceense* strain Marseille-
100 Q0843$^T$ failed to be identified by our systematic MALDI-TOF MS screening, suggesting that
101 the corresponding species was not in the database - https://www.mediterranee-
102 infection.com/acces-ressources/base-de-donnees/urms-data-base/. **(Figure 1)** Moreover,
103 *Aeromicrobium phoceense* strain Marseille-Q0843$^T$ exhibited a 16S rRNA sequence
104 similarity value of 99.46% with *Aeromicrobium choanae* strain 9H-4 (GenBank accession no.
105 NR_156062.1), the phylogenetically closest bacterium standing in nomenclature **(Figure 2).**
106 A digital DNA–DNA hybridization analysis between the novel organism and the
107 *Aeromicrobium choanae* strain 9H-4 type strain revealed an identity of 52.6 % [49.9 - 55.3%]
108 and OrthoANI software parameter provided a value of 93.67 %.

109

110 **Phenotypic characteristics**

111 Growth of *Aeromicrobium phoceense* strain Marseille-Q0843$^T$ was initially isolated by direct
112 seeding of 50µL of sample on Columbia Agar with 5% Sheep Blood media (BioMérieux,
113 France) incubated in aerobiosis at 31°C (known to approach the mean physiological skin
114 temperature). Colonies from strain Marseille-Q0843$^T$ showed a yellow pigmentation and no
115 hemolysis. Bacterial cells were Gram-positive, non-motile, irregular rods with a size of 0.3-
116 0.5 x 1.0-1.4 µm determined by electronic scanning microscopy **(Figure 3).** Strain Marseille-
117 Q0843$^T$ is strictly aerobic. Optimum pH of this bacteria is 7.5 and the optimal temperature
118 growth range 31°C to 45°C. The sporulation test (20 minutes at 80°C) was negative. Using
119 API strips, positive reactions were shown for Potassium nitrate, Esculin ferric citrate,
120 Alkaline phosphatase, Esterase (C4), Esterase Lipase (C8), Leucine arylamidase, Cystine
121 arylamidase, Acid phosphatase, Naphthol-AS-BI-phosphohydrolase, $\beta$-galactosidase, $\alpha$ -
122 glucosidase, $\beta$-glucosidase, D-fructose, D-saccharose. All other reactions tested were
123 negative. In addition, this bacterium shows catalase positive and oxidase negative. The results
124 are summarized in **Table 1**. **Table 2** compares the characteristics of *Aeromicrobium*
125 *phoceense* strain Marseille-Q0843$^T$ with other bacterial species.

126

127

128 **Genome properties**

129 The genome size of strain Marseille-Q0843$^{T}$ was 3.270.508 bp long with a 70.96 % G+C
130 content. The genome assembly of this strain was achieved on 3 contigs. Of 3.235 predicted
131 genes, 3.161were protein-coding genes and 51 were RNAs (2 16S rRNA, 2 additional 5S
132 rRNAs, 2 additional 23S rRNAs, 45 tRNAs AND 3 ncRNAS). **(Figure 4)** The *in silico*
133 resistome of the strain Marseille-Q0843 shows no genes with high identity percentage, neither
134 virulence gene nor plasmid were found. Finally, dDDH and OrthoANI analysis among closely
135 related species shown similarity index from 21.8% to 93.67% respectively. **(Table 3 and**
136 **figure 5)**

## Discussion and conclusion

In the past 8 years, culturomic approach induce the discovery of more than 500 bacterial species [1, 27]. Using the taxono-genomics concept *i.e*. the combination of the genomic and phenotypic properties of a putative new taxon [27], we have characterized a new bacterial species representing a new species within the family *Nocardioidaceae* found on a forehead 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 Sequences Database

*Aeromicrobium phoceense* strain Marseille-Q0843$^T$, was deposited in CSUR collections under accession CSUR-Q0843. The 16S rRNA and genome sequences are available in GenBank under accession numbers MT764256.1 and JACEOG000000000.1, respectively.

