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1 **Draft genome and description of *Chryseobacterium manosquense* strain Marseille-Q2069^T**
2 **sp.nov., a new bacterium isolated from human healthy skin**

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21

1 **Draft genome and description of *Chryseobacterium manosquense* strain Marseille-**
2 **Q2069^T *sp.nov.*, a new bacterium isolated from human healthy skin**

3

4 **Abstract**

5 In 2019, by culturing a skin swab from the hand of a 25-years-old healthy woman through the
6 culturomic method, we isolated the new bacterial strain Marseille-Q2069^T = CSUR-Q2069.
7 Matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS)
8 failed to identify this isolate. Analysis of the 16S rRNA gene and Genome-to-Genome
9 comparison suggested that this taxon belongs to a novel bacterial species within the family
10 *Flavobacteriaceae* in the phylum *Bacteroidetes*. We described here the main phenotypic
11 characteristics, genome sequence and annotation of *Chryseobacterium manosquense* strain
12 Marseille-Q2069^T, a new member of the *Chryseobacterium* genus, that we propose as type
13 strain.

14 **Keywords: Bacteria, *Chryseobacterium manosquense*, culturomics, genome, species, sp.**
15 **nov., taxono-genomics**

16 **Introduction**

17 The genus *Chryseobacterium* counts 144 species [1] isolated from ubiquitous sources. As an
18 illustration, *C. anthropi* strain NF 1366, *C. treverense* strain IMMIB L-1519 [2,3], *C.*
19 *hainfense* strain H38 [4], *C. montanum* strain WG4 [5] and *C. pallidum* strain 26-3St2b [6]
20 were respectively isolated from human clinical specimen but also raw milk, soil or even in
21 beer-bottling plant environment. Strain Marseille-Q2069 was isolated using the culturomics
22 approach, based on the use of a large panel of culture conditions in order to describe the
23 microbial composition of a sample by high-throughput culture. [7–9] A taxono-genomics
24 approach including matrix-assisted laser desorption-ionization time-of-flight mass
25 spectrometry (MALDI-TOF MS), phylogenetic analysis, main phenotypic description and
26 genome sequencing was used to describe this new bacterial species [7,10].

27

28 **Materiel and method**

29 **Strain isolation and phenotypic tests**

30 Strain Marseille-Q2069 was initially isolated by direct seeding of seeding of 50µL of sample
31 on a homemade R2A (Reasoner's 2A agar) incubated in aerobiosis at 31°C, MALDI-TOF
32 mass spectrometry (MS) protein analysis was carried out using a Microflex spectrometer
33 (Bruker Daltonics, Bremen, Germany) [8]. Spectra from strain Marseille-Q2069 were
34 imported into the MALDI BioTyper software (version 3.0, Bruker) and analyzed by standard
35 pattern matching (with default parameter settings). The study was validated by the ethics
36 committee ID-RCB: 2019-A01508-49. Different growth temperatures (21, 28, 30, 37, 45 and
37 56°C), atmosphere conditions, anaerobic, aerobic and microaerophilic (CampyGEN, Oxoid,
38 USA), pH (5,5; 6.5; 7.5; 8.5) and NaCl concentration (5, 10, 15 g/L) were tested. API ZYM,
39 API 20NE and API 50 CH strips (BioMérieux, Marcy L'Etoile, France) were used to evaluate
40 the biochemical properties of the strain according to the manufacturer's instructions. For
41 scanning electronic microscopy, a colony was collected from agar and immersed into a 2.5 %
42 glutaraldehyde fixative solution. The slide was gently washed in water; air dried and
43 examined with approximately 60 centimeters in height and 33 cm in width to evaluate
44 bacterial structure on a TM4000 microscope (Hitachi, Tokyo, Japan). Motility test was
45 performed using the semi solid TCC media as described by Tittsler *et al.*[11]

46

47 **Genome sequencing**

48 Genomic DNA (gDNA) of strain Marseille-Q2069^T was extracted in two steps: a mechanical
49 treatment was first performed by glass beads acid washed (G4649-500g Sigma) using a

