Draft genome and description of *Chryseobacterium manosquense* strain Marseille-Q2069^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 hand of a 35-year-old healthy woman using culturomics methods, we isolated the new bacterial strain Marseille-Q2069^T =CSUR-Q2069. Matrix-assisted desorption/ionization time-of-flight mass spectrometry 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 *Flavobacteriaceae in* the phylum *Bacteroidetes*. We described here the main phenotypic characteristics, genome sequence and annotation of *Chryseobacterium manosquense* strain Marseille-Q2069^T, a new member of the *Chryseobacterium* genus, that we propose as type strain.

Keywords: Bacteria, *Chryseobacterium manosquense*, culturomics, genome, sp. nov., species, taxonogenomics

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Introduction

The genus *Chryseobacterium* contains 144 species [1] isolated from ubiquitous sources. As an illustration, *Chryseobacterium anthropi* strain NF 1366, *Chryseobacterium treverense* strain IMMIB L-1519 [2,3], *Chryseobacterium hainfense* strain H38 [4], *Chryseobacterium montanum* strain WG4 [5] and *Chryseobacterium pallidum* strain 26-3St2b [6] were respectively isolated from not only human clinical specimens (the first two), but also from raw milk, soil and a beer-bottling plant. Strain Marseille-Q2069 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 [7–9]. 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 new bacterial species [7,10].

Materials and method

Strain isolation and phenotypic tests

Strain Marseille-Q2069 was initially isolated by direct seeding of seeding of 50 µL of sample on a homemade R2A (Reasoner's 2A agar) incubated in aerobiosis at 31°C, MALDI-TOF MS protein analysis was carried out using a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [8]. Spectra from strain Marseille-Q2069 were imported into the MALDI BioTyper software (version 3.0, Bruker) and analysed by standard pattern matching (with default parameter settings). The study was validated by the ethics committee (ID-RCB: 2019-A01508-49). Different growth temperatures (21°C, 28°C, 30°C, 37°C, 45°C and 56°C), atmosphere conditions (anaerobic, aerobic and microaerophilic; CampyGEN, Oxoid Ltd, Basingstoke, England, UK, Lenexa, KS, USA), pH (5.5, 6.5, 7.5 and 8.5) and NaCl concentrations (5, 10 and 15 g/L) 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 on a TM4000 microscope (Hitachi, Tokyo, Japan) approximately 60 cm in height and 33 cm in width to evaluate the bacterial structure. Motility test was performed using

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the semi solid TCC media as described by Tittsler and Sandholzer [11].

Genome sequencing

Genomic DNA of strain Marseille-Q2069^T was extracted in two steps: a mechanical treatment was performed first using acid-washed glass beads (G4649-500g; Sigma, St Louis, MO, USA) using a FastPrep-24™ 5G Grinder (mpBio, Santa Ana, CA, USA) at maximum speed (6.5) for 90 seconds. Then, after 30 minutes of lysozyme incubation at 37°C, DNA was extracted on the EZ1 biorobot (Qiagen, Hilden, Germany) with an EZ1 DNA tissues kit. The elution volume was 50 µL. Genomic DNA of strain Marseille-Q2069^T was quantified by a Qubit assay with a 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 with 23 other genomic projects prepared with the Nextera XT DNA sample prep kit (Illumina Inc.). To prepare the paired-end library, dilution was performed to obtain 1 ng of each genome as input. 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 normalized on specific beads according to the Nextera XT protocol (Illumina Inc.). 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-hour run in 2 × 250-bp. Total information of 9.20 Gb was obtained from a 1063/mm^2 cluster density with a cluster passing quality control filters of 95.7%. Within this run, the index representation for strain Marseille-Q2069^T was determined to index 4.15%. The 20 050 916 paired-end reads were filtered according to the read qualities.

Genome annotation and genome comparison

Genome annotation was obtain through the NCBI Prokaryotic Genome Annotation Pipeline [12]. The genome sequence data were uploaded to the Type (Strain) Genome Server (TYGS), a free bioinformatics platform available at https://tygs.dsmz.de, for whole-genome-based taxonomic analysis [13]. Determination of closest type strain genomes was performed in two complementary ways: first, all user genomes were compared against all type strain genomes available in the TYGS database