## Transparency declaration

None to declare


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259 **Figure.1.** MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies of
260 strain Marseille-Q0843$^T$ were compared and a reference spectrum was generated.

261 **Figure 2:** 16s rRNA-based phylogenetic tree highlighting the position of *Aeromicrobium*
262 *phoceense* sp., nov., strain Marseille-Q0843$^T$(red) relative to other closely related bacterial
263 taxa. Sequences were aligned using Muscle v3.8.31 with default parameters and phylogenetic
264 relationship inferred using the Maximum Likelihood method, with 1,000 bootstrap replicates,
265 within the MEGA software version 7.0

266 **Figure 3:** Scanning electron microscopy of *Aeromicrobium phoceense* sp. nov., strain
267 Marseille-Q0843$^T$ using a Tabletop microscope TM 4000plus (Hitachi, Tokyo, Japan). The
268 scale bar represents 5 µm.

269 **Figure 4:** Graphical circular map of the genome from strain Marseille-Q0843$^T$ obtained by
270 CG view tool. [27]

271 **Figure.5:** Heatmap generated with OrthoANI values calculated using the OAT software
272 between *Aeromicrobium phoceense* sp., nov., strain Marseille-Q0843$^T$ and other closely
273 related species standing in nomenclature.

274 **Table 1:** Differential characteristics of *Aeromicrobium phoceense* strain Marseille-Q0843$^T$ and other closely related species standing in
275 nomenclature

| Properties | *A. phoceense* **Marseille-Q0843** | *A. alkaliterrae* **KSL-107** | *A.flavum* **TYLN1** | *A. tamlense* **SSW1-57** | *A.choanae* **9H-4** | *A. panaciterrae* **Gsoil 161T** |
|---|---|---|---|---|---|---|
| Cell size (µm ) | 0.3-0.5 x 1.0-1.4 µm | 0.3–0.56x0.8–1.4 µm | 0.2–0.4 µm x 0.3–1.2 µm | 0.4–0.66 x 0.8–1.2 to 0.56 x 3.8–4.8 µm | 1.5µm | 0.2–0.4 mm |
| Oxygen requirement | + | + | facultevaly | facultevaly | + | + |
| Gram Strain | + | + | + | + | + | + |
| Motility | - | - | - | - | - | - |
| Endospore formation | - | - | na | - | - | - |
| Optimum temperature for growth (°C) | 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 : soils, herbage, sea water | air sample | dried seaweed sample | soil, air, plants, ascidians, human faces ans the marine environment | soil |

276

277 **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-Q0843T. 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 | BOXBERGER Manon, 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 Blood media (BioMérieux, France) at 31°C |
| Gram strain | postive |
| Cell shape | irregular rods |
| Cell size | 0.3-0.5 x 1.0-1.4 µm |
| Motility | Non-Motile |
| Sporulation | Non-sporulating |
| Colony morphology | |
| Temperature range | 31-45°C |
| Temperature optimum | 31°C |
| Relationship to O2 | Necessary |
| O2 requirement | Strictly aerobe |
| Oxidase | Negative |
| Catalase | Positive |

