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1 Complete Genome and Description of *Corynebacterium incognitum* Sp.Nov., A New

2 Bacterium within the *Corynebacterium* genus

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- 17

18 Abstract

19 In 2020, as part of the diagnosis in IHU-Méditerrannée Infection Institute in Marseille (France) we isolated the new bacterial strain Marseille-3630^T from a 7-year-old girl blood 20 21 specimen (= CSUR: Q3630). Matrix-assisted desorption ionization-time of flight mass 22 spectrometry (MALDI-TOF MS) failed to identify this isolate. Analysis of the 16S rRNA 23 gene and Genome-to-Genome comparison suggested that this taxon belongs to a novel 24 bacterial species within the family Corynebacteriaceae in the phylum Actinobacteria. We 25 described here its main phenotypic characteristics, genome sequence and annotation of Corynebacterium incognitum strain Marseille-3630^T, a new member of the Corynebacterium 26 27 genus, that we propose as type strain.

28 Keywords: bacteria, Corynebacterium, human, genome, species, sp. nov., taxono-

29 genomics

1 Introduction

2 The genus Corynebacterium (Gr. fem. n. korynê, a club; L. neut. n. bacterium, a rod, and in 3 biology a bacterium (so called because the first ones observed were rod-shaped); N.L. neut. 4 n. Corynebacterium, a club bacterium) belongs to the large family Corynebacteriaceae and 5 was first introduced into literature by Lehmann and Neumann in 1896. [1] The genus 6 Corynebacterium currently counts 177 species [2], some of them are of medical, veterinary or 7 biotechnological interest [3]. Species within this genus are ubiquitous and are potentially 8 pathogens. As an illustration, toxin production by C. diphtheriae is involved in diphtheria, 9 dreadful historical disease in the 19th century and in the first half of the 20th century. This study contributes to the taxonomical and clinical knowledge of this genus by describing a 10 novel species, strain Marseille-Q3630, isolated as part of a microbiological workup of a 11 12 patient in IHU-Méditerrannée Infection Institute in Marseille (France). We aimed at 13 comparing strain Marseille-Q3630 to its closely related phylogenetic neighbors and proposed 14 to establish for this strain the species name *Corynebacterium incognitum* sp. nov.

15 Material and method

16 Strain isolation

17 *Corynebacterium incognitum* strain Marseille-Q3630^T was isolated after 50μL of liquid
18 aerobic blood culture bottle (BACT/ALERT®, bioMérieux, Marcy l'Etoile, France) was
19 seeded on Columbia Agar with 5% Sheep Blood media (Biomérieux, Marcy l'Etoile, France)
20 and maintained à 37°C for 24 hours. The strain is then routinely cultivated on Columbia Agar

21 with 5% Sheep Blood media ((bioMérieux, Marcy l'Etoile, France)

22 incubated in aerobiosis at 37°C for 24 hours.

23

24 Strain identification

25 Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry 26 (MS protein analysis was carried out using a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) and spectra from strain Marseille-O3630^T were imported into the MALDI 27 28 BioTyper software (version 3.0, Bruker, Germny) and analyzed by standard pattern matching 29 (with default parameter settings). As MALDI-TOF analyze failed to identify this organism by 30 [4] (https://www.mediterranee-infection.com/accesanalyzing against our database 31 ressources/base-de-donnees/urms-data-base/.), the genome was sequenced as described in the 32 following section. BlastN tool from NCBI were performed to compare the 16S best hits 33 sequence from strain Marseille-Q3630 against the 16S database [5]. Phylogenetic trees for the 34 16s RNA and rpoB gene was obtained using the Maximum Likelihood method and Kimura 2-35 parameter within the MEGA 7 software. [6]