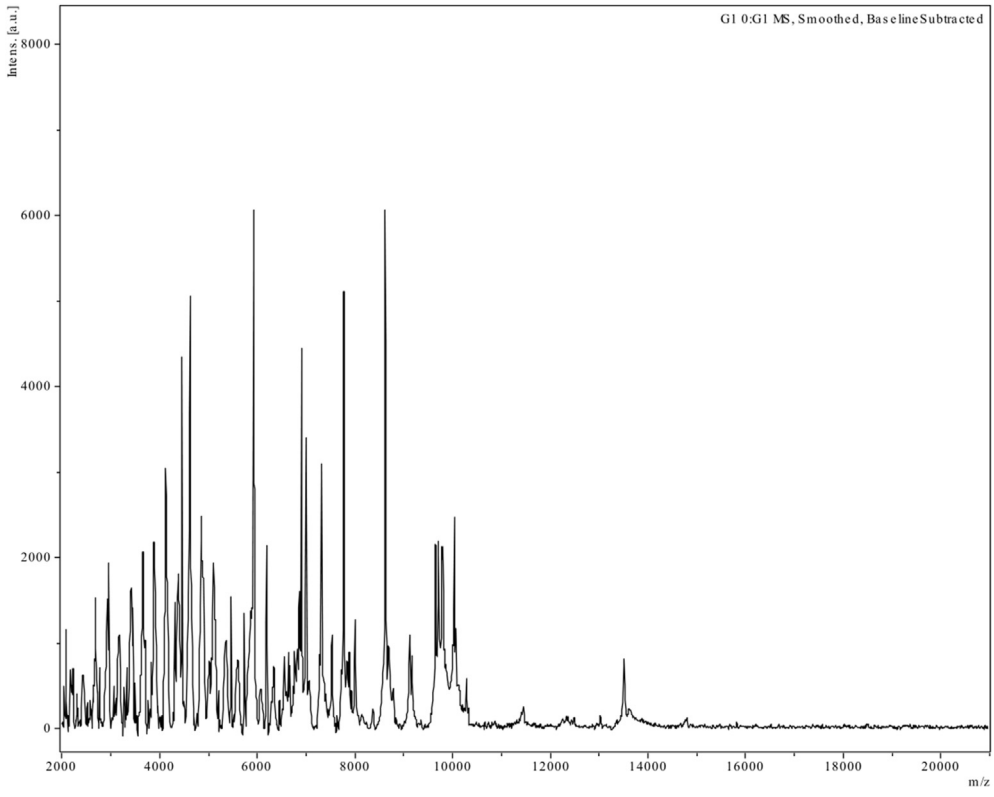

FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies of strain Marseille-Q2069^T were compared and a reference spectrum was generated.