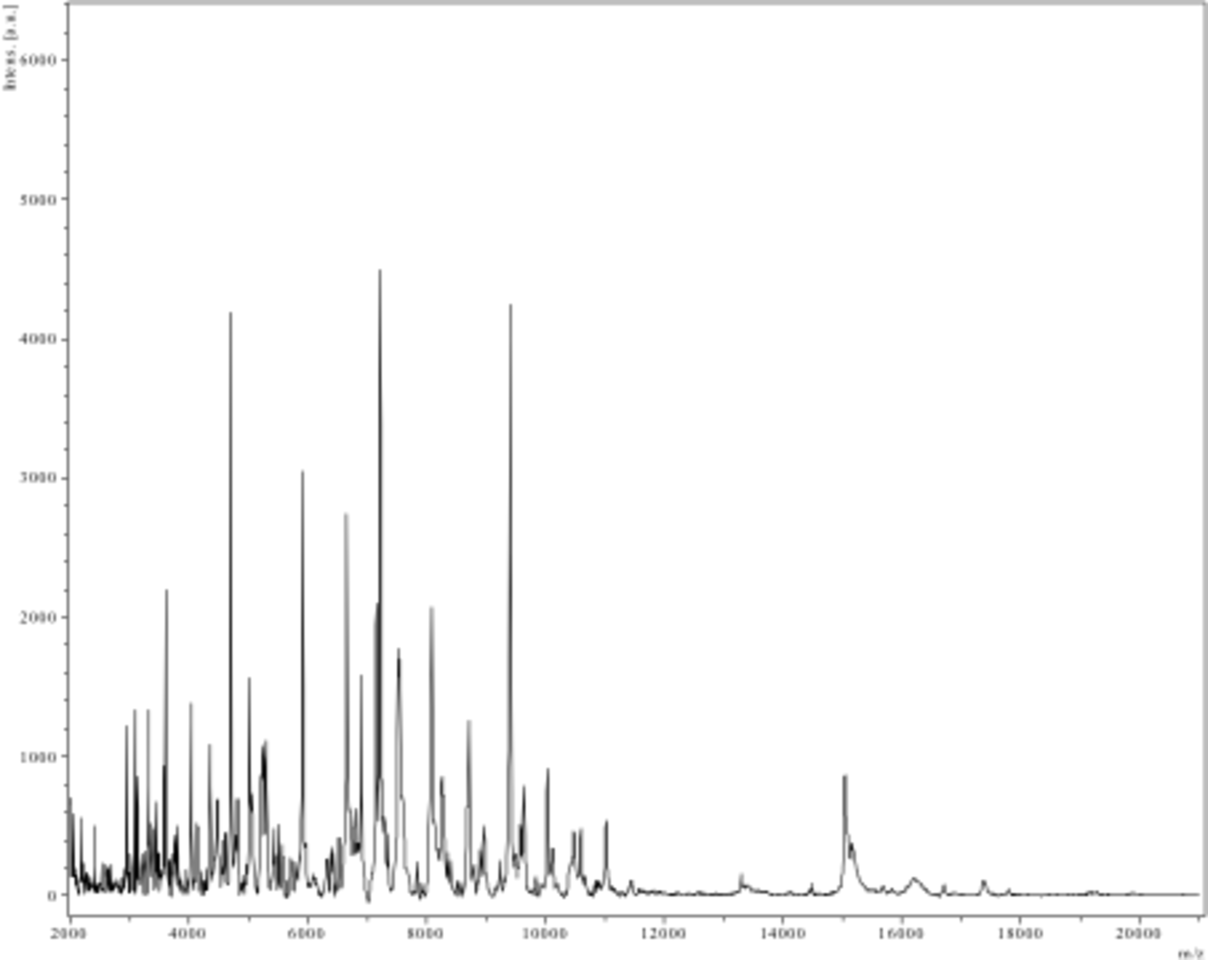
278

279

280 **Table 3:** Digital DNA–DNA hybridization values obtained by sequence comparison of all
281 studied genomes using GGDC server. (formula 2a)
282

| **Type Strain Genome Server** | | | | |
|---|---|---|---|---|
| **Query strain** | **Subject strain** | **dDDH ( %)** | **C.I. (%)** | **G+C content difference (in %)** |
| 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 |

