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1 **Draft genome and description of *Aeromicrobium phoceense* strain *Marseille-Q0843*<sup>T</sup> sp.**  
2 **nov., a new bacterium isolated from human healthy skin**

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21

1 **Abstract**

2 In 2019, by culturing a skin swab from the back of the hand of a 61-year-old healthy woman  
3 through the culturomic method, we isolated the new bacterial strain Marseille-Q0843<sup>T</sup> (=   
4 CSUR-Q0843). Matrix-assisted desorption ionization–time of flight mass spectrometry  
5 (MALDI-TOF MS) failed to identify this isolate. Analysis of the 16S rRNA gene and  
6 Genome-to-Genome comparison suggested that this taxon belongs to a novel bacterial species  
7 within the family in *Nocardioidaceae* the phylum *Actinobacteria*. We described here its main  
8 phenotypic characteristics, genome sequence and annotation of *Aeromicrobium phoceense*  
9 strain Marseille-Q0843<sup>T</sup>, a new member of the *Aeromicrobium* genus, that we propose as type  
10 strain.

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

12 **Introduction**

13 The genus *Aeromicrobium* counts 22 species [1], most of them were isolated from diverse  
14 samples of the environment such as air [2], soils and water [3–12] or associated with plants  
15 and birds [13,14]. Only one species were isolated from human, *Aeromicrobium massiliense*  
16 [15]. *Aeromicrobium phoceense* strain Marseille-Q0843<sup>T</sup> was isolated using the culturomics  
17 approach, based on the use of a large panel of culture conditions in order to describe the  
18 microbial composition of a sample by high-throughput culture [16–19] A taxono-genomics  
19 approach including matrix-assisted laser desorption-ionization time-of-flight mass  
20 spectrometry (MALDI-TOF MS), phylogenetic analysis, main phenotypic description and  
21 genome sequencing, was used to describe this species [20,21]. The genome of *Aeromicrobium*  
22 *phoceense* strain Marseille-Q0843<sup>T</sup> is 3.270.508 bp long with 70.96% G+C content. This new  
23 bacterium is most closely related to *Aeromicrobium choanae* strain 9H-4 16S with a 16S  
24 rRNA sequence similarity value of 99.46%. Genomic comparison using the OrthoANI  
25 parameter provided a value of 93.67 % and a dDDH value of 21.8% [19.5 - 24.2] with  
26 *Aeromicrobium choanae* strain 9H-4. Based on these data, we propose *Aeromicrobium*  
27 *phoceense* strain Marseille-Q0843<sup>T</sup>, a new member of the *Aeromicrobium* genus, as type  
28 strain.

29

30

## 31 **Materials and methods**

### 32 **Strain isolation and phenotypic tests**

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

43

### 44 **Phenotypic characterization**

45 Different growth temperatures (21, 28, 30, 37, 45 and 56°C), atmosphere conditions,  
46 anaerobic, aerobic and microaerophilic (CampyGEN, Oxoid, USA) and pH (5.5; 6.5; 7.5; 8.5)  
47 were tested. API ZYM, API 20NE and API 50 CH strips (BioMérieux, Marcy L'Etoile,  
48 France) were used to evaluate the biochemical properties of the strain according to the  
49 manufacturer's instructions. For scanning electronic microscopy, a colony was collected from  
50 agar and immersed into a 2.5 % glutaraldehyde fixative solution. The slide was gently washed  
51 in water; air dried and examined with approximately 60 centimeters in height and 33 cm in  
52 width to evaluate bacterial structure on a TM4000 microscope. (Hitachi High-Technologies  
53 Corp., Tokyo, Japan). Sporulation test was performed by applying a heat choc to the bacteria  
54 20 minutes at 80°C. Motility test was performed using the semi solid TCC media as described  
55 by Tittsler *et al.*[22]

56

### 57 **Genome sequencing**

58 Genomic DNA (gDNA) of *Aeromicrobium phoceense* strain Marseille-Q0843<sup>T</sup> was extracted  
59 in two steps: a mechanical treatment was first performed by glass beads acid washed (G4649-  
60 500g Sigma) using a FastPrep-24<sup>TM</sup> 5G Grinder (mpBio) at maximum speed (6.5) for 90s.  
61 Then after 30 minutes lysozyme incubation at 37°C, DNA was extracted on the EZ1 biorobot  
62 (Qiagen) with EZ1 DNA tissues kit. The elution volume is 50µL. gDNA of *Aeromicrobium*  
63 *phoceense* strain Marseille-Q0843 was quantified by a Qubit assay with the high sensitivity  
64 kit (Life technologies, Carlsbad, CA, USA) to 0.2 ng/µl. Genomic DNA was next sequenced

