Draft genome and description of Corynebacterium haemomassiliense strain Marseille-Q3615(T) sp. nov., a new bacterium isolated from a 59-year-old man with chronic obstructive pulmonary disease symptoms

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Abstract

In 2020, as part of the diagnosis in IHU-Méditerrannée Infection Institute in Marseille (France), a blood specimen was obtained from a from a 59-year-old man suffering from chronic obstructive pulmonary disease symptoms, from which we isolated the new bacterial *Corynebacterium haemomassiliense* strain Marseille-Q3615^{T}. 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 *Corynebacteriaceae* in the phylum *Actinobacteria*. We described here the main phenotypic characteristics, genome sequence and annotation of *Corynebacterium haemomassiliense* strain Marseille-Q3615^{T}, a new member of the *Corynebacterium* genus, that we propose as type strain.

Keywords: Bacteria, *Corynebacterium*, human, genome, species, sp. nov., taxono-genomics

Introduction

The genus *Corynebacterium* counts 173 species [1], some of them are of medical, veterinary or biotechnological interest [2]. *Corynebacterium haemomassiliense* strain Marseille-Q3615^T was isolated as part of the diagnosis in IHU-Méditerrannée Infection Institute in Marseille (France). 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 [3,4]. The genome of *Corynebacterium haemomassiliense* strain Marseille-3615^T is 2.578.128 bp long with 65.28% G+C content. This new bacterium is most closely related to *Corynebacterium pilbarense strain DSM 45350* with a 16S rRNA sequence identity value of 99.12 %. Furthermore, genomic comparison using the OrthoANI parameter provided a value of 73.65 % with *Corynebacterium striatum* strain KC-NA01 (NZ_CP014634.1) and a dDDH value of 43.3 % with *Corynebacterium afermentans* strain DSM 44280, closest species standing in nomenclature.

Material and method

Strain isolation and phenotypic tests

Corynebacterium haemomassiliense strain Marseille-Q3615T was initially isolated from a liquid aerobic hemoculture bottle (BACT/ALERT®, bioMérieux, Marcy l'Etoile, France) incubated 24h at 37°C, and is routinely cultivated on Columbia Agar with 5% Sheep Blood media (BioMérieux, Marcy L'Etoile, France) incubated in aerobiosis at 37°C. MALDI-TOF mass spectrometry (MS) protein analysis was carried out using a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [8]. Spectra from strain Marseille-Q3615^T **(Fig.1)** 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 of the Institut Fédératif de Recherche IFR48. Different growth temperatures (20; 31.5; 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 Coryne 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, Tokyo, Japan). Motility test was performed using the semi solid TCC media as described by Tittsler *et al.*[5]

Genome sequencing

Genomic DNA (gDNA) of *Corynebacterium haemomassiliensis* strain Marseille-Q3615^T was extracted the EZ1 biorobot (Qiagen) with EZ1 DNA tissues kit.after a mechanical and enzymaic pretreatment – respectively by glass beads acid washed (G4649-500g Sigma) using a FastPrep-24™ 5G Grinder (mpBio) and lysozyme incubation at 37°C . gDNA was next sequenced on the MiSeq Technology (Illumina Inc, San Diego, CA, USA) with the paired end strategy using the Nextera XT DNA sample prep kit (Illumina). Purification step was performed using AMPure XP beads (Beckman Coulter Inc, Fullerton, CA, USA) and libraries were normalized according the Nextera XT protocol (Illumina). They were pooled into a single library for sequencing on the MiSeqAutomated cluster generation and paired end sequencing with dual index reads were performed in a single 39-hours run in 2x250-bp. Total

information of 4.8 Gb was obtained from a 511/mm^2 cluster density with a cluster passing quality control filters of 90.7%. Within this run, the index representation for *Corynebacterium haemomassiliensis* Marseille- Q3615^T was determined to index 4.8%. The 9 843 335 paired end reads were filtered according to the read qualities.

Phylogeny, Genome annotation and genome comparison

Assembly was performed using SPAdes software v3.10 using default parameters. [6] Genome annotation was obtain through the NCBI Prokaryotic Genome Annotation Pipeline [7]. By extracting the sequence,a 16S rRNA based phylogenetic tree was obtained using the Maximum Likelihood method parameter within the MEGA 7 software [11]. The Genome-to Genome Distance Calculator (GGDC) web server (http://ggdc.dsmz.de) was used to estimate the overall identity among compared genomes and to replace the wet-lab DNA–DNA hybridization (DDH) by a digital DDH (dDDH) [15, 16]. The degree of genomic identity of *Corynebacterium haemomassiliense* strain Marseille-Q3015^T with closely related species was estimated using the OrthoANI software [8]. Antibiotic resistance genes and presence of pathogenesis-related proteins was investigated using the ABRicate tool and CARD, Resfinder, VFDB and PlasmidFinder databases of the Online Galaxy platform. [9]