36

37 Phenotypic tests

38 Different growth temperatures (20-56°C), atmosphere conditions (anaerobic, aerobic and 39 microaerophilic), using atmosphere generators (CampyGEN, Oxoid, USA) and pH (5,5-8.5) 40 were tested. API ZYM, API Coryne and API 50 CH strips (BioMérieux, Marcy L'Etoile, 41 France) were used to evaluate the biochemical properties of the strain according to the 42 manufacturer's instructions. Morphology was analyzed by scanning electronic 43 microscopy.,Briefly, a colony was collected from agar and immersed into a 2.5 % 44 glutaraldehyde fixative solution. The slide was gently washed in water; air dried and 45 examined to evaluate bacterial structure on a TM4000 microscope (Hitachi, Tokyo, Japan). 46 Motility test was performed using the semi solid TCC media [7].

47

48 Genome sequencing

Genomic DNA (gDNA) of Corynebacterium incognitum strain Marseille-Q3630^T was 49 50 extracted with the EZ1 biorobot (Qiagen) with EZ1 DNA tissues kit after a mechanical and 51 chemical (lysozyme) pretreatment. gDNA was next sequenced on the MiSeq Technology 52 (Illumina Inc, San Diego, CA, USA) with the paired end strategy and was prepared with the 53 Nextera XT DNA sample prep kit (Illumina). Normalized libraries were pooled into a single 54 library for sequencing on the MiSeq. Total information of 6.03 Gb was obtained from a 628 55 K/mm2 cluster density with a cluster passing quality control filters of 96.38. The 12 164 746 56 paired end reads were filtered according to the read qualities. In order to improve the genome 57 quality, an Oxford Nanopore approach was performed on 1D gDNA sequencing for the MinIon device using SQK-LSK109 kit. Library was constructed from 1 µg genomic DNA 58 59 without fragmentation and end repair. 753 actives pores were detected for the sequencing and 60 the workflow WIMP was chosen for bioinformatic analysis in live. After 3.5 hours as run time 61 and end life of the flow cell, 61.140 reads as raw data were generated.

62

63 Genome annotation and genome comparison

64 Genome assembly was proceed using SPAdes software v3.10 [8]. Genome annotation was 65 obtained through the NCBI Prokaryotic Genome Annotation Pipeline [9]. The TYGS online 66 platform was used to determinate the closely related type strains. Determination of closest 67 type strain genomes was done in two complementary ways: First, the Marseille-Q3630 68 genome was compared against all type strain genomes available in the TYGS database via the 69 MASH algorithm, a fast approximation of intergenomic relatedness [10], and the ten type 70 strains with the smallest MASH distances chosen per user genome. Second, an additional set 71 of ten closely related type strains was determined via the 16S rDNA gene sequences. These 72 were extracted from the user genomes using RNAmmer [11] and each sequence was 73 subsequently BLASTed [5] against the 16S rDNA gene sequence of each of the currently 74 14.130 type strains available in the TYGS database. This was used as a proxy to find the best 75 50 matching type strains (according to the bitscore) for each user genome and to subsequently 76 calculate precise distances using the Genome BLAST Distance Phylogeny approach (GBDP) 77 under the algorithm 'coverage' and distance formula d5 [12]. These distances were finally 78 used to determine the 10 closest type strain genomes for each of the user genomes. The 79 Genome-to Genome Distance Calculator Digital DDH values and confidence intervals were 80 calculated using the recommended settings of the GGDC 2.1 The degree of genomic 81 similarity based on the Ortholog group of genes of Corynebacterium incognitum strain Marseille-Q3630 with closely related species was estimated using the OrthoANI software[13].