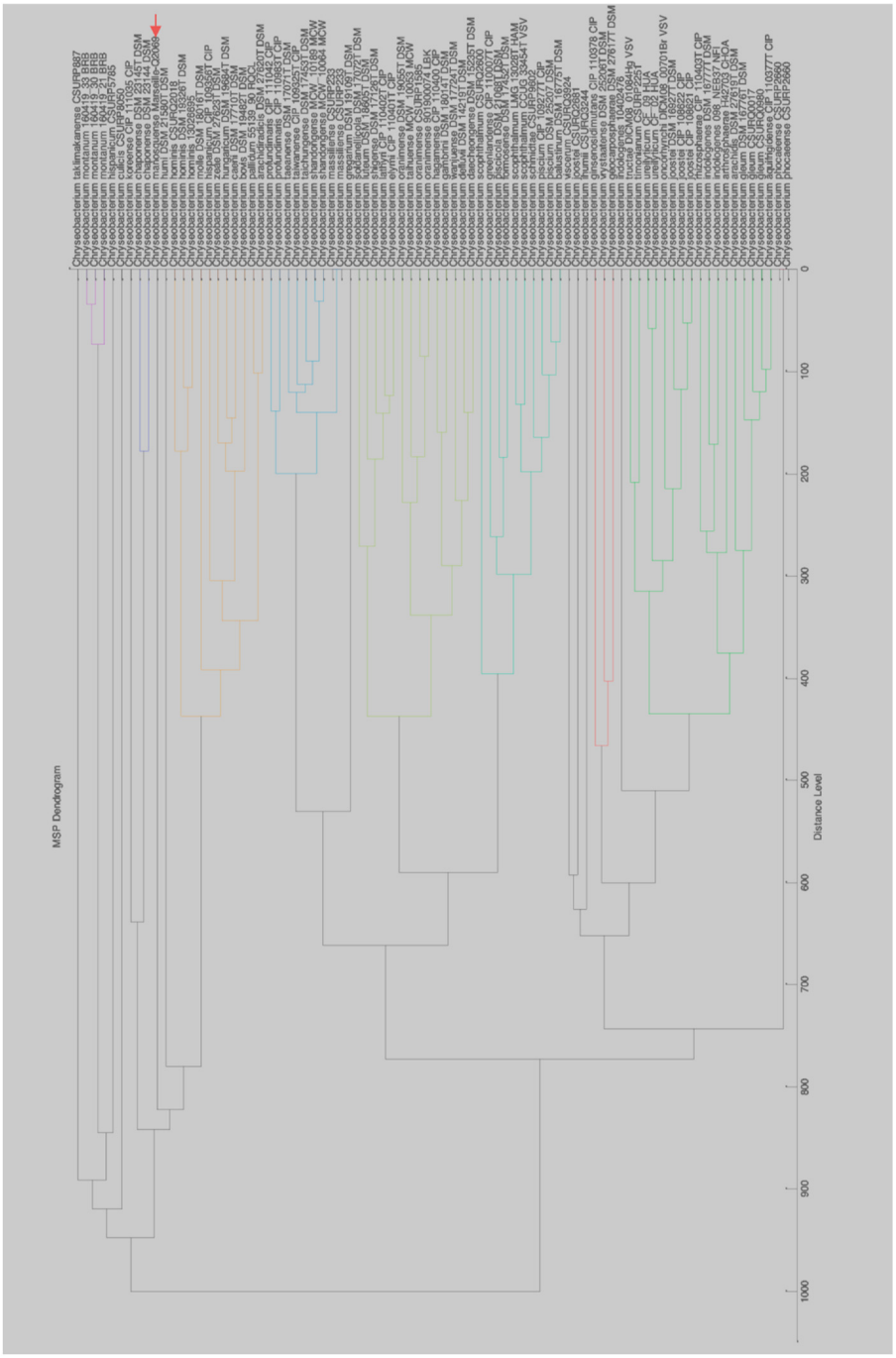

FIG. 2. MALDI-TOF MS dendrogram highlighting the position of *Chryseobacterium manosquense* sp. nov. within the *Chryseobacterium* genus.

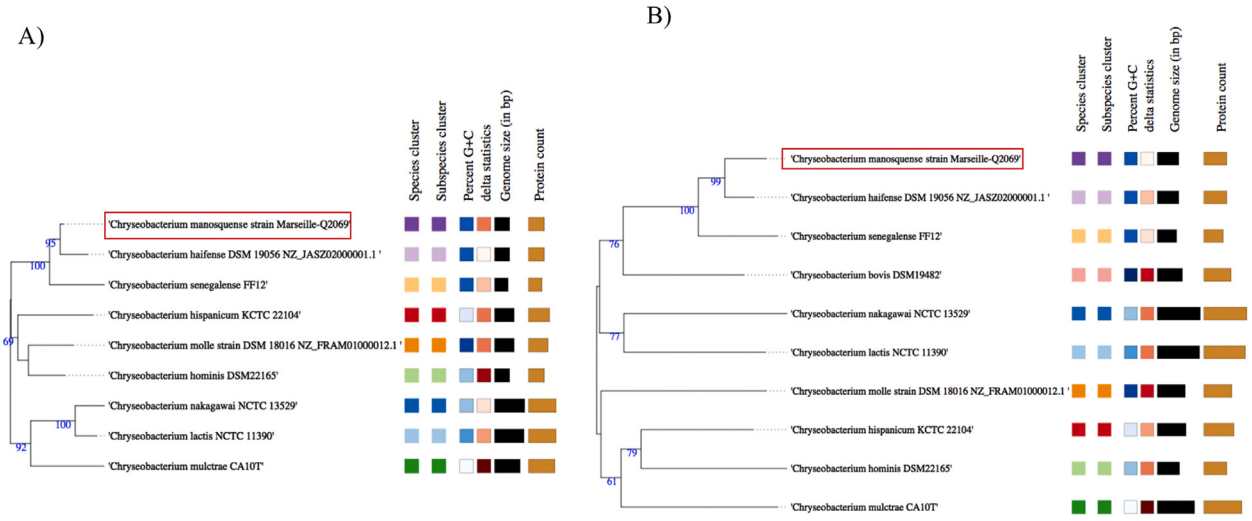

FIG. 3. (a) 16s rRNA-based phylogenetic tree and (b) whole-genome-based phylogenetic tree highlighting the position of *Chryseobacterium manosquense* sp. nov., strain Marseille-Q2069, relative to other closely related bacterial taxa. Trees are generated with FastME 2.1.6.1 [23] from Genome BLAST Distance Phylogeny (GBDP) distances calculated from genome sequences our 16S sequences. The branch lengths are scaled in terms of GBDP distance formula d5. The numbers above branches are GBDP pseudo-bootstrap support values > 60% from 100 replications, with an average branch support of 66.6%. The tree was rooted at the midpoint [24].