Results

Strain identification and classification

Corynebacterium haemomassiliense strain Marseille-Q3615^T was isolated from a blood specimen of a 59-year-old man suffering from chronic obstructive pulmonary disease symptoms. This strain 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/. By analyzing its conserved sequences, *Corynebacterium haemomassiliense* strain Marseille-Q3615^T exhibited a 99.12% 16S gene sequence identity with *Corynebacterium pilbarense* strain DSM 45350 (NR_116953.1), the phylogenetically closest bacterium standing in nomenclature and a 95.60% *rpoB* gene - shown to be more discriminant for *Corynebacterium* species, validating the <96.6% identity cuttoff described by Khamis et al. [10]- sequence identity with *Corynebacterium ureicelerivorans* strain DSM 45051 (CP_009215.1) **(Fig.2).** Digital DNA–DNA hybridization analysis between the novel organism with *Corynebacterium afermentans* strain DSM 44280 type strain revealed an identity of only 43.3 % and OrthoANI parameter

provided a value of 73.65 % with *Corynebacterium aurismucosum* strain ATCC 700975. These both values are below the species delineation cut off. [11]

Phenotypic characteristics

Colonies from strain Marseille-Q3615^T showed a white pigmentation and no hemolysis. Bacterial cells were Gram-positive, non-motile, rod-shaped bacilli with a size of 1.8 x 0.2 µm determined by electronic scanning microscopy **(Fig. 3)**. Strain Marseille-Q3615^T is a facultative aerobe. Optimal growth medium pH and NaCl concentration is comprised between 5,5-8,5 and 10-15 g/L respectively. The sporulation test (20 minutes at 80°C) was negative. Using an API strips (BioMérieux, Marcy L'Etoile, France) positive reactions were shown for Pyrazinecarboxamide, 2-naphthyl-phosphate, D-glucose, D-ribose, D-saccharose (sucrose), Alkaline phosphatase, Esterase (C4), Esterase Lipase (C8), Acid phosphatase, Naphthol-AS-BI-phosphohydrolase, N-acetyl-β-glucosaminidase. All other reactions tested were negative. In addition, this bacterium shows catalase positive and oxidase negative. **Table.1** synthetized the main characteristic of the strain compared to its relative species standing in nomenclature.

Genome properties

The genome size of strain Marseille-Q3615^T is 2,578,128-bp long with a 65.28% G+C content. The genome *de novo* assembly of this strain was achieved on 4 contigs. **(Fig. 4)** Of the 2.431 predicted genes, 2,365 were protein-coding genes and 66 were RNAs (4 16S rRNA, 4 additional 5S rRNAs, 4 additional 23S rRNAs 3 ncRNA and 51 tRNAs). The genome properties and distribution of genes into COGs functional categories are detailed in **Table 2.** The *in silico* resistome of the strain Marseille-Q3615^T shows 3 genes *erm(X)_4*, *tet(W)_4* and *cmx_1*with high identity percentage (94.85%, 99.01% and 99.83% respectively) that could be involved in tetracycline and chloramphenicol resistance. Neither associated plasmid nor virulence factor was found. OrthoANI parameter provided a value of 73.65 % with *Corynebacterium striatum* strain KC-NA01 **(Fig.5)** and a dDDH value of 43.3 % with *Corynebacterium afermentans* strain DSM 44280.

Discussion and conclusion

Using the taxono-genomics concept *i.e.* the combination of the genomic and phenotypic properties of a putative new taxon, we have characterized a new bacterial species representing a new species within the family *Corynebacteriaceae* found in human.**(Table.3)** It was named as *Corynebacterium haemomassiliense* strain Marseille-3615T. Gr. fem. n. *korynê*, a club; L. neut. n. *bacterium*, a rod, and in biology a bacterium (so called because the first ones observed were rod-*shaped); N.L. neut. n. Corynebacterium, a club bacterium. Haemomassiliense, blood (Lat.* transliteration *haema)* referring to the nature of the specimen. *massiliense*, 'to Massilia,' the antic name of Marseille, France, where the strain was isolated.

Deposit in culture collections and Sequences Database

Corynebacterium haemomassiliense strain Marseille-Q3615^{T}, was deposited in CSUR collection under accession CSUR-Q3615. This Whole Genome Shotgun project has been deposited at GenBank under the accession JACDTZ000000000. 16S gene sequence has been deposited under the accession MT772001.

List of abbreviation

API -Analytical Profile Index
CARD - Comprehensive Antibiotic Resistance Database
CSUR - Collection de Souches de l'Unité de Rickettsies
dDDH - digital DDH
DDH - DNA–DNA hybridization
DNA - Deoxyribonucleic acid
DSM-Z - Deutsche Sammlung von Mikroorganismen und Zellkulturen
GGDC - Genome-to-Genome Distance Calculator
MALDI-TOF MS - Matrix Assisted Laser Desorption Ionization - Time of Flight - Mass Spectrometry
NCBI -National Center for Biotechnology Information
OrthoANI - Ortho - Average Nucleotide Identity
rRNAs - Ribosomal ribonucleic acid
VFDB -Virulence Factors DataBase

Transparency declaration

None to declare

Funding sources

MB PhD grant is supported by the collaboration between M&L Laboratories and Aix Marseille University referenced PVM: 2018-200**.** This study was supported by the French State managed by the National Research Agency under the "Investissements d'avenir (Investments for the Future)" program under the reference ANR-10-IAHU-03 (Méditerranée Infection) and by the Région Provence-Alpes-Côte-d'Azur and the European funding FEDER PRIMI.