- 84
- 85 Results

86 Strain identification and classification

87 *Corynebacterium incognitum* strain Marseille-Q3630^T was isolated from a 7-year-old girl that 88 was consulting pediatric service for an acute febrile illness. Blood was collected and analyzed 89 in aerobic blood culture bottle (BACT/ALERT®, bioMérieux, Marcy l'Etoile, France). Corynebacterium incognitum strain Marseille-Q3630^T failed to be identified by our 90 91 systematic MALDI-TOF MS screening, suggesting that the corresponding species was not in 92 the database. (Figure.1) Moreover, Corynebacterium incognitum strain Marseille-Q3630^T 93 97.16% rRNA exhibited а 16S sequence similarity with Corynebacterium $tuberculostearicum^{T}$ (GenBank accession NR_028975.1), the phylogenetically closest 94 95 bacterium standing in nomenclature and a 88.03% rpoB gene identity with Corynebacterium 96 aurimucosum ATCC 700975 (extracted from the genome sequence CP001601.1) - shown to 97 be more discriminant for Corynebacterium species, validating the <96.6% identity cutoff 98 described by Khamis et al. [14]. (Figure.2). Digital DNA-DNA hybridization analysis 99 between the novel organism and Corynebacterium diphtheria subsp. lausannense 100 CHUV2995T exhibited value of 25.4%. Furthermore, genomic comparison using the 101 OrthoANI parameter provided a value of 72.06% Corynebacterium ureicelerivorans strain 102 IMMIB RIV-2301^T. (Table.1 and Supplementary file1)

103

104 **Phenotypic characteristics**

Colonies from strain Marseille-Q3630^T showed a white pigmentation and no hemolysis. 105 106 Bacterial cells were gram-positive, non-motile, rod shaped, with a mean size of 0.5 x 0.9 µm determined by scanning electronic microscopy (Figure.3). Strain Marseille-Q3630^T is 107 108 facultatively aerobic. Optimal growth medium temperature, pH and NaCl concentration is 109 comprised between 31-37°C, 5.5-8.5 and 10-15g/L respectively. The sporulation test (20 110 minutes at 80°C) is negative. Using API strips, positive reactions were shown for Pyrazine-111 carboxamide, 2-naphthyl-phosphate, D-glucose, Alkaline phosphatase, Esterase Lipase (C8), 112 Naphthol-AS-BI-phosphohydrolase, D-fructose. All other reactions tested were negative. In 113 addition, this bacterium shows catalase positive and oxidase negative. These results are 114 summarized in **Table.2**

116 **Genome properties**

- 117 The assembly was achieved in a single contig with a coverage value 22.017x. The genome is
- 118 2.348.605 bp long with a 62.44% G+C content. The genome assembly of this strain was
- achieved on a single contig with a N_{50} value of 22.017. Of the 2.178 predicted genes, 2.083
- 120 were protein-coding genes and 63 were RNAs (3 16S rRNA, 4 5S rRNAs, 3 23S rRNAs, 50
- 121 tRNAs, and 3 ncRNA). (Supplementary file 2). The genome properties and distribution of
- 122 genes into COGs functional categories are detailed in **Supplementary file.3**. The *in silico*
- 123 resistome of the strain Marseille-Q3630 shows $erm(X)_4$ gene with 94.74% identity
- 124 percentage, notably involved in erythromycin resistance.

125 Discussion and conclusion

126 Using the taxono-genomics concept *i.e.*, the combination of the genomic and phenotypic 127 properties of a putative new taxon [15], we have characterized a new bacterial species 128 representing a new species within the family Corynebacteriaceae found in human. Although 129 it was found in a blood culture of a 7-year-old girl, Corynebacterium sp are known to be 130 associated with such contaminated samples. It is important to keep in mind that 131 Corynebacterium incognitun could also be such skin contaminant.[16] This strain is most closely related to Corynebacterium tuberculostearicum T with a 16S rRNA sequence 132 similarity value of 97.16%. Furthermore, genomic comparison using the OrthoANI parameter 133 provided a value of 72.06% with *Corynebacterium ureicelerivorans*^T and a dDDH value of 134 135 25.4 % with Corynebacterium diphtheria subsp. lausannense CHUV2995T Indeed, although 136 they are not universal cutoff, a value lower than 70% of DDH and 95-96% of ANI are 137 common indicators for a new species discovery, this new strain shows a *rpoB* gene identity 138 with Corynebacterium aurimucosum of 88.03%, validating the <96.6% identity cutoff. [14] 139 Taken altogether, these parameters have prompt us to propose Corynebacterium incognitum strain Marseille-Q3630^T as a new member of *Corynebacterium* genus. Gr. fem. n. korynê, a 140 141 club; L. neut. n. bacterium, a rod, and in biology a bacterium (so called because the first ones 142 observed were rod-shaped); N.L. neut. n. Corynebacterium, a club bacterium. Incognitum, 143 lat. incognitus « id. », der. cognitus, known. (Table.3)