50 FastPrep-24™ 5G Grinder (mpBio) at maximum speed (6.5) for 90s. Then after 30 minutes
51 lysozyme incubation at 37°C, DNA was extracted on the EZ1 biorobot (Qiagen) with EZ1
52 DNA tissues kit. The elution volume is 50µL. gDNA of strain Marseille-Q2069^T was
53 quantified by a Qubit assay with the high sensitivity kit (Life technologies, Carlsbad, CA,
54 USA) to 0.2 ng/µl. Genomic DNA was next sequenced on the MiSeq Technology (Illumina
55 Inc, San Diego, CA, USA) with the paired end strategy and was barcoded in order to be
56 mixed respectively with 23 other genomic projects prepared with the Nextera XT DNA
57 sample prep kit (Illumina). To prepare the paired end library, dilution was performed to
58 require 1ng of each genome as input to prepare the paired end library. The « tagmentation »
59 step fragmented and tagged the DNA. Then limited cycle PCR amplification (12 cycles)
60 completed the tag adapters and introduced dual-index barcodes. After purification on AMPure
61 XP beads (Beckman Coulter Inc, Fullerton, CA, USA), the libraries were then normalized on
62 specific beads according to the Nextera XT protocol (Illumina). Normalized libraries were
63 pooled into a single library for sequencing on the MiSeq. The pooled single strand library was
64 loaded onto the reagent cartridge and then onto the instrument along with the flow cell.
65 Automated cluster generation and paired end sequencing with dual index reads were
66 performed in a single 39-hours run in 2x250-bp. Total information of 9.20 Gb was obtained
67 from a 1063/mm² cluster density with a cluster passing quality control filters of 95.7%.
68 Within this run, the index representation for strain Marseille-Q2069^T was determined to index
69 4.15%. The 20 050 916 paired end reads were filtered according to the read qualities.

70

71 **Genome annotation and genome comparison**

72 Genome annotation was obtain through the NCBI Prokaryotic Genome Annotation Pipeline
73 [12]. The genome sequence data were uploaded to the Type (Strain) Genome Server (TYGS),
74 a free bioinformatics platform available under <https://tygs.dsmz.de>, for a whole genome-based
75 taxonomic analysis [13]. Determination of closest type strain genomes was done in two
76 complementary ways: first, all user genomes were compared against all type strain genomes
77 available in the TYGS database via the MASH algorithm, a fast approximation of
78 intergenomic relatedness[14], and, the ten type strains with the smallest MASH distances
79 chosen per user genome. Second, an additional set of ten closely related type strains was
80 determined via the 16S rDNA gene sequences. These were extracted from the user genomes
81 using RNAmmer [15] and each sequence was subsequently BLASTed [16] against the 16S
82 rDNA gene sequence of each of the currently 12983 type strains available in the TYGS
83 database. This was used as a proxy to find the best 50 matching type strains (according to the

84 bitscore) for each user genome and to subsequently calculate precise distances using the
85 Genome BLAST Distance Phylogeny approach (GBDP) under the algorithm 'coverage' and
86 distance formula d5 [17]. These distances were finally used to determine the 10 closest type
87 strain genomes for each of the user genomes. All pairwise comparisons among the set of
88 genomes were conducted using GBDP and accurate intergenomic distances inferred under the
89 algorithm 'trimming' and distance formula d5. 100 distance replicates were calculated each.
90 Digital DDH values and confidence intervals were calculated using the recommended settings
91 of the GGDC2. Complementarily, the degree of genomic similarity of strain Marseille-Q2069
92 with closely related species was estimated using the OrthoANI software with default
93 parameters [18]. Antibiotic resistance genes and presence of pathogenesis-related proteins
94 was investigated using the ABRicate tools, CARD[19] and VFDB [20] using the Online
95 Galaxy platform. [21]
96

97 **Results**

98 **Strain identification and classification**

99 Strain Marseille-Q2069 was isolated from the hand skin swab of a 25-year-old
100 healthy. Strain Marseille-Q2069 failed to be identified by our systematic MALDI-TOF MS
101 screening, suggesting that the corresponding species was not in the database -
102 <https://www.mediterranee-infection.com/acces-ressources/base-de-donnees/urms-data-base/>.
103 Strain Marseille-Q2069 reference spectra was generated (**Figure.1**) and analyzed within all
104 the IHU databases available *Chryseobacterium sp* spectra and does not belong to any known
105 cluster. (**Figure.2**) Moreover, strain Marseille-Q2069^T exhibited a 99.02% 16S rRNA
106 sequence similarity *Chryseobacterium haifense strain H38* type strain (GenBank accession
107 no. NR_044167.1), the phylogenetically closest bacterium standing in nomenclature.
108 (**Figure.3. A**) Furthermore, a digital DNA–DNA hybridization analysis between these two
109 organisms revealed a maximum identity similarity of only 65.6% (**Figure.3. B and Table.1**).
110 and an OrthoANI parameter provided a value of 95.73%. (**Figure.4**) Taken altogether these
111 results confirm the status of this strain as a new member of the *Chryseobacterium* genus for
112 which the name *Chryseobacterium manosquense* strain Marseille-Q2069^T is proposed.