Acknowledgements

The authors are indebted to Ludivine Brechard for sequencing the genome and the platform of electron microscopy of IHU -MI for the electron micrographs.

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Figure.1 MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies of *Corynebacterium haemomassiliense* strain Marseille-Q3615^T were compared and a reference spectrum was generated

Figure 2: A. *16S* rRNAgene (A) and *rpoB* gene (B) based phylogenetic trees highlighting the position of *Corynebacterium haemomassiliense* sp., nov., strain Marseille-Q3615 (red) relative to other closely related bacterial taxa. Sequences were aligned using Muscle v3.8.31 with default parameters and phylogenetic relationship inferred using the Maximum Likelihood method, with 1,000 bootstrap replicates, within the MEGA software version 7.

Figure.3: Scanning electron microscopy of *Corynebacterium haemomassiliense*. nov., strain Marseille-Q3615^T using a Tabletop microscope TM 4000plus (Hitachi, Tokyo, Japan). The scale bar represents 5 µm.

Figure.4: Graphical circular map of the genome from strain Marseille-Q3615^T obtained by CG view tool. [12]

Figure.5: Heatmap generated with OrthoANI values calculated using the OAT software between *Corynebacterium haemomassiliense*., nov., strain Marseille-Q3615^T and other closely related species standing in nomenclature.

Table.1: Differential characteristics of *Corynebacterium haemomassiliense* strain Marseille-Q3615^{T} and closest species standing in nomenclature

Properties	***C. haemonassiliense*** **Marseille-Q3615**	***C. pilbarense*** **IMMIB WACC 658**	**C. ureicelerivorans IMMIB RIV-2301**	***C. mucifaciens*** **CCUG 36878**	***C. coyleae*** **DSM 44184**	***C. ihumii*** **GD7**
Cell diameter (um)	1.8 x 0.2 µm	0.5–2.0 µm			1mm	0.7 µm
Oxygen requirement	Facultative	Facultative	Facultative	Facultative	Facultative	Facultative
Gram Strain	-	+	+	+	+	+
Motility	-	-	-	-	-	-
Endospore formation	na	-	-	-	-	-
Optimum temperature for growth (°C)	31.5-56°C	na	na	na	na	37
Production of :						
Alkaline phosphatase	+	+	+	+	+	+
Catalase	+	+	+	+	+	+
Oxidase	-	-	-	-	na	-
alpha-Glucosidase	-	-	-	-	-	-
Béta-galactosidase	-	-	-	-	-	-
Acid from:						
N-Acetylglucosamine	+	-	-	-	-	+
L-arabinose	-	-	+	-	-	+
D-ribose	+	+	+	variable	+	+
D-mannose	-	na	na	+	+	+
D-mannitol	-	-	-	-	-	+
D-glucose	+	+	+	+	+	+
D-fructose	-	na	na	+	+	+

D-maltose	-	-	-	-	-	+
D-lactose	-	-	na	-	-	+
G+C content (mol%)	65,28%	na	na	64 mol%	62 mol%	65.1 mol%
Habitat	Human healthy skin	ankle aspirate from a male patient	blood culture	Human clinical material	Human clinical specimens	Fecal flora of a 62 yo male patient

234 **Table 2:** Numbers of genes of strain Marseille-Q3615^T associated with the 25 general COG
235 functional categories

INFORMATION STORAGE AND PROCESSING	
[J] Translation, ribosomal structure and biogenesis	175
[A] RNA processing and modification	1
[K] Transcription	135
[L] Replication, recombination and repair	113
[B] Chromatin structure and dynamics	0
CELLULAR PROCESSES AND SIGNALING	
[D] Cell cycle control, cell division, chromosome partitioning	31
[Y] Nuclear structure	0
[V] Defense mechanisms	61
[T] Signal transduction mechanisms	73
[M] Cell wall/membrane/envelope biogenesis	99
[N] Cell motility	8
[Z] Cytoskeleton	0
[W] Extracellular structures	1
[U] Intracellular trafficking, secretion, and vesicular transport	15
[O] Posttranslational modification, protein turnover, chaperones	82
[X] Mobilome: prophages, transposons	35
METABOLISM	
[C] Energy production and conversion	95
[G] Carbohydrate transport and metabolism	117
[E] Amino acid transport and metabolism	169
[F] Nucleotide transport and metabolism	72
[H] Coenzyme transport and metabolism	107
[I] Lipid transport and metabolism	68
[P] Inorganic ion transport and metabolism	131
[Q] Secondary metabolites biosynthesis, transport and catabolism	34
POORLY CHARACTERIZED	
[R] General function prediction only	143
[S] Function unknown	98