65 on the MiSeq Technology (Illumina Inc, San Diego, CA, USA) with the paired end strategy  
66 and was barcoded in order to be mixed respectively with 18 other genomic projects prepared  
67 with the Nextera XT DNA sample prep kit (Illumina). To prepare the paired end library,  
68 dilution was performed to require 1ng of each genome as input to prepare the paired end  
69 library. The « tagmentation » step fragmented and tagged the DNA. Then limited cycle PCR  
70 amplification (12 cycles) completed the tag adapters and introduced dual-index barcodes.  
71 After purification on AMPure XP beads (Beckman Coulter Inc, Fullerton, CA, USA), the  
72 libraries were then normalized on specific beads according to the Nextera XT protocol  
73 (Illumina). Normalized libraries were pooled into a single library for sequencing on the  
74 MiSeq. The pooled single strand library was loaded onto the reagent cartridge and then onto  
75 the instrument along with the flow cell. Automated cluster generation and paired end  
76 sequencing with dual index reads were performed in a single 39-hours run in 2x250-bp. Total  
77 information of 7.33 Gb was obtained from a 763 K/mm<sup>2</sup> cluster density with a cluster passing  
78 quality control filters of 77.80%. Within this run, the index representation for *Aeromicrobium*  
79 *phoceense* strain Marseille-Q0843<sup>T</sup> was determined to index 3.54%. The 14 825 474 paired  
80 end reads were filtered according to the read qualities.

81

## 82 **Genome annotation and genome comparison**

83 Genome assembly was performed on Spades software (v.3.10). [23] Genome annotation was  
84 obtain through the NCBI Prokaryotic Genome Annotation Pipeline [24]. A phylogenetic tree  
85 was obtained using the Maximum Likelihood method and Kimura 2-parameter within the  
86 MEGA 7 software on the annotated-genome-extracted 16S RNA gene sequence [11]. The  
87 Genome-to Genome Distance Calculator (GGDC) web server (<http://ggdc.dsmz.de> ) was used  
88 to estimate the overall similarity among compared genomes and to replace the wet-lab DNA–  
89 DNA hybridization (DDH) by a digital DDH (dDDH) [15, 16]. The degree of genomic  
90 similarity of *Aeromicrobium phoceense* strain Marseille-Q0843<sup>T</sup> with closely related species  
91 was estimated using the OrthoANI software [25]. Antibiotic resistance genes, presence of  
92 pathogenesis-related proteins and plasmid was investigated using the ABRicate [26] tools on  
93 Online Galaxy platform by using Resfinder, CARD , NCBI Bacterial Antimicrobial  
94 Resistance Reference Genes, PlasmidFinder and VFDB databases.

95

## 96 **Results**

### 97 **Strain identification and classification**

98 *Aeromicrobium phoceense* strain Marseille-Q0843<sup>T</sup> 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<sup>T</sup> 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-](https://www.mediterranee-infection.com/acces-ressources/base-de-donnees/urms-data-base/)  
102 [infection.com/acces-ressources/base-de-donnees/urms-data-base/](https://www.mediterranee-infection.com/acces-ressources/base-de-donnees/urms-data-base/). (**Figure 1**) Moreover,  
103 *Aeromicrobium phoceense* strain Marseille-Q0843<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> with other bacterial species.

126

127

### 128 **Genome properties**

129 The genome size of strain Marseille-Q0843<sup>T</sup> 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, 3.161 were 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**)



137 **Discussion and conclusion**

138 In the past 8 years, culturomic approach induce the discovery of more than 500 bacterial  
139 species [1, 27]. Using the taxono-genomics concept *i.e.* the combination of the genomic and  
140 phenotypic properties of a putative new taxon [27], we have characterized a new bacterial  
141 species representing a new species within the family *Nocardioideae* found on a forehead  
142 human skin. It was named as *Aeromicrobium phoceense* strain Marseille-Q0843<sup>T</sup>.  
143 *Aer.o.mi.cro'bi.um* Gr. masc. n. *aer* (gen. *aeros*), air; N.L. neut. n. *microbium*, microbe; N.L.  
144 neut. n. *Aeromicrobium*, aerobic microbe. *Me.di.ter.ra.ne.en'sis*, L. masc. adj.,  
145 *mediterraneensis*, 'of *Mediterraneum*,' the Latin name of the Mediterranean Sea by which  
146 Marseille is located and the bacteria isolated.