via the MASH algorithm, a fast approximation of intergenomic relatedness [14], and the ten type strains with the smallest MASH distances were chosen per user genome. Second, an additional set of ten closely related type strains was determined via the 16S rDNA gene sequences. These were extracted from the user genomes using RNAmmer [15] and each sequence was subsequently BLASTed [16] against the 16S rDNA gene sequence of each of the currently 12 983 type strains available in the TYGS database. This was used as a proxy to find the best 50 matching type strains (according to the bitscore) for each user genome and to subsequently calculate precise distances using the Genome BLAST Distance Phylogeny approach (GBDP) under the algorithm 'coverage' and distance formula d5 [17]. These distances were finally used to determine the ten closest type strain genomes for each of the user genomes. All

TABLE 1. Digital DNA–DNA hybridization values obtained by sequence comparison of all studied genomes using TYGS comparison server using the second formula

Query strain	Subject strain	dDDH (in %)	95% CI (%)	G + C content difference (%)
Chryseobacterium manosquense strain Marseille-Q2069'	*Chryseobacterium haifense DSM 19056*	65.6	62.7–68.4	0.07
Chryseobacterium manosquense strain Marseille-Q2069'	*Chryseobacterium senegalense FF12*	45.3	42.7–47.8	0.25
Chryseobacterium manosquense strain Marseille-Q2069'	*Chryseobacterium hispanicum KCTC 22104*	28	25.6–30.5	2.36
Chryseobacterium manosquense strain Marseille-Q2069'	*Chryseobacterium bovis DSM19482*	26.7	24.3–29.2	1.3
Chryseobacterium manosquense strain Marseille-Q2069'	*Chryseobacterium molle DSM 18016*	24.6	22.3–27.1	0.54
Chryseobacterium manosquense strain Marseille-Q2069'	*Chryseobacterium pallidum DSM 18015*	24	21.7–26.4	0.77
Chryseobacterium manosquense strain Marseille-Q2069'	*Chryseobacterium mulctrae CA10T*	23.9	21.6–26.3	3.13
Chryseobacterium manosquense strain Marseille-Q2069'	*Chryseobacterium hominis DSM22165*	23.8	21.5–26.2	1.32
Chryseobacterium manosquense strain Marseille-Q2069'	*Chryseobacterium nakagawai NCTC 13529*	23.7	21.4–26.2	1.53
Chryseobacterium manosquense strain Marseille-Q2069'	*Chryseobacterium lactis NCTC 11390*	23.5	21.2–26.0	0.83

Abbreviations: dDDH, digital DNA–DNA hybridization; TYGS, Type (Strain) Genome Server.

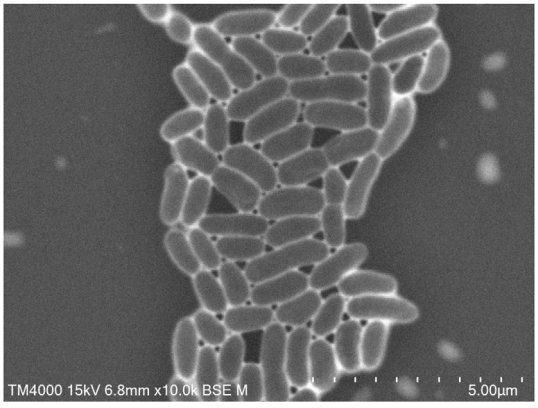

FIG. 4. Scanning electron microscopy of *Chryseobacterium manosquense* sp. nov., strain Marseille-Q2069T, using a Tabletop microscope TM 4000 plus (Hitachi, Tokyo, Japan). The scale bar represents 5 µm.