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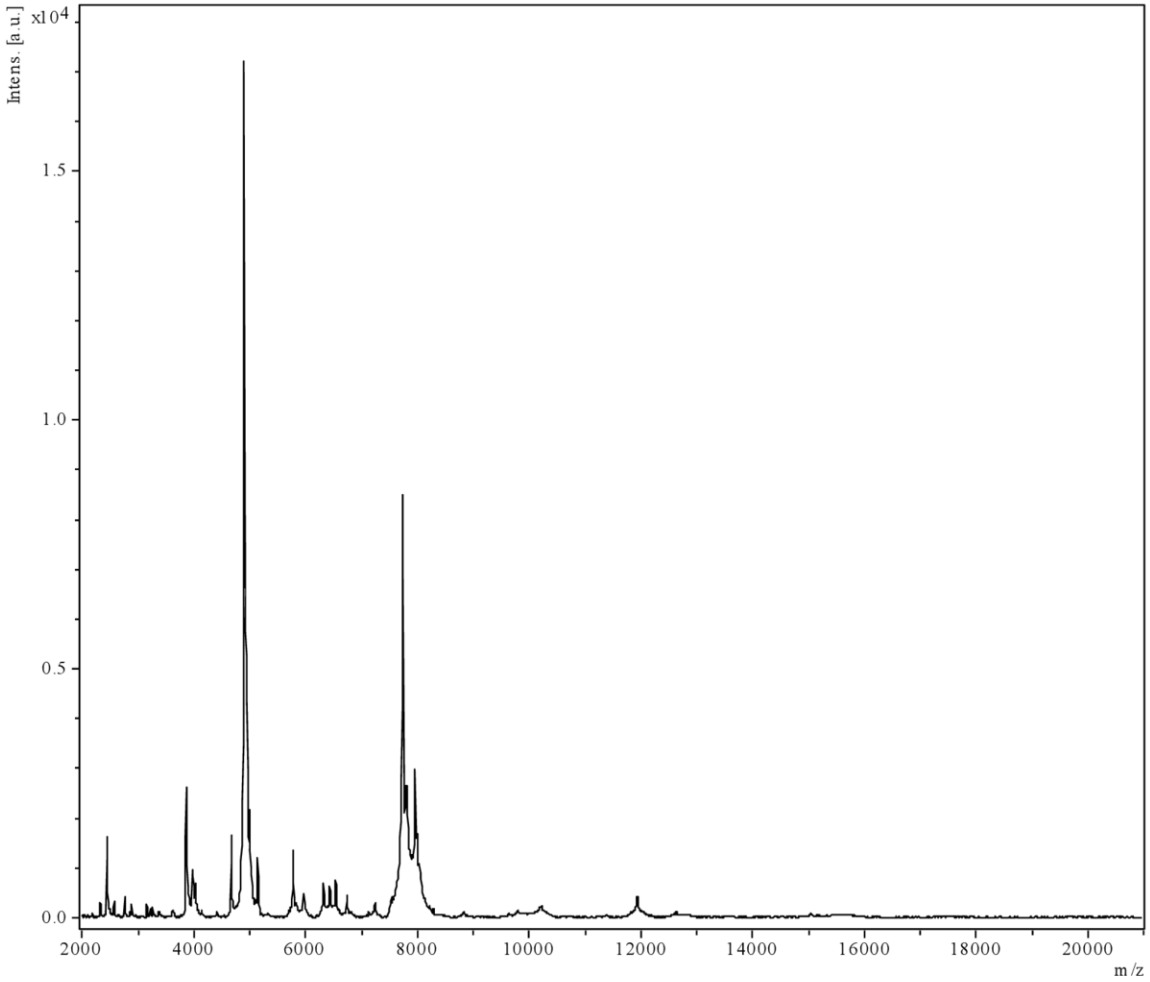
237

238 **Table 3:** Description of *Corynebacterium haemomassiliense* sp. nov. strain Marseille-Q3615^{T}

Type of description	**New description**
Species name	*haemonassiliense*
Genus name	*Corynebacterium*
Specific epithet	*Corynebacterium*
Species Status	sp. nov
Species etymology	*Corynebacterium haemomassiliense* strain Marseille-3615T. Gr. fem. n. *korynê*, a club; L. neut. n. *bacterium*, a rod, and in biology a bacterium (so called because the first ones observed were rod-*shaped); N.L. neut. n. Corynebacterium, a club bacterium. Haemomassiliense, blood (Lat.* transliteration *haema)* referring to the nature of the specimen. *massiliense*, 'to Massilia,' the antic name of Marseille, France, where the strain was isolated.
Authors	Manon Boxberger, Angéline Antezack, Sibylle Magnien, Nadim Cassir, Bernard La Scola
Designation of the type strain	Marseille-Q3615
Strain collection number	CSUR
16S rRNA gene accession number	MT772001
Genome accession number	JACDTZ000000000
Genome status	Whole genome
Genome size	2.578.128 bp
GC%	65.28%
Country of origin	France
Date of isolation	2019
Source of isolation	Human healthy skin
Conditions used for standard cultivation	Columbia Agar with 5% Sheep Blood media (BioMérieux, Marcy L'Etoile, France)