147

148 **Deposit in culture collections and Sequences Database**

149 *Aeromicrobium phoceense* strain Marseille-Q0843<sup>T</sup>, was deposited in CSUR collections  
150 under accession CSUR-Q0843. The 16S rRNA and genome sequences are available in  
151 GenBank under accession numbers MT764256.1 and JACEOG000000000.1, respectively.

152

153 **Transparency declaration**

154 None to declare

155

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167

168

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258

259 **Figure.1.** MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies of  
260 strain Marseille-Q0843<sup>T</sup> 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<sup>T</sup>(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<sup>T</sup> using a Tabletop microscope TM 4000plus (Hitachi, Tokyo, Japan). The  
268 scale bar represents 5  $\mu$ m.

269 **Figure 4:** Graphical circular map of the genome from strain Marseille-Q0843<sup>T</sup> 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<sup>T</sup> and other closely  
273 related species standing in nomenclature.

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

Properties	<i>A. phoceense</i>	<i>A. alkaliterrae</i>	<i>A. flavum</i>	<i>A. tamlense</i>	<i>A. choanae</i>	<i>A. panaciterrae</i>
	Marseille-Q0843	KSL-107	TYLN1	SSW1-57	9H-4	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
<b>Production of :</b>						
Alkaline phosphatase	+	-	-	+	+	na
Catalase	+	+	+	+	+	-
Oxidase	-	-	+	-	-	-
α-Glucosidase	+	+	+	+	-	na
β-galactosidase	+	-	-	-	+	na
<b>Acid from:</b>						
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
<b>Habitat</b>	Human	various : soils, herbage, sea water	air sample	dried seaweed sample	soil, air, plants, ascidians, human faces ans the marine environment	soil

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277 **Table 2** : Description of *Aeromicrobium phoceense* strain Marseille-Q0843<sup>T</sup>

Type of description	New description
Species name	<i>phoceense</i>
Genus name	<i>Aeromicrobium</i>
Specific epithet	Aeromicrobium
Species Status	sp.nov
Species etymology	<i>Aeromicrobium phoceense</i> 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 Mediterranean,' 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	<a href="#">MT764256.1</a>
Genome accession number	<a href="#">JACEOG000000000.1</a>
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 O <sub>2</sub>	Necessary
O <sub>2</sub> requirement	Strictly aerobe
Oxidase	Negative
Catalase	Positive