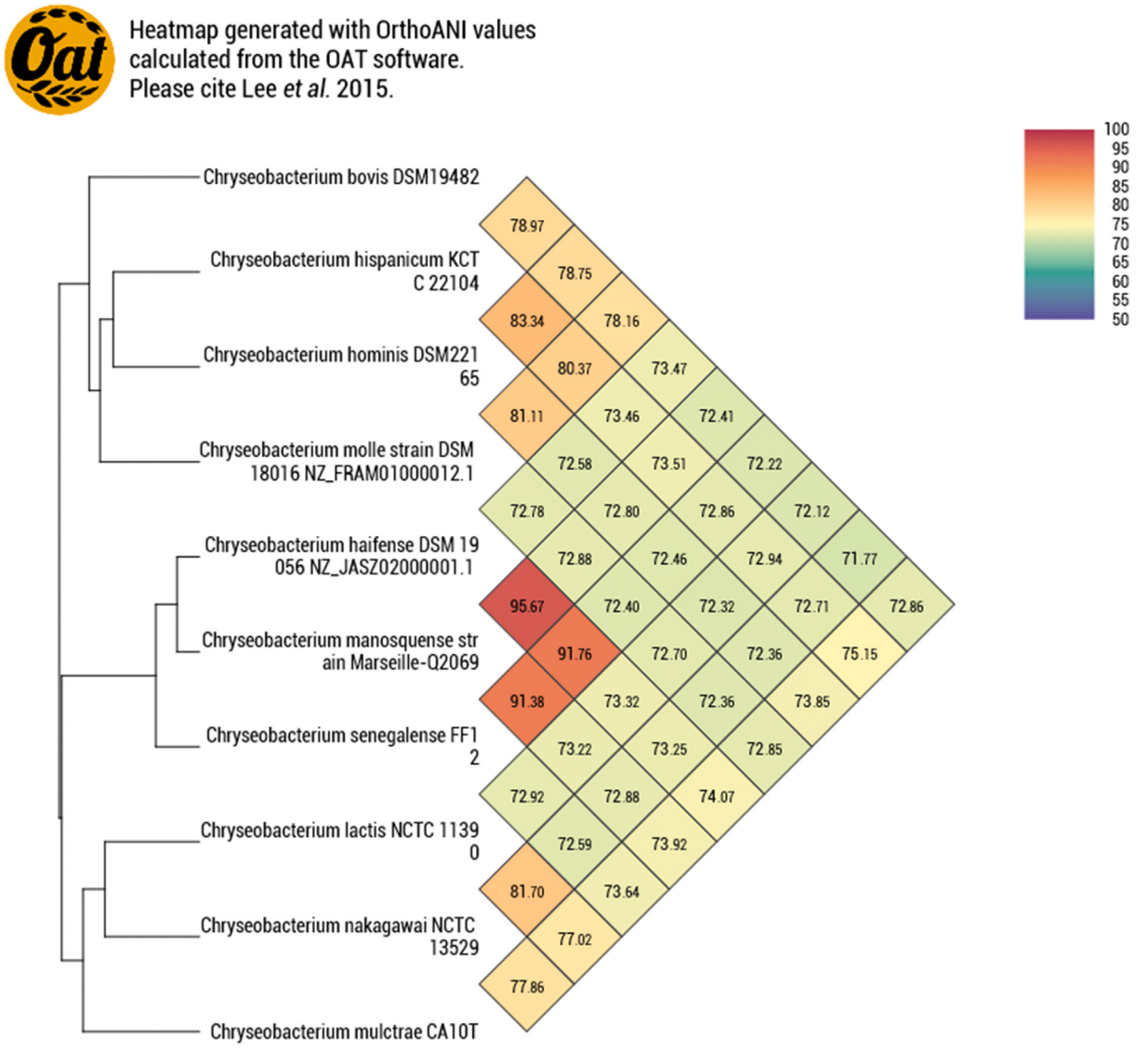

FIG. 5. Heatmap generated with OrthoANI values calculated using the OAT software between *Corynebacterium manosquense* sp. nov., strain Marseille-Q2069, and other closely related species with standing in nomenclature.

pairwise comparisons among the set of genomes were conducted using GBDP and accurate intergenomic distances were inferred under the algorithm 'trimming' and distance formula d5. One hundred distance replicates were calculated each. Digital DNA–DNA hybridization values and confidence intervals were calculated using the recommended settings of the GGDC2. Complementarily, the degree of genomic similarity of strain Marseille-Q2069 with closely related species was estimated using the OrthoANI software with default parameters [18]. Antibiotic resistance genes and presence of pathogenesis-related proteins was investigated using the ABRicate tools, CARD [19] and VFDB [20] using the Online Galaxy platform [21].

Results

Strain identification and classification

Strain Marseille-Q2069 was isolated from the hand skin swab of a 35-year-old healthy woman. Strain Marseille-Q2069 failed to

TABLE 2. Differential characteristics of *Chryseobacterium manosquense* strain Marseille-Q2069 and its most closely related species standing in nomenclature