283


Intens. [a.u.]
6000
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2000
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12000
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18000
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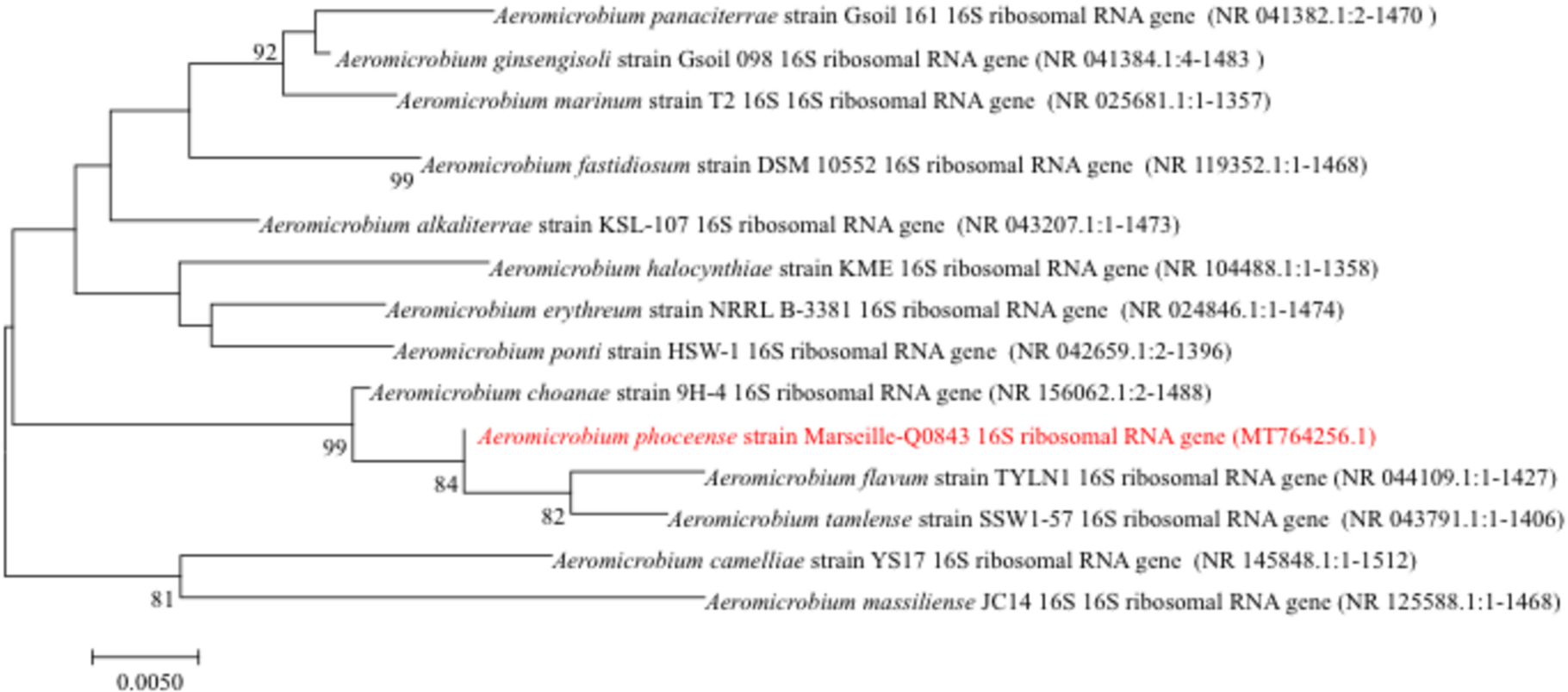
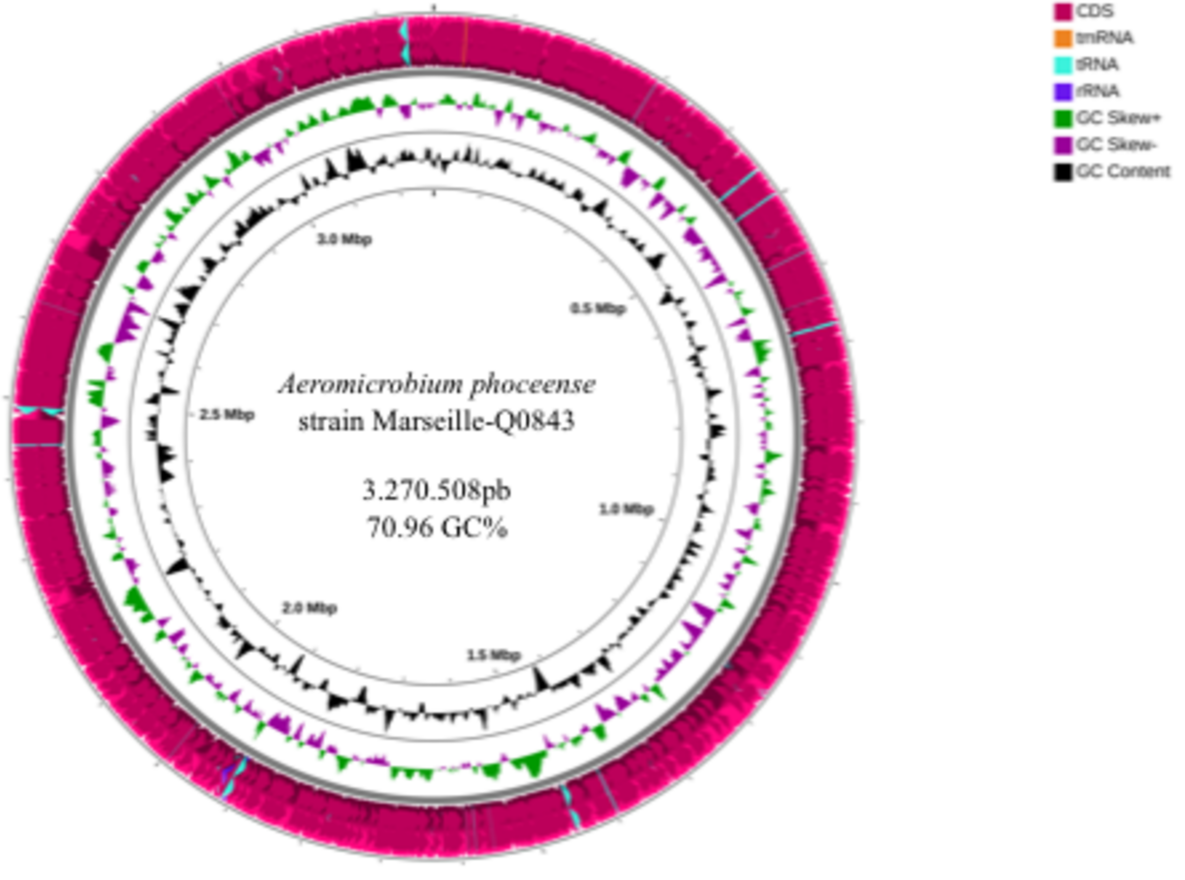
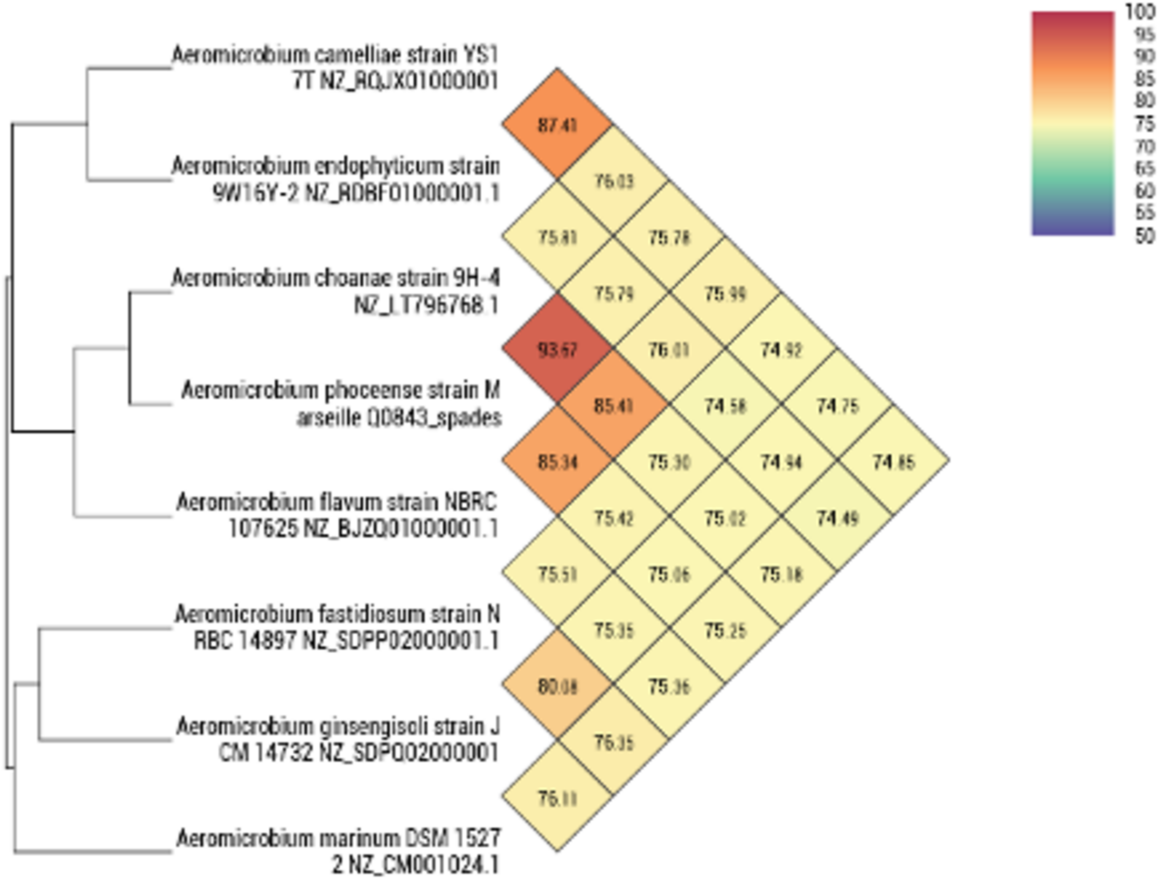
m/z