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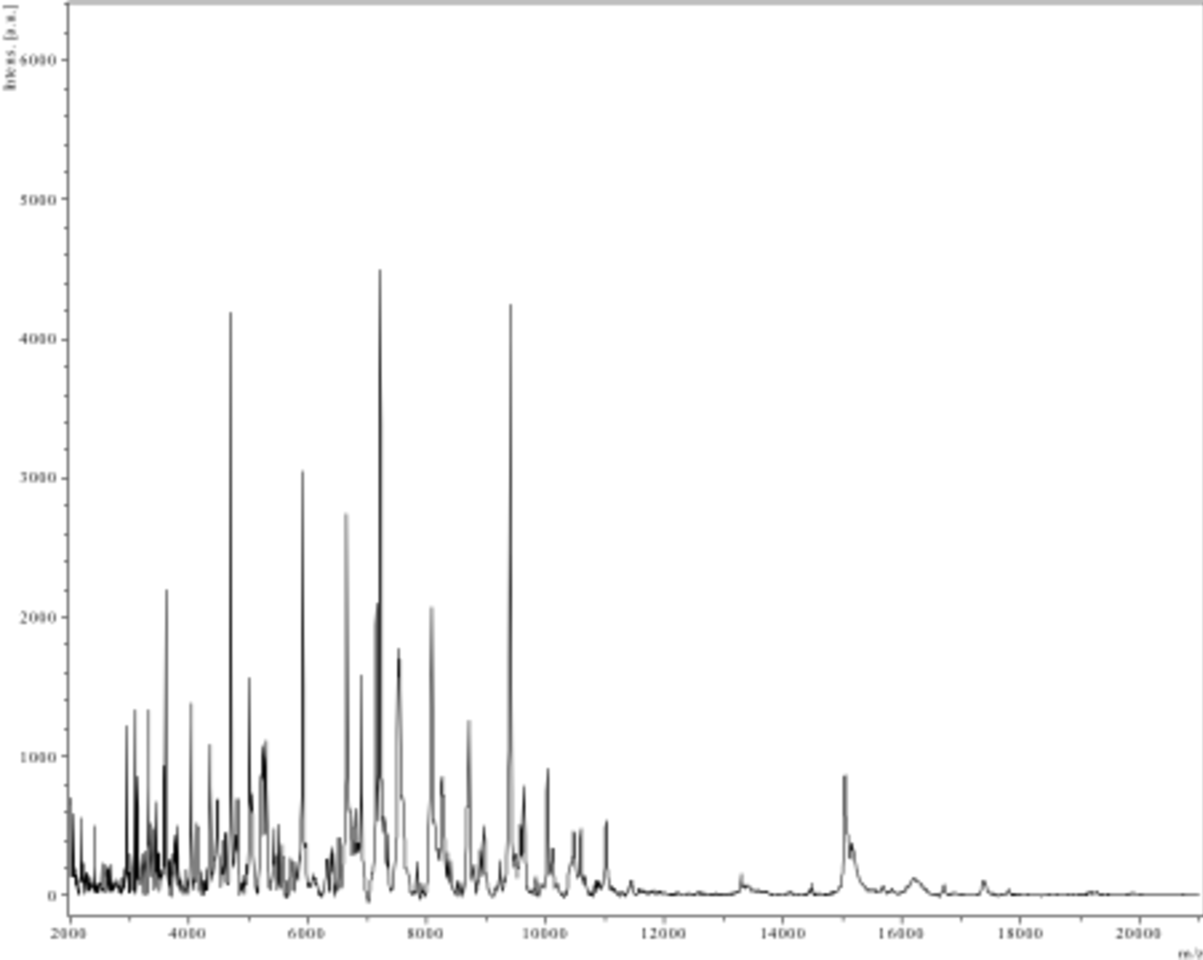
279

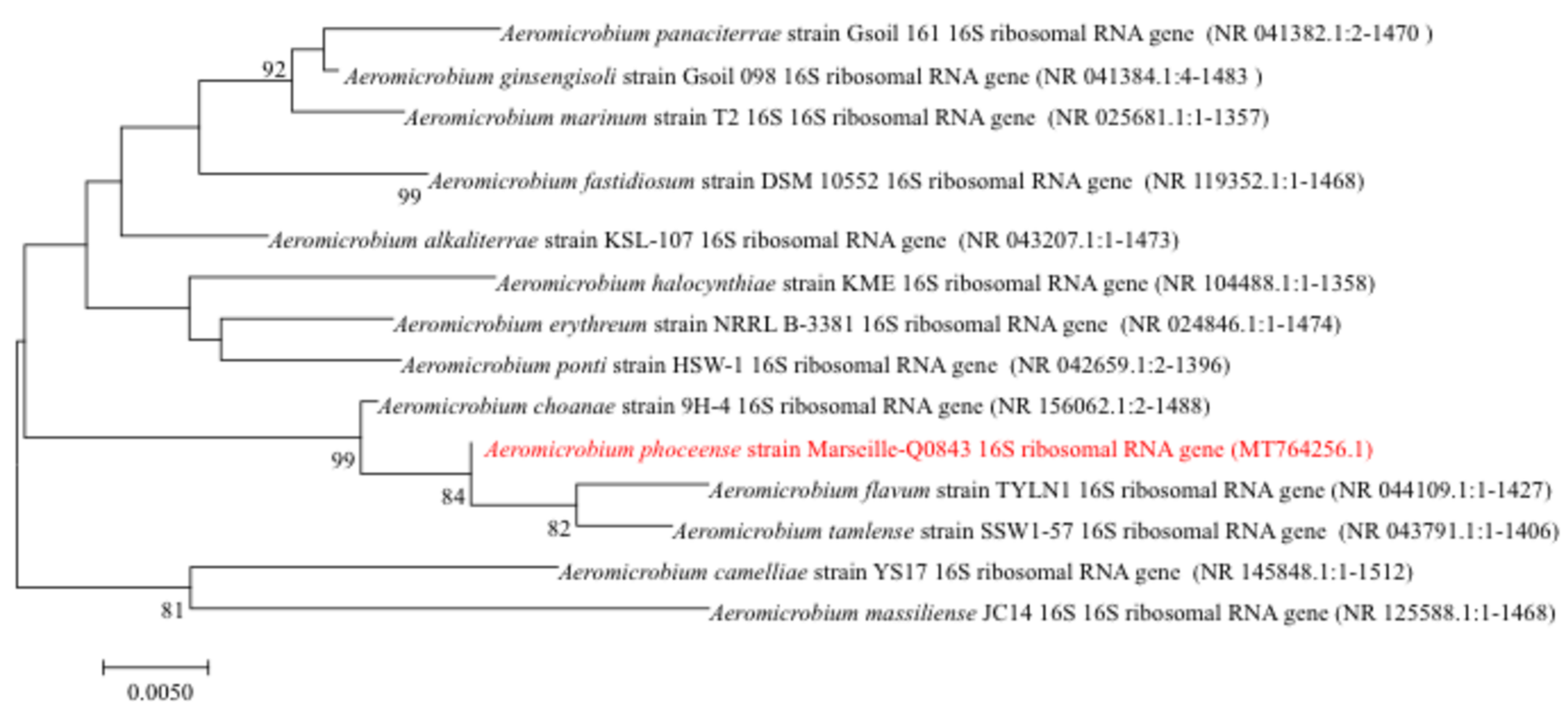
280 **Table 3:** Digital DNA–DNA hybridization values obtained by sequence comparison of all  
 281 studied genomes using GGDC server. (formula 2a)  
 282