144

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

- 146 *Corynebacterium incognitum* strain Marseille-Q3630^T, was deposited in CSUR collections 147 under accession CSUR-Q3630. The 16S rRNA and genome sequences available in GenBank
- under accession numbers MT772002 and GCA_014217255.1 respectively.
- 149

150 **Ethics Committee**

- 151 The study was validated by the ethics committee of the Institut Fédératif de Recherche IFR48.
- 152
- 153 Transparency declaration
- 154 None to declare
- 155
- 156 Funding

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- 169 **Reference**
- 170
- 171 [1] Lehmann Karl Bernhard, Royal College of Physicians of Edinburgh. Atlas und
- 172 Grundriss der Bakteriologie und Lehrbuch der speziellen bakteriologischen Diagnostik. vol. 1.
- 173 Munchen : Lehmann,; 1896.
- 174 [2] LPSN PAC. List of Prokaryotic names with Standing in Nomenclature (bacterio.net),
- 175 20 years on. International Journal of Systematic and Evolutionary Microbiology
- 176 2018;68:1825–9. https://doi.org/10.1099/ijsem.0.002786.
- 177 [3] Oliveira A, Oliveira LC, Aburjaile F, Benevides L, Tiwari S, Jamal SB, et al. Insight
- 178 of Genus Corynebacterium: Ascertaining the Role of Pathogenic and Non-pathogenic Species.
- 179 Front Microbiol 2017;8:1937. https://doi.org/10.3389/fmicb.2017.01937.
- 180 [4] Grégory D, Chaudet H, Lagier J-C, Raoult D. How mass spectrometric approaches
- 181 applied to bacterial identification have revolutionized the study of human gut microbiota.
- 182 Expert Rev Proteomics 2018;15:217–29. https://doi.org/10.1080/14789450.2018.1429271.
- 183 [5] Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K. BLAST+:
- architecture and applications. BMC Bioinformatics 2009;10. https://doi.org/10.1186/14712105-10-421.
- 186 [6] Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis
- 187 Version 7.0 for Bigger Datasets. Mol Biol Evol 2016;33:1870–4.
- 188 https://doi.org/10.1093/molbev/msw054.
- 189 [7] Tittsler RP, Sandholzer LA. The Use of Semi-solid Agar for the Detection of Bacterial
 190 Motility. J Bacteriol 1936;31:575–80.
- 191 [8] Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al.
- 192 SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell
- 193 Sequencing. Journal of Computational Biology 2012;19:455–77.
- 194 https://doi.org/10.1089/cmb.2012.0021.
- 195 [9] Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L.
- 196 NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 2016;44:6614–24.
- 197 https://doi.org/10.1093/nar/gkw569.
- 198 [10] Ondov BD, Treangen TJ, Melsted P, Mallonee AB, Bergman NH, Koren S. Mash: fast
- 199 genome and metagenome distance estimation using MinHash. Genome Biol 2016;17.
- 200 https://doi.org/10.1186/s13059-016-0997-x.
- 201 [11] Lagesen K, Hallin P, Rødland EA, Stærfeldt H-H, Rognes T, Ussery DW. RNAmmer:
- 202 consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Research