113

114 **Phenotypic characteristics**

115 Growth of *Chryseobacterium manosquense* strain Marseille-Q2069^T was initially isolated by
116 direct seeding of 50µL of sample on a homemade modified R2A (Reasoner's 2A agar)
117 incubated in aerobiosis at 31°C. Colonies from strain Marseille-Q2069^T showed a white/beige
118 pigmentation and no hemolysis. They were circular with a diameter of 0.5 to 1.5 mm.
119 Bacterial cells were Gram-negative, non-motile rods with a length of about 1.30 µm and a
120 width of about 0.5 µm, as determined by electronic scanning microscopy (**Figure 5**). Strain
121 Marseille-Q2069^T is strictly aerobic. Optimal growth medium pH and NaCl concentration is 7
122 and 10-15g/L respectively. The sporulation test (20 minutes at 80°C) was negative. Using an
123 API strips, positive reactions were shown for Potassium nitrate, L-Tryptophane, Esculin ferric
124 citrate, Alkaline phosphatase, Esterase (C4), Esterase Lipase (C8), Leucine arylamidase,
125 Valine arylamidase, Cystine arylamidase, Trypsin, α-chymotrypsin, Acid phosphatase,
126 Naphthol-AS-BI-phosphohydrolase, α-glucosidase, glycerol, D-glucose, D-fructose, D-
127 mannose, D-maltose, D-lactose, D-saccharose and Amidon. All other reactions tested were
128 negative. In addition, this bacterium shows catalase and oxidase positive. These results are
129 summarized in **Table.2**

130

131 **Genome properties**

132 The genome size of strain Marseille-Q2069 was 2,944,264-bp long with a 36.9% G+C
133 content. The genome assembly of this strain was achieved on a chromosome. Of the 2,807
134 predicted genes, 2,667 were protein-coding genes and 57 were RNAs (4 16S rRNA, 4
135 additional 5S rRNAs, 4 additional 23S rRNAs and 42 tRNAs) (**Figure.6**). The distribution of
136 genes into COGs functional categories for strain Marseille-Q2069^T and cl other closely
137 related bacterial taxa is detailed in **Table 3**. Analysis of the Clusters of Orthologous Groups
138 (COGs) categories shows that the Translation, ribosomal structure and biogenesis elements
139 and the Cell wall/membrane/envelope biogenesis elements of the strain *Marseille-Q2069*
140 appear to be the more numerous putative function (by COGs) (153 in category [J], 129 in
141 category [M] respectively). Through this analysis, we can see that the repartition of all COG
142 categories is similar across these species. (**Figure.7**) The *in silico* resistome of the strain
143 Marseille-Q2069 and the search for virulence factors [20] of this strain shows no genes with
144 high identity percentage.

145

146 **Discussion and conclusion**

147 In the past 8 years, culturomic approach induce the discovery of more than 500 bacterial
148 species. [7]. Using the taxono-genomics concept *i.e.* the combination of the genomic and
149 phenotypic properties of a putative new taxon [22], we have characterized a new bacterial
150 species representing a new species within the family *Flavobacteriaceae* found on a human
151 hand skin. Analysis of the genome sequence revealed a maximal DDH value of 65.6% and an
152 OrthoANI value of 95.73% with the closer species standing in nomenclature
153 *Chryseobacterium haifense* strain H38, that are under the admitted delineation cutoff and thus
154 confirm the status of this strain as a new member of the *Chryseobacterium* genus. It was
155 named *Chryseobacterium manosquense* strain Marseille-Q2069^T. (**Table.4**) Gr. adj. *chruseos*,
156 golden; L. neut. n. bacterium, a small rod; N.L. neut. n. *Chryseobacterium*, a yellow rod.
157 *Manosquense*, translitt. L. adj., ‘of *Manosque*,’ referring to the place where M&L
158 Laboratories (one of the founders) are located.

159

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

161 *Chryseobacterium manosquense* strain Marseille-Q2069^T, was deposited in CSUR
162 collections under accession CSUR-Q2069. The 16S rRNA and genome sequences are
163 available in GenBank under accession numbers MT795957 and CP060203 respectively.

164

165 **Transparency declaration**

166 None to declare

167

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178

179 **Reference**

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258

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

261

262 **Figure.2:** MALDI-TOF MS dendrogram highlighting the position of *Chryseobacterium*
263 *manosquense* sp., nov within *Chryseobacterium* genus.