Properties	*Chryseobacterium manosquense* Marseille-Q2069	*Chryseobacterium anthropi* NF 1366	*Chryseobacterium hainfense* H38	*Chryseobacterium treverense* IMMIB L-1519	*Chryseobacterium montanum* WG4	*Chryseobacterium pallidum* 26-3St2b
Cell diameter (µm)	1.3 × 0.5	2–4 × 0.5–1	0.6–0.9	1.4–2.7 × 60.5–0.6	0.7–1.2 × 0.4–0.6	2.0 × 0.7
Oxygen requirement	+	Facultative	Facultative	+	+	Facultative
Gram stain	—	—	—	—	—	—
Motility	—	—	—	—	—	—
Endospore formation	—	na	na	—	—	—
Optimum temperature for growth	na	30	32	20	37	37
Production of:						
Alkaline phosphatase	+	+	+	+	+	na
Catalase	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+
α-glucosidase	+	na	na	+	—	+
β-galactosidase	—	+	+	—	—	na
Acid from:						
N-acetylglucosamine	—	na	na	—	—	—
L-arabinose	—	+	—	—	—	+
D-ribose	—	na	na	—	+	na
D-mannose	+	—	+	+	+	—
D-mannitol	—	—	—	—	—	+
D-glucose	+	+	+	+	+	+
D-fructose	+	+	+	—	—	na
D-maltose	+	+	+	—	+	+
D-lactose	—	—	+	—	+	+
G + C content (mol%)	36.9	39	37.8	na	37.7	37.8
Habitat	Human healthy skin	Human clinical specimens	Raw milk	Human clinical source	Mountain soil	Beer-bottling plants

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/. Strain Marseille-Q2069 reference spectra were generated (Fig. 1) and analysed within all the IHU databases available for *Chryseobacterium* sp. spectra; the spectra did not belong to any known cluster (Fig. 2). Moreover, strain Marseille-Q2069^T exhibited a 99.02% 16S rRNA sequence similarity with *Chryseobacterium haifense* strain H38 type strain (GenBank accession no. NR_044167.1), the phylogenetically closest bacterium with standing in nomenclature (Fig. 3a). Furthermore, a digital DNA–DNA hybridization analysis between these two organisms revealed a maximum identity similarity of only 65.6% (Fig. 3b and Table 1), and an OrthoANI parameter provided a value of 95.73% (Fig. 4). Taken together, these results confirm the status of this strain as a new member of the *Chryseobacterium* genus for which the name *Chryseobacterium manosquense* strain Marseille-Q2069^T is proposed.

Phenotypic characteristics

Growth of *Chryseobacterium manosquense* strain Marseille-Q2069^T was initially isolated by direct seeding of 50 µL of sample on a homemade modified R2A (Reasoner's 2A agar) incubated aerobically at 31°C. Colonies from strain Marseille-Q2069^T showed a white/beige pigmentation and no haemolysis. They were circular with a diameter of 0.5–1.5 mm. Bacterial cells were Gram-negative, non-motile rods with a length of about 1.30 µm and a width of about 0.5 µm, as determined by electronic scanning microscopy (Fig. 5). Strain Marseille-Q2069^T is strictly aerobic. Optimal growth medium pH was 7 and optimal NaCl concentration was 10–15 g/L. The sporulation test (20 minutes at 80°C) was negative. Using API strips, positive reactions were shown for potassium nitrate, L-tryptophan, esculin ferric citrate, alkaline phosphatase, esterase

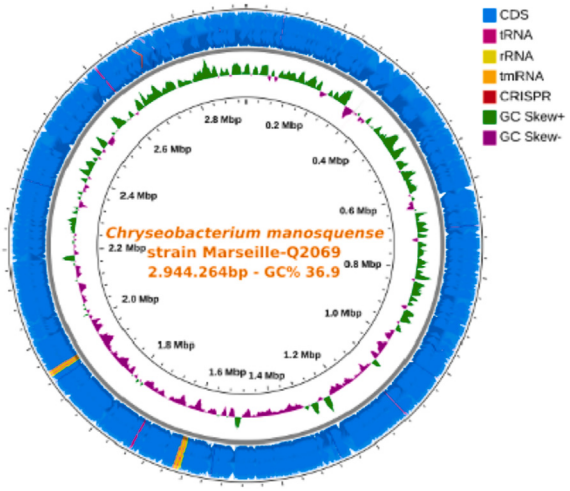

FIG. 6. Graphical circular map of the genome from strain Marseille-Q2069^T obtained by CGView Server online tool [25].