Aeromicrobium panaciterrae strain Gsoil 161 16S ribosomal RNA gene (NR 041382.1:2-1470 )
92
Aeromicrobium ginsengisoli strain Gsoil 098 16S ribosomal RNA gene (NR 041384.1:4-1483 )
Aeromicrobium marinum strain T2 16S 16S ribosomal RNA gene (NR 025681.1:1-1357)
Aeromicrobium fastidiosum strain DSM 10552 16S ribosomal RNA gene (NR 119352.1:1-1468)
99
Aeromicrobium alkaliterrae strain KSL-107 16S ribosomal RNA gene (NR 043207.1:1-1473)
Aeromicrobium halocynthiae strain KME 16S ribosomal RNA gene (NR 104488.1:1-1358)
Aeromicrobium erythreum strain NRRL B-3381 16S ribosomal RNA gene (NR 024846.1:1-1474)
Aeromicrobium ponti strain HSW-1 16S ribosomal RNA gene (NR 042659.1:2-1396)
Aeromicrobium choanae strain 9H-4 16S ribosomal RNA gene (NR 156062.1:2-1488)
99
Aeromicrobium phoceense strain Marseille-Q0843 16S ribosomal RNA gene (MT764256.1)
84
Aeromicrobium flavum strain TYLN1 16S ribosomal RNA gene (NR 044109.1:1-1427)
82
Aeromicrobium tamlense strain SSW1-57 16S ribosomal RNA gene (NR 043791.1:1-1406)
Aeromicrobium camelliae strain YS17 16S ribosomal RNA gene (NR 145848.1:1-1512)
81
Aeromicrobium massiliense JC14 16S 16S ribosomal RNA gene (NR 125588.1:1-1468)
0.0050

SU5000 10.0kV 4.7mm x9.00k BSE-ALL
5.00µm


CDS
tmRNA
tRNA
rRNA
GC Skew+
GC Skew-
GC Content
3.0 Mbp
0.5 Mbp
2.5 Mbp
1.0 Mbp
2.0 Mbp
1.5 Mbp
Aeromicrobium phoceense
strain Marseille-Q0843
3.270.508pb
70.96 GC%

Heatmap generated with OrthoANI values calculated from the OAT software.
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