Gram stain	+
Cell shape	irregular rods
Cell size	1.8 x 0.2 µm
Motility	-
Sporulation	-
Colony morphology	White, smooth
Temperature range	20-56°C
Temperature optimum	31.5-56°C
Relationship to O2	facultative
O2 for strain testing	+
Oxidase	-
Catalase	+

239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26
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73
17
69
100
Corynebacterium mucifaciens CIP 105129 strain DMMZ 2278 16S ribosomal RNA (NR 026396.1:1-1405)
Corynebacterium ureicelerivorans strain IMMIB RIV-2301 16S ribosomal RNA (NR 042558.1:1-1404)
Corynebacterium sp nov Marseille-Q3615
Corynebacterium pilbarense strain IMMIB WACC-658 16S ribosomal RNA (NR 116953.1:1-1409)
Corynebacterium ihumii strain GD7 16S ribosomal RNA (NR 144708.1:1-1419)
Corynebacterium afermentans strain CIP 103499 16S ribosomal RNA (NR 026214.1:1-1326)
Corynebacterium coyleae strain DSM 44184 16S ribosomal RNA (NR 044905.1:1-1414)
Corynebacterium jeddahense strain JCB 16S ribosomal RNA (NR 144695.2:3-1437)
Corynebacterium camporealensis strain CRS-51 16S ribosomal RNA (NR 029326.1:2-1401)