TABLE 3. Detailed functional classes of predicted genes according to the clusters of orthologous groups of proteins of *Chryseobacterium manosquense* sp. nov. and other closely related bacterial taxa

Description	COG	*C. manosquense*	*C. bovis*	*C. haifense*	*C. hispanicum*	*C. senegalense*
RNA processing and modification	[A]	0	0	0	0	0
Chromatin structure and dynamics	[B]	0	0	0	0	0
Energy production and conversion	[C]	86	93	81	95	77
Cell cycle control, mitosis and meiosis	[D]	19	21	19	21	19
Amino acid transport and metabolism	[E]	91	104	89	114	82
Nucleotide transport and metabolism	[F]	40	42	41	47	40
Carbohydrate transport and metabolism	[G]	51	73	56	90	49
Co-enzyme transport and metabolism	[H]	70	80	73	88	72
Lipid transport and metabolism	[I]	64	74	63	85	61
Translation	[J]	153	151	152	152	151
Transcription	[K]	77	101	74	112	56
Replication, recombination and repair	[L]	91	96	81	93	82
Cell wall/membrane biogenesis	[M]	129	146	120	160	128
Cell motility	[N]	8	9	8	9	6
Post-translational modification, protein turnover, chaperones	[O]	65	79	61	77	66
Inorganic ion transport and metabolism	[P]	73	102	77	106	78
Secondary metabolites biosynthesis, transport and catabolism	[Q]	11	13	11	12	10
General function prediction only	[R]	64	74	61	93	66
Function unknown	[S]	37	44	37	42	29
Signal transduction mechanisms	[T]	52	59	48	61	47
Intracellular trafficking and secretion	[U]	16	16	17	15	14
Defence mechanisms	[V]	44	69	50	64	31
Extracellular structures	[W]	0	0	0	0	0
Mobilome: prophages, transposons	[X]	26	16	11	22	20
Nuclear structure	[Y]	0	0	0	0	0
Cytoskeleton	[Z]	0	0	0	0	0

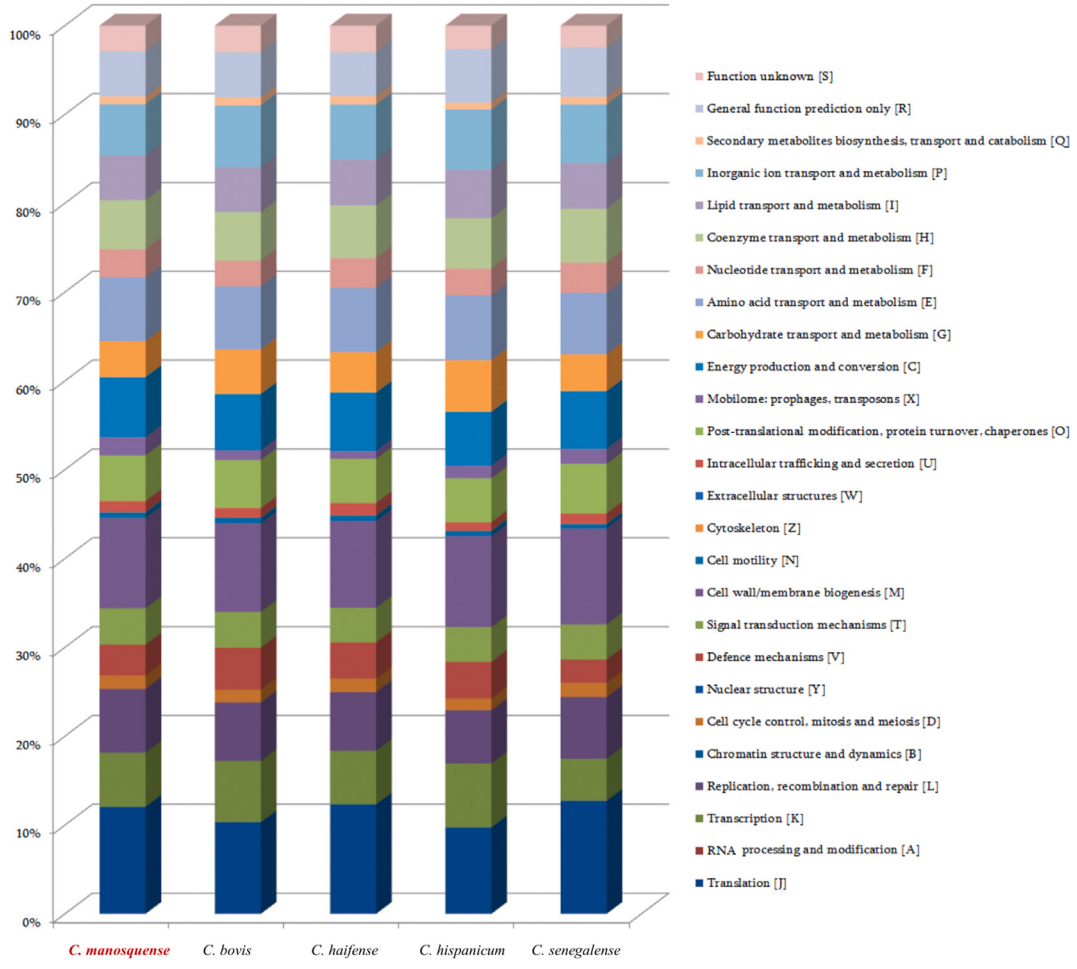

FIG. 7. Distribution of functional classes of predicted genes according to the clusters of orthologous groups of proteins of *Chryseobacterium manosquense* sp. nov. and other closely related bacterial taxa.