264

265 **Figure 3: A)**16s rRNA-based phylogenetic tree **B)** Whole-genome based phylogenetic tree
266 highlighting the position of *Chryseobacterium manosquense* sp., nov., strain Marseille-Q2069
267 relative to other closely related bacterial taxa. Trees are generated with FastME 2.1.6.1 [23]
268 from GBDP distances calculated from genome sequences our 16S sequences. The branch
269 lengths are scaled in terms of GBDP distance formula d5. The numbers above branches are
270 GBDP pseudo-bootstrap support values > 60 % from 100 replications, with an average branch
271 support of 66.6 %. The tree was rooted at the midpoint. [24]

272

273 **Figure 4:** Scanning electron microscopy of *Chryseobacterium manosquense* sp. nov., strain
274 Marseille-Q2069^T using a Tabletop microscope TM 4000plus (Hitachi, Tokyo, Japan). The
275 scale bar represents 5 μm.

276

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

280

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

283

284 **Figure 7:** Distribution of functional classes of predicted genes according to the clusters of
285 orthologous groups of proteins of *Chryseobacterium manosquense* sp. nov. other closely
286 related bacterial taxa

287 **Table.1** Digital DNA–DNA hybridization values obtained by sequence comparison of all studied genomes using TYGS comparison server using
 288 the 2nd formula.

Query strain	Subject strain	dDDH (in %)	C.I. (in %)	G+C content difference (in %)
<i>Chryseobacterium manosquense</i> strain Marseille-Q2069'	<i>Chryseobacterium haifense</i> DSM 19056	65,6	[62.7 - 68.4]	0,07
<i>Chryseobacterium manosquense</i> strain Marseille-Q2069'	<i>Chryseobacterium senegalense</i> FF12	45,3	[42.7 - 47.8]	0,25
<i>Chryseobacterium manosquense</i> strain Marseille-Q2069'	<i>Chryseobacterium hispanicum</i> KCTC 22104	28	[25.6 - 30.5]	2,36
<i>Chryseobacterium manosquense</i> strain Marseille-Q2069'	<i>Chryseobacterium bovis</i> DSM19482	26,7	[24.3 - 29.2]	1,3
<i>Chryseobacterium manosquense</i> strain Marseille-Q2069'	<i>Chryseobacterium molle</i> DSM 18016	24,6	[22.3 - 27.1]	0,54
<i>Chryseobacterium manosquense</i> strain Marseille-Q2069'	<i>Chryseobacterium pallidum</i> DSM 18015	24	[21.7 - 26.4]	0,77
<i>Chryseobacterium manosquense</i> strain Marseille-Q2069'	<i>Chryseobacterium mulctrae</i> CA10T	23,9	[21.6 - 26.3]	3,13
<i>Chryseobacterium manosquense</i> strain Marseille-Q2069'	<i>Chryseobacterium hominis</i> DSM22165	23,8	[21.5 - 26.2]	1,32
<i>Chryseobacterium manosquense</i> strain Marseille-Q2069'	<i>Chryseobacterium nakagawai</i> NCTC 13529	23,7	[21.4 - 26.2]	1,53
<i>Chryseobacterium manosquense</i> strain Marseille-Q2069'	<i>Chryseobacterium lactis</i> NCTC 11390	23,5	[21.2 - 26.0]	0,83

289

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

	<i>C. manosquense</i>	<i>C. anthropi</i>	<i>C.hainfense</i>	<i>C.treverense</i>	<i>C. montanum</i>	<i>C. pallidum</i>
Properties	Marseille-Q2069	NF 1366	H38	IMMIB L-1519	WG4	26-3St2b
Cell diameter (µm)	1.3 x 0.5 µm	2–4 µm x 0.5–1 µm	0.6–0.9 µm	1.4–2.760.5–0.6 µm	0.7–1.2 µm x 0.4–0.6 µm	2.0 µm x 0.7 µm
Oxygen requirement	+	Facultative	Facultative	+	+	Facultative
Gram Strain	-	-	-	-	-	-
Motility	-	-	-	-	-	-
Endospore formation	-	na	na	-	-	-
Optimum temperature for growth (°C)	na	30°C	32°C	20°C	37°C	37°C
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 mol%	39 mol%	37.8 mol%	na	37.7 mol%	37.8 mol%
Habitat	Human healthy skin	Human Clinical Specimens	Raw milk	Human Clinical Source	Mountain Soil	Beer-bottling plants