Corynebacterium glaucum strain IMMIB R-5091 16S ribosomal RNA (NR 028971.1:1-1397)
Corynebacterium timonense strain 5401744 16S ribosomal RNA (NR 044181.1:1-1412)
Corynebacterium mycetoides strain NCTC 9864 16S ribosomal RNA (NR 037078.1:1-1402)
Corynebacterium tuscaniense strain ISS-5309 16S ribosomal RNA (NR 043093.1:1-1415)
Corynebacterium appendicis strain IMMIB R-3491 16S ribosomal RNA (NR 028951.1:1-1379)
Corynebacterium godavarianum strain PRD07 16S ribosomal RNA (NR 159267.1:1-1397)
Corynebacterium gottingense strain 99221/2016 16S ribosomal RNA (NR 159075.1:1-1400)
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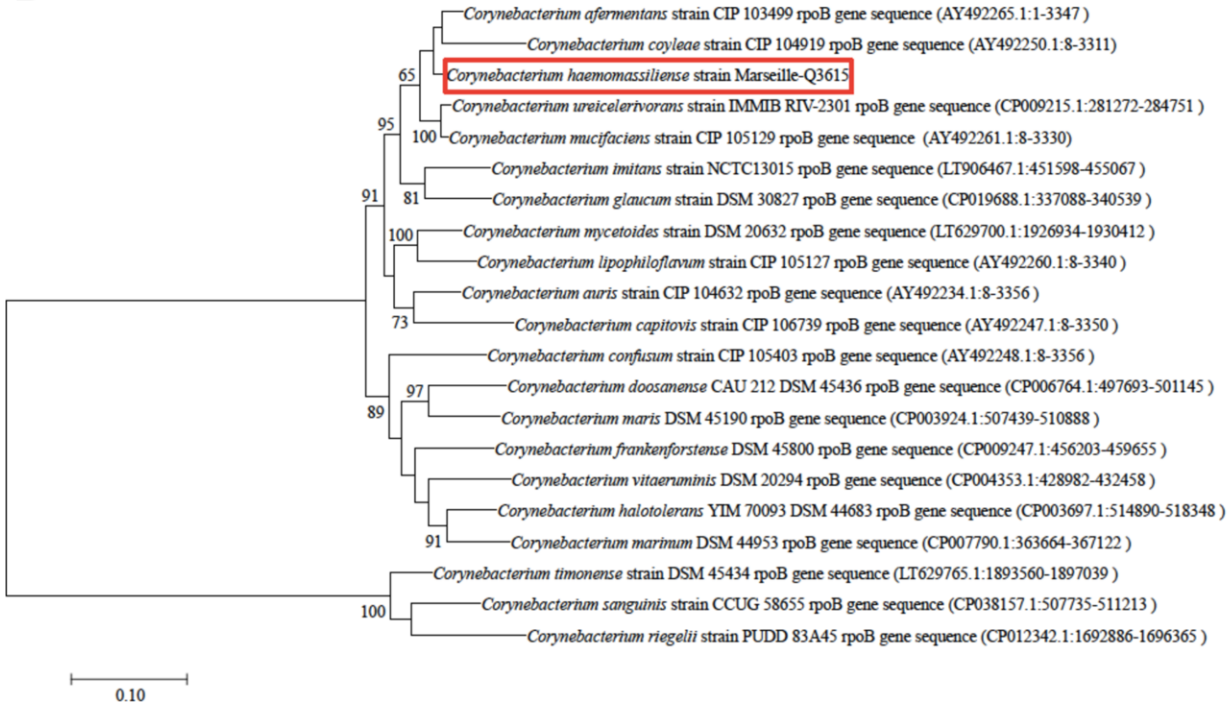
B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Corynebacterium capitovis strain CIP 106739 rpoB gene sequence (AY492247.1:8-3350)
Corynebacterium confusum strain CIP 105403 rpoB gene sequence (AY492248.1:8-3356)
Corynebacterium doosanense CAU 212 DSM 45436 rpoB gene sequence (CP006764.1:497693-501145)
Corynebacterium maris DSM 45190 rpoB gene sequence (CP003924.1:507439-510888)
Corynebacterium frankenforstense DSM 45800 rpoB gene sequence (CP009247.1:456203-459655)