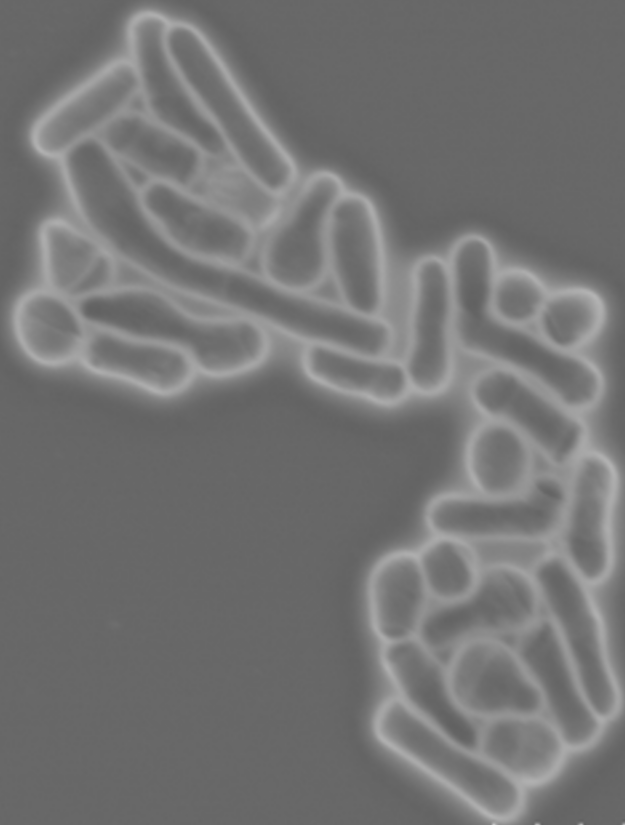
<b>Type Strain Genome Server</b>				
<b>Query strain</b>	<b>Subject strain</b>	<b>dDDH ( %)</b>	<b>C.I. (%)</b>	<b>G+C content difference (in %)</b>
Q0843 PE1MN1 spades'	<i>Aeromicrobium choanae</i> 9H-4	52,6	[49.9 - 55.3]	0,05
<i>Aeromicrobium phoceense</i> strain Marseille-Q0843	<i>Aeromicrobium tamlense</i> DSM 19087	31,9	[29.5 - 34.4]	0,29
<i>Aeromicrobium phoceense</i> strain Marseille-Q0843	<i>Aeromicrobium flavum</i> NBRC 107625	29,2	[26.8 - 31.7]	0,18
<i>Aeromicrobium phoceense</i> strain Marseille-Q0843	<i>Aeromicrobium erythreum</i> ATCC 51598	20,4	[18.2 - 22.9]	1,17
<i>Aeromicrobium phoceense</i> strain Marseille-Q0843	<i>Aeromicrobium massiliense</i> JC14	20,2	[18.0 - 22.7]	1,69
<i>Aeromicrobium phoceense</i> strain Marseille-Q0843	<i>Aeromicrobium camelliae</i> YS17	20,2	[17.9 - 22.6]	1,59
<i>Aeromicrobium phoceense</i> strain Marseille-Q0843	<i>Aeromicrobium endophyticum</i> 9W16Y-2	20,1	[17.9 - 22.5]	2,06
<i>Aeromicrobium phoceense</i> strain Marseille-Q0843	<i>Aeromicrobium ginsengisoli</i> JCM14732	19,8	[17.6 - 22.2]	1,12
<i>Aeromicrobium phoceense</i> strain Marseille-Q0843	<i>Aeromicrobium marinum</i> DSM 15272	19,7	[17.5 - 22.2]	0,15
<i>Aeromicrobium phoceense</i> strain Marseille-Q0843	<i>Nocardioides marinisabuli</i> DSM 18965	19,5	[17.4 - 21.9]	2,21
<i>Aeromicrobium phoceense</i> strain Marseille-Q0843	<i>Nocardioides jensenii</i> NBRC 14755	19,3	[17.2 - 21.7]	2,04