TABLE 4. Description of *Chryseobacterium manosquense* sp. nov., strain Marseille-Q2069^T

Species name	*manosquense*
Genus name	*Chryseobacterium*
Specific epithet	*Chryseobacterium*
Species status	sp.nov.
Species etymology	Gr. adj. *chruseos*, golden; L. neut. n. bacterium, a small rod; N.L. neut. n. *Chryseobacterium*, a yellow rod. *Manosquense*, translitt. L. adj., 'of *Manosque*,' referring to the provenance of the sample.
Authors	Manon Boxberger, Mariem Ben Khedher, Sibylle Magnien, Nadim Cassir, Bernard La Scola
Designation of the type strain	Marseille-Q2069
Strain collection number	CSUR-Q2069
16S rRNA gene accession number	https://www.ncbi.nlm.nih.gov/nuccore/MT795957
Genome accession number	CP060203
Genome status	Complete
Genome size	2 944 264 bp
GC%	36.9
Country of origin	Marseille, France
Date of isolation	2019
Source of isolation	Human healthy skin
Growth medium, incubation	R2A (Reasoner's 2A agar)
Conditions used for standard cultivation	31°C in aerobiosis
Gram stain	Negative
Cell shape	Rods
Cell size	1.3 × 0.5 µm
Motility	Non-motile
Sporulation	Non-sporulating
Colony morphology	Circular
Temperature range	21°C–56°C
Temperature optimum	31°C
Relationship to O_2	Aerobe
O_2 for strain testing	Strictly aerobic
Oxidase	+
Catalase	+

(C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, glycerol, D-glucose, D-fructose, D-mannose, D-maltose, D-lactose, D-saccharose and starch. All other reactions tested were negative. In addition, this bacterium was catalase and oxidase positive. These results are summarized in Table 2.

Genome properties

The genome size of strain Marseille-Q2069 was 2 944 264-bp with a 36.9% G + C content. The genome assembly of this strain was achieved on a chromosome. Of the 2807 predicted genes, 2667 were protein-coding genes and 57 were RNAs (four 16S rRNA, four additional 5S rRNAs, four additional 23S rRNAs and 42 tRNAs) (Fig. 6). The distribution of genes into clusters of orthologous groups (COGs) functional categories for strain Marseille-Q2069^T and other closely related bacterial taxa is detailed in Table 3. Analysis of the COG categories shows that the translation, ribosomal structure and biogenesis elements and the cell wall/membrane/envelope biogenesis elements of strain Marseille-Q2069 appear to be the more numerous putative function (by COGs) (153 in category J, 129 in category M, respectively). Through this analysis, we can see that the repartition of all COG categories is similar across these species (Fig. 7). The *in silico* resistome of the strain Marseille-Q2069 and the search for virulence factors [20] of this strain shows no genes with high identity percentage.

Discussion

In the past 8 years, the culturomics approach has led to the discovery of more than 500 bacterial species [7]. Using the taxonogenomics concept, i.e. the combination of the genomic and phenotypic properties of a putative new taxon [22], we have characterized a new bacterial species representing a new species within the family *Flavobacteriaceae* found on human hand skin. Analysis of the genome sequence revealed a maximum DNA–DNA hybridization value of 65.6% and an OrthoANI value of 95.73% with the closer species with standing in nomenclature *Chryseobacterium haifense* strain H38; these are below the admissible delineation cut-off and so confirm the status of this strain as a new member of the *Chryseobacterium* genus. It was named *Chryseobacterium manosquense* strain Marseille-Q2069^T (Table 4): Gr. adj. *chruseos*, golden; L. neut. n. bacterium, a small rod; N.L. neut. n. *Chryseobacterium*, a yellow rod. *Manosquense*, translitt. L. adj., 'of *Manosque*', referring to the place where M&L Laboratories (one of the founders) are located.

Deposit in culture collections and sequences database

Chryseobacterium manosquense strain Marseille-Q2069T, was deposited in the CSUR collections under Accession number CSUR-Q2069. The 16S rRNA and genome sequences are available in GenBank under accession numbers https://www.ncbi.nlm.nih.gov/nuccore/MT795957 and CP060203, respectively.

Conflicts of interest

None to declare.

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