Corynebacterium vitaeruminis DSM 20294 rpoB gene sequence (CP004353.1:428982-432458)
Corynebacterium halotolerans YIM 70093 DSM 44683 rpoB gene sequence (CP003697.1:514890-518348)
Corynebacterium marinum DSM 44953 rpoB gene sequence (CP007790.1:363664-367122)
Corynebacterium timonense strain DSM 45434 rpoB gene sequence (LT629765.1:1893560-1897039)
Corynebacterium sanguinis strain CCUG 58655 rpoB gene sequence (CP038157.1:507735-511213)
Corynebacterium riegelii strain PUDD 83A45 rpoB gene sequence (CP012342.1:1692886-1696365)
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100
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100
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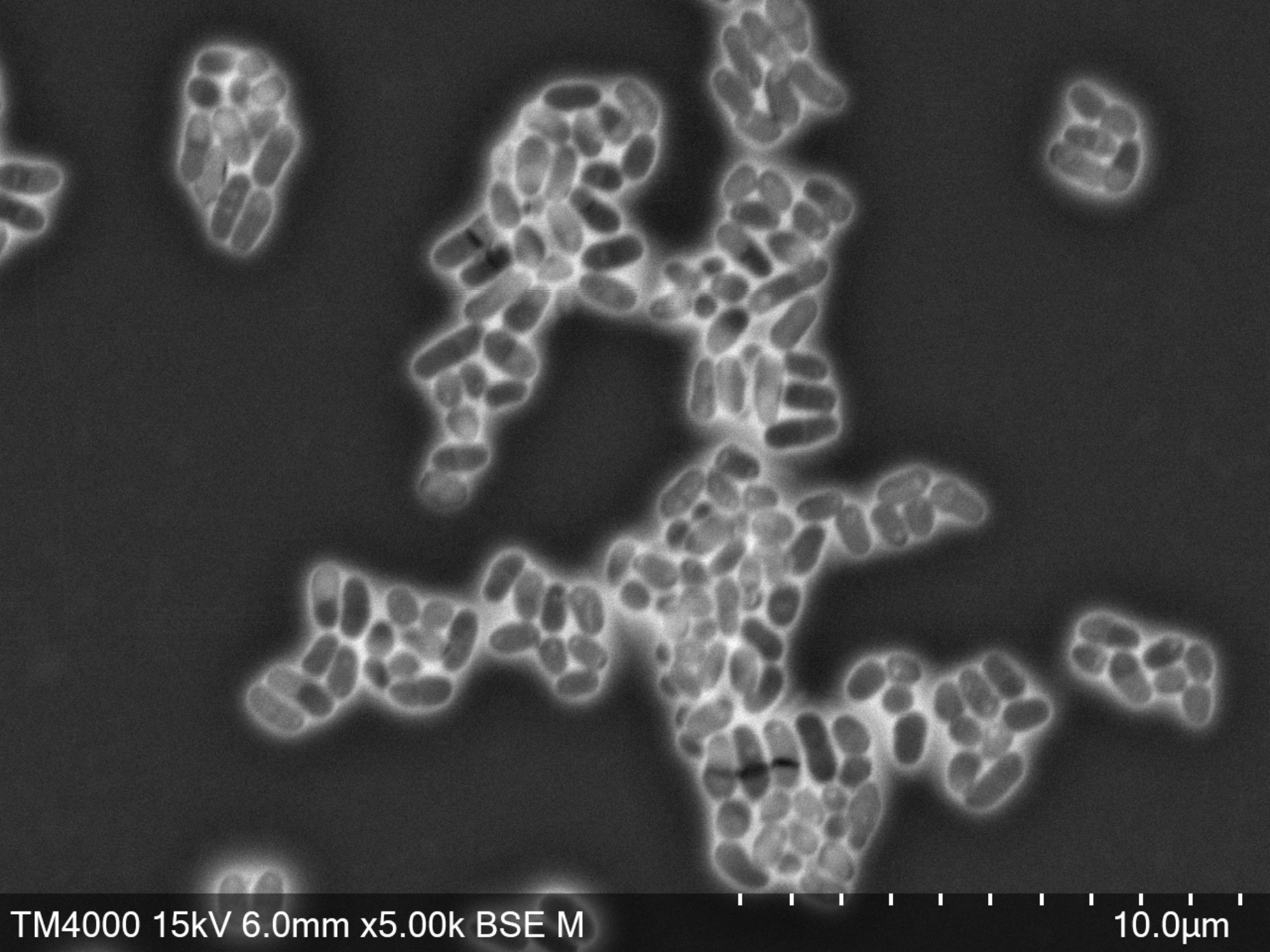
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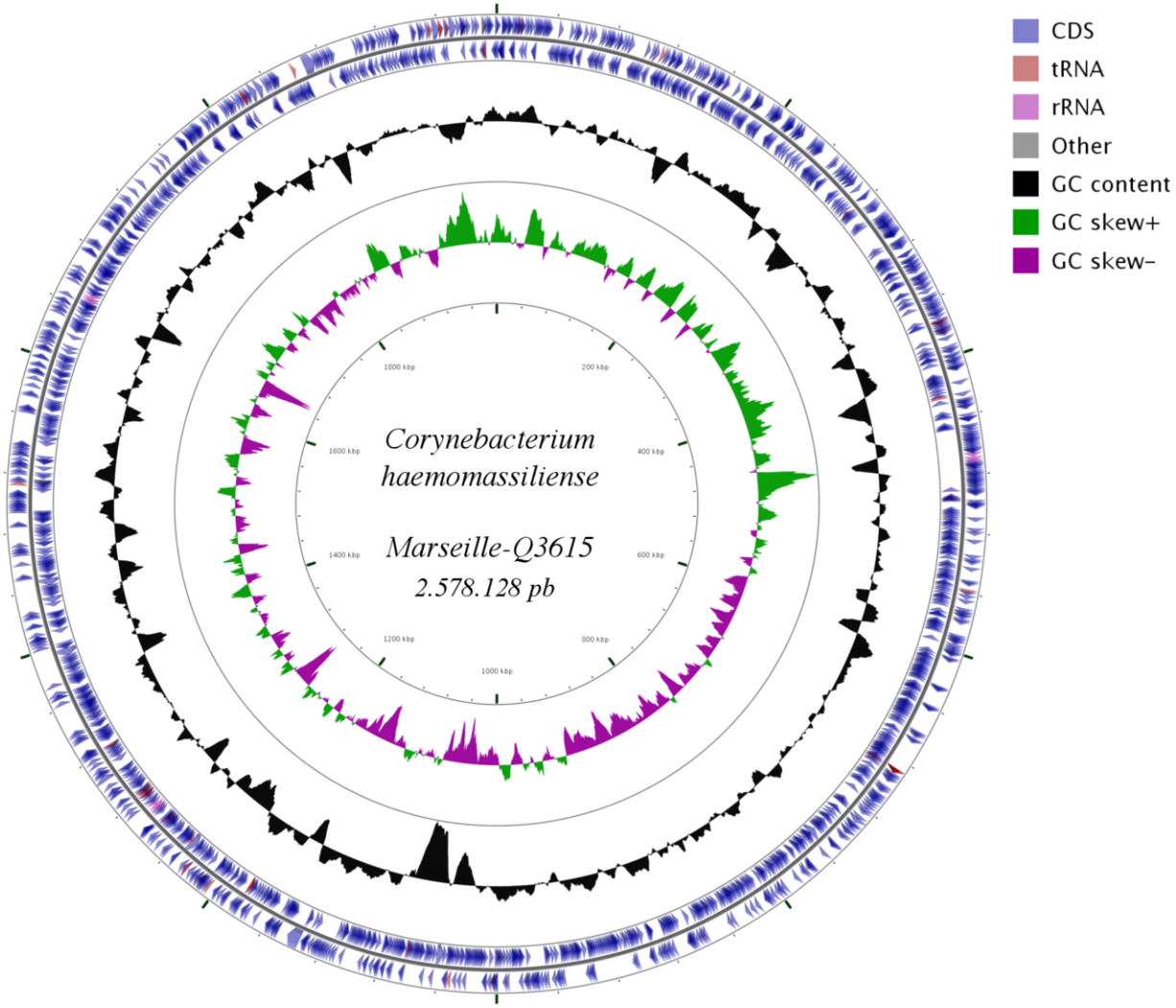
CDS
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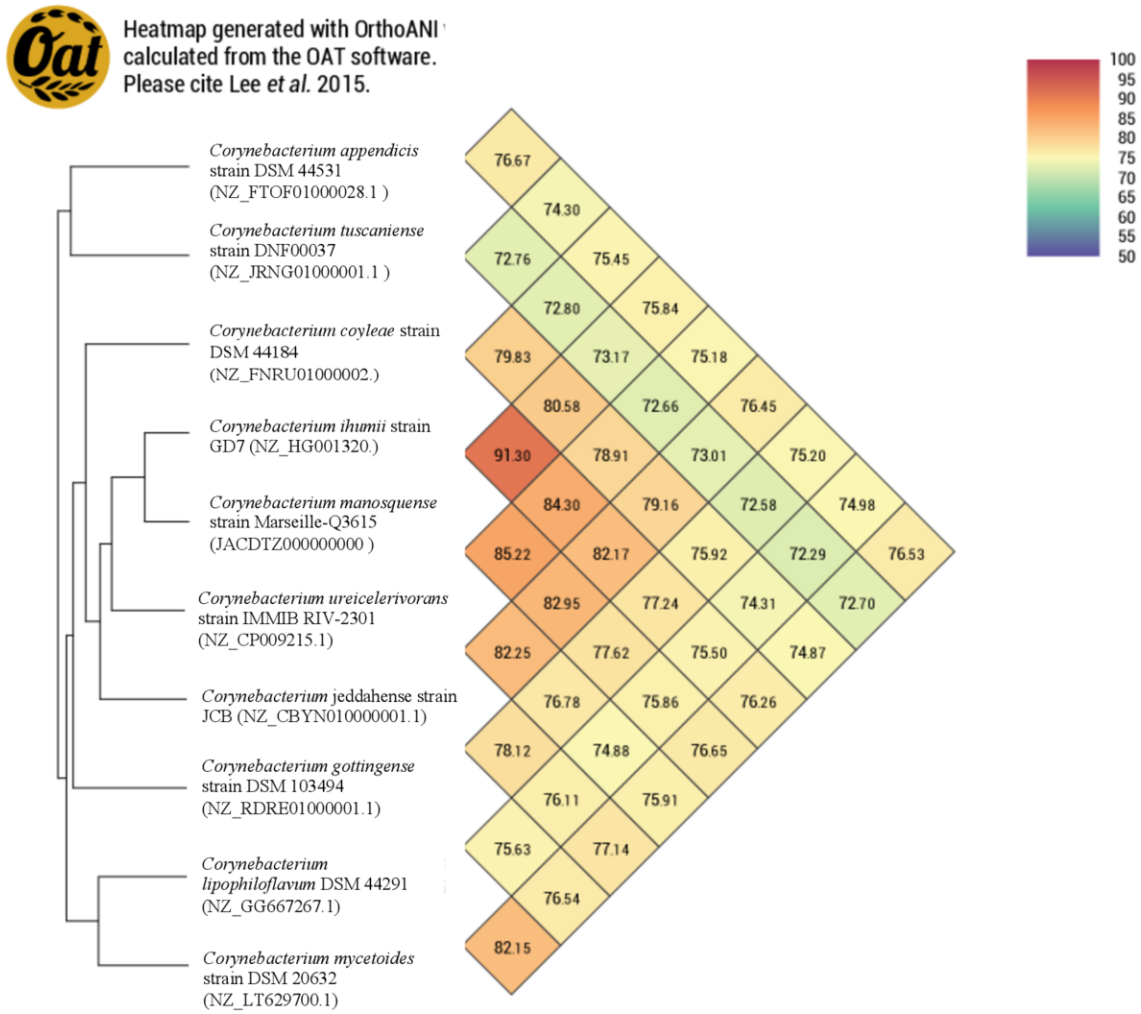
Other
GC content
GC skew+
GC skew−
Corynebacterium haemomassiliense
Marseille-Q3615
2.578.128 pb
200 kbp
400 kbp
600 kbp
800 kbp
1000 kbp
1200 kbp
1400 kbp
1600 kbp
1800 kbp