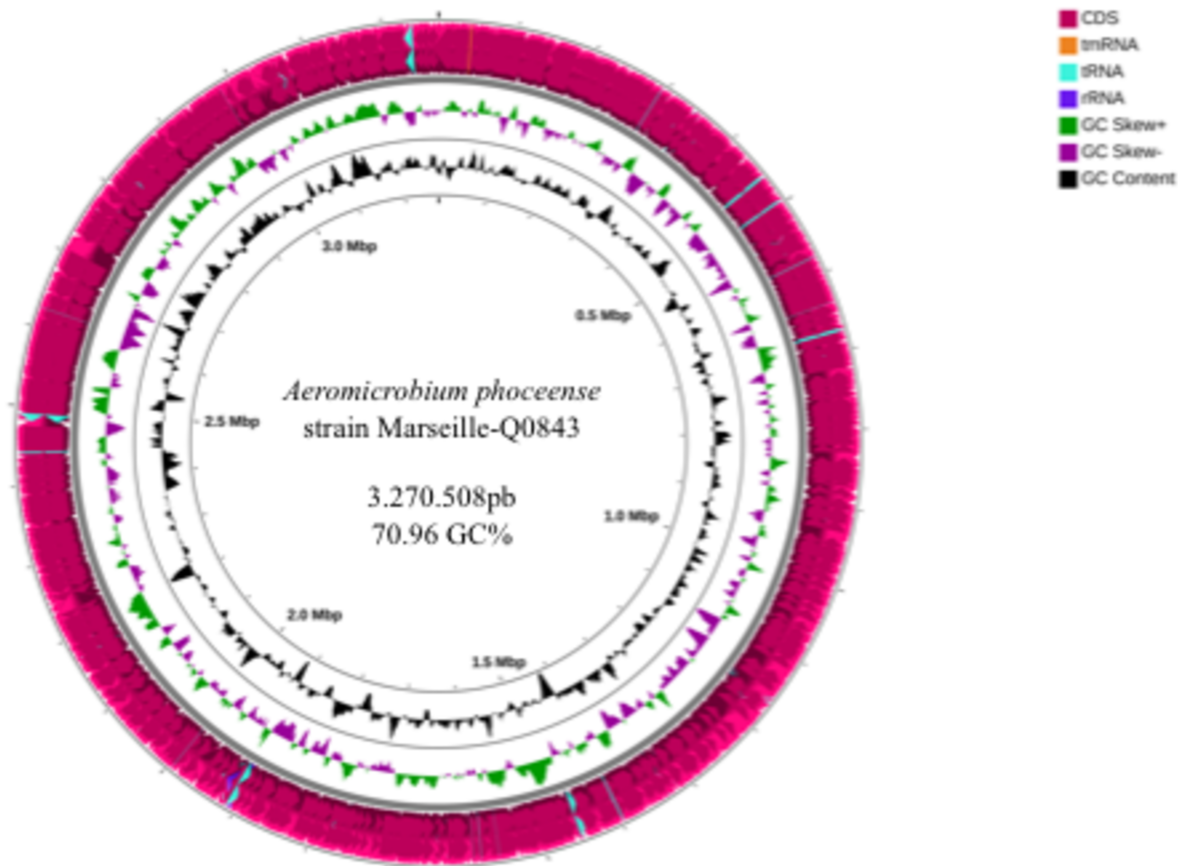
283













Heatmap generated with OrthoANI values  
calculated from the OAT software.  
Please cite Lee *et al.* 2015.