- 203 2007;35:3100-8. https://doi.org/10.1093/nar/gkm160.
- 204 [12] Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species
- 205 delimitation with confidence intervals and improved distance functions. BMC Bioinformatics
- 206 2013;14. https://doi.org/10.1186/1471-2105-14-60.
- 207 [13] Lee I, Ouk Kim Y, Park S-C, Chun J. OrthoANI: An improved algorithm and software
- 208 for calculating average nucleotide identity. International Journal of Systematic and
- 209 Evolutionary Microbiology 2016;66:1100–3. https://doi.org/10.1099/ijsem.0.000760.
- 210 [14] Khamis A, Raoult D, La Scola B. rpoB Gene Sequencing for Identification of
- 211 Corynebacterium Species. Journal of Clinical Microbiology 2004;42:3925–31.
- 212 https://doi.org/10.1128/JCM.42.9.3925-3931.2004.
- 213 [15] Abdallah RA, Beye M, Diop A, Bakour S, Raoult D, Fournier P-E. The impact of
- 214 culturomics on taxonomy in clinical microbiology. Antonie van Leeuwenhoek
- 215 2017;110:1327–37. https://doi.org/10.1007/s10482-017-0871-1.
- 216 [16] Doern GV, Carroll KC, Diekema DJ, Garey KW, Rupp ME, Weinstein MP, et al.
- 217 Practical Guidance for Clinical Microbiology Laboratories: A Comprehensive Update on the
- 218 Problem of Blood Culture Contamination and a Discussion of Methods for Addressing the
- 219 Problem. Clin Microbiol Reviews 2019;33:CMR.00009-19, e00009-19.
- 220 https://doi.org/10.1128/CMR.00009-19.
- 221 [17] Grant S JR, P. The CGView Server: a comparative genomics tool for circular
- genomes. Nucleic Acids Research 2008;36:W181-4. https://doi.org/10.1093/nar/gkn179.
- 223

224	Figure.1 MALDI	-TOF MS ref	ference	mass spectrum. Sp	ectra fi	rom 12 indi	vidua	l c	olonies o	of
225	Corynebacterium	incognitum	strain	Marseille-Q3630 ^T	were	compared	and	a	reference	ce
226	spectrum was gene	erated								

227

Figure 2: A. *16S* rRNAgene (A) and *RpoB* gene (B) based phylogenetic trees highlighting the position of *Corynebacterium incognitum* sp., nov., strain Marseille-Q3630 (red) relative to other closely related bacterial taxa. Best hits of BlastN megablast was added to the relative pointed by the TYGS software. 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

234

Figure 3: Scanning electron microscopy of *Corynebacterium incognitum* sp. nov., strain Marseille-Q3630^T using a Tabletop microscope TM 4000plus (Hitachi, Tokyo, Japan). The scale bar represents $5 \,\mu\text{m}$

238

Table1: Orthologous Average Nucleotide Identity from OrthoANI software and Digital
 DNA–DNA hybridization values obtained by sequence comparison of all studied genomes
 using TYGS comparison server using the 2nd formula

242

243 **Table.2** Differential characteristics of *Corynebacterium incognitum* strain Marseille- Q3630^T

- 244 and closest species standing in nomenclature
- 245

246 **Table.3:** Description of *Corynebacterium incognitum* sp. nov. strain Marseille-Q3630^T

- 247
- 248

Table.1: Digital DNA–DNA hybridization values obtained by sequence comparison of all