Heatmap generated with OrthoANI
calculated from the OAT software.
Please cite Lee et al. 2015.
100
95
90
85
80
75
70
65
60
55
50
Corynebacterium appendicis strain DSM 44531 (NZ_FTOF01000028.1)
Corynebacterium tuscaniense strain DNF00037 (NZ_JRNG01000001.1)
Corynebacterium coyleae strain DSM 44184 (NZ_FNRU01000002.)
Corynebacterium ihumii strain GD7 (NZ_HG001320.)
Corynebacterium manosquense strain Marseille-Q3615 (JACDTZ000000000)
Corynebacterium ureicelerivorans strain IMMIB RIV-2301 (NZ_CP009215.1)
Corynebacterium jeddahense strain JCB (NZ_CBYN010000001.1)
Corynebacterium gottingense strain DSM 103494 (NZ_RDRE01000001.1)
Corynebacterium lipophiloflavum DSM 44291 (NZ_GG667267.1)
Corynebacterium mycetoides strain DSM 20632 (NZ_LT629700.1)
76.67
74.30 72.76
75.45 72.80 75.84
79.83 73.17 75.18
80.58 72.66 76.45
91.30 78.91 73.01 75.20
84.30 79.16 72.58 74.98
85.22 82.17 75.92 72.29 76.53
82.95 77.24 74.31 72.70
82.25 77.62 75.50 74.87
76.78 75.86 76.26
78.12 74.88 76.65
76.11 75.91
75.63 77.14
76.54
82.15