250 studied genomes using TYGS comparison server using the 2nd formula

6 6	-	Ũ			
Subject strain	OrthoANI value (Supplementary Fig.1)	dDDH (in %) with Corynebacterium incognitum strain Marseille-Q3630T	Confidence Interval (in %)	G+C content difference (in %)	
Corynebacterium diphtheriae subsp. lausannense CHUV2995	69.3094	25,4	[23.1 - 27.9]	8,49	
Corynebacterium rouxii FRC0190 T	69.6315	25,1	[22.8 - 27.6]	9,21	
Corynebacterium mustelae DSM 45274	68.4165	24,6	[22.3 - 27.1]	9,86	
Corynebacterium diphtheriae NCTC 11397	69.5648	24,5	[22.2 - 27.0]	8,91	
Corynebacterium belfantii FRC0043	69.4349	24,1	[21.8 - 26.6]	8,81	
Corynebacterium pseudotuberculosis ATCC 19410	68.5154	23,8	[21.4 - 26.2]	10,25	
Corynebacterium	Not found in public				
pseudotuberculosis DSM 20689	database	23,8	[21.4 - 26.2]	10,25	
Corynebacterium ureicelerivorans DSM 45051	72.0657	23,6	[21.3 - 26.0]	2,57	
Corynebacterium ulcerans NCTC 7910	68.928	23,6	[21.3 - 26.1]	9,12	
Corynebacterium jeikeium ATCC 43734	71.0532	23,6	[21.3 - 26.0]	0,8	

	C. incognitum	C. tuberculostearicum	C. tuscaniense	C. macgninleyi	C. simulans	C. accolens
Properties	Marseille-Q3630	Medalle X	ISS-5309	JCL-2	DSM 44415	CIP 104783
Oxygen requirement	Facultative	Facultative	+	Facultative	Facultative	Facultative
Gram Strain	+	+	+	+	+	+
Motility	-	-	-	-	-	-
Endospore formation	-	-	-	na	-	-
Optimum temperature for growth (°C)	31-37°C	na	na	37°C	na	37°C
Production of :						
Alkaline phosphatase	+	+	+	+	+	-
Catalase	+	+	+	+	+	+
Oxidase	-	-	na	-	na	-
α-Glucosidase	-	-	-	-	-	+
β-galactosidase	-	-	-	-	-	-
Acid from:						
N-Acetylglucosamine	-	+	-	-	-	+
L-arabinose	-	na	na	+	-	na
D-ribose	-	+	-	+	+	+
D-mannose	-	+	na	na	+	+
D-mannitol	-	-	-	+	-	-
D-glucose	+	+	+	+	+	+
D-fructose	+	+	na	+	+	+
D-maltose	-	-	+	-	-	-
D-lactose	-	-	-	-	-	-
Genome size	2,348,605	2,453,172	2,263,530	2,419,073	2,598,702	2,465,636

Table.2 Differential characteristics of *Corynebacterium incognitum* strain Marseille- Q3630^T and closest species standing in nomenclature.

Isolation source Human healthy skin Clinical and food samples	Blood cultuires of a patient with endocarditis	Bacterial flora of certain human body sites	Clinical samples	Clinical and food samples	
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Type of description	New description
Species name	incognitum
Genus name	Corynebacterium
Specific epithet	Corynebacterium
Species Status	sp nov
Species etymology	Corynebacterium incognitum strain Marseille-Q3630T.
	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. Incognitum, lat.
	incognitus « id. », der. cognitus, known.
Authors	Manon Boxberger, Angéline Antezack, Sibylle
	Magnien, Nadim Cassir, Bernard La Scola
Designation of the type strain	Marseille Q3630
Strain collection number	CSUR:3630
16S rRNA gene accession number	<u>MT772002</u>
Genome accession number	<u>GCA_014217255.1</u>
Genome status	Whole genome
Genome size	2,348,605-bp
GC%	62.44%

Table.3: Description of *Corynebacterium incognitum* sp. nov. strain Marseille-Q3630^T

Country of origin	France
Date of isolation	2020
Source of isolation	Human healthy skin
Growth medium, incubation	Columbia Agar with 5% Sheep Blood media
	(Biomérieux, France), 37°C, Aerobiosis, 1day
Gram stain	Positive
Cell shape	Rod shaped
Cell size	0.9 x 0.5 μm
Motility	-
Sporulation	-
Colony morphology	White
Temperature range	21°C-56°C
Temperature optimum	37°C
Relationship to O2	Facultative
O2 for strain testing	Anaerobiosis, microaerophilic, aerobiosis
Oxidase	Negative
Catalase	Positive

255 =