291 **Table 3:** Detailed functional classes of predicted genes according to the clusters of
 292 orthologous groups of proteins of *Chryseobacterium manosquense* sp. nov. other closely
 293 related bacterial taxa

<i>Description</i>	<i>CO</i> <i>G</i>	<i>C.manosquen</i> <i>se</i>	<i>C.bov</i> <i>is</i>	<i>C.</i> <i>haifense</i>	<i>C.</i> <i>hispanicum</i>	<i>C.</i> <i>senegalense</i>
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
Coenzyme 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
Posttranslational 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
Defense 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

294

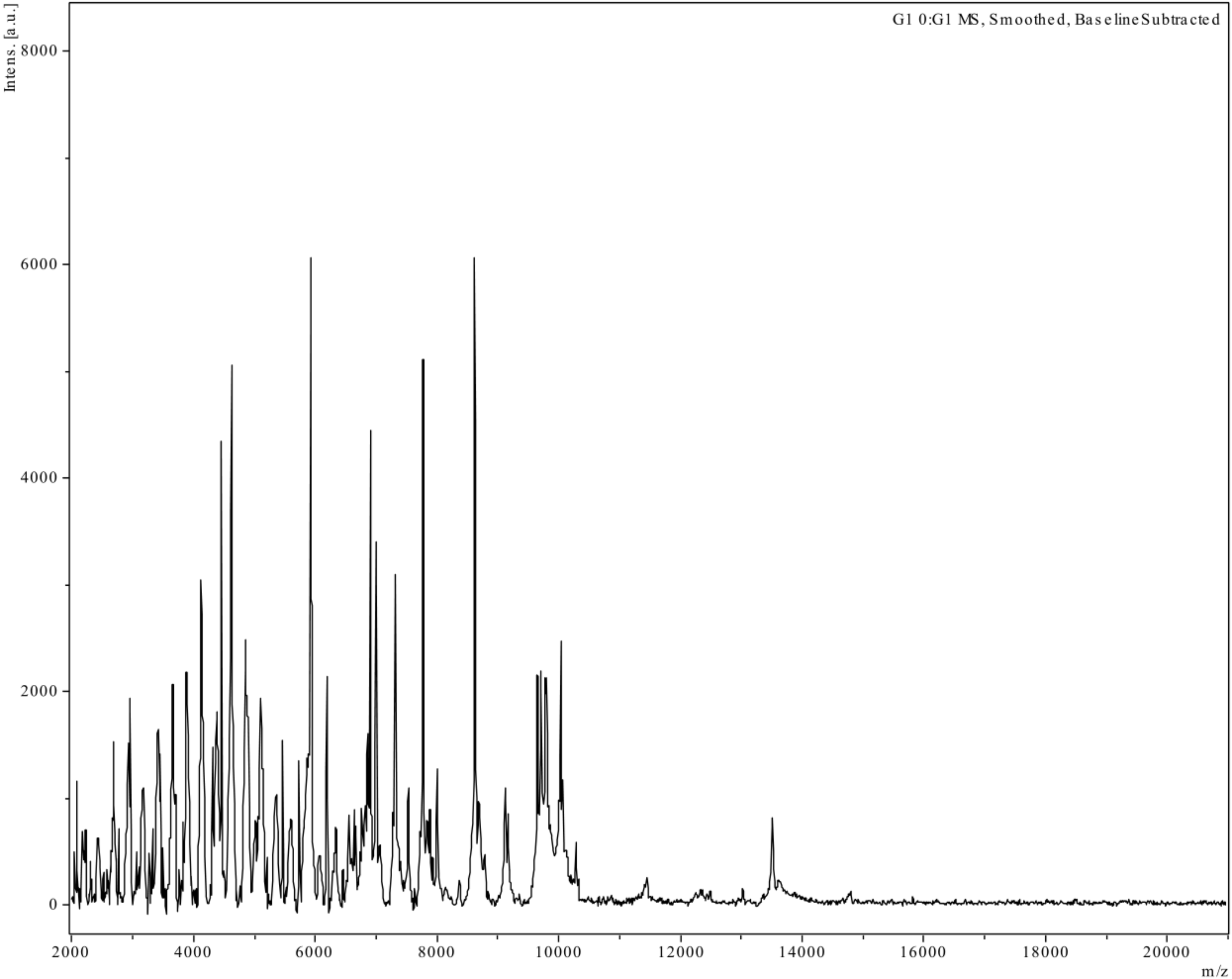
295

296 **Table.4.** Description of *C. manosquense* sp. nov. strain Marseille-Q2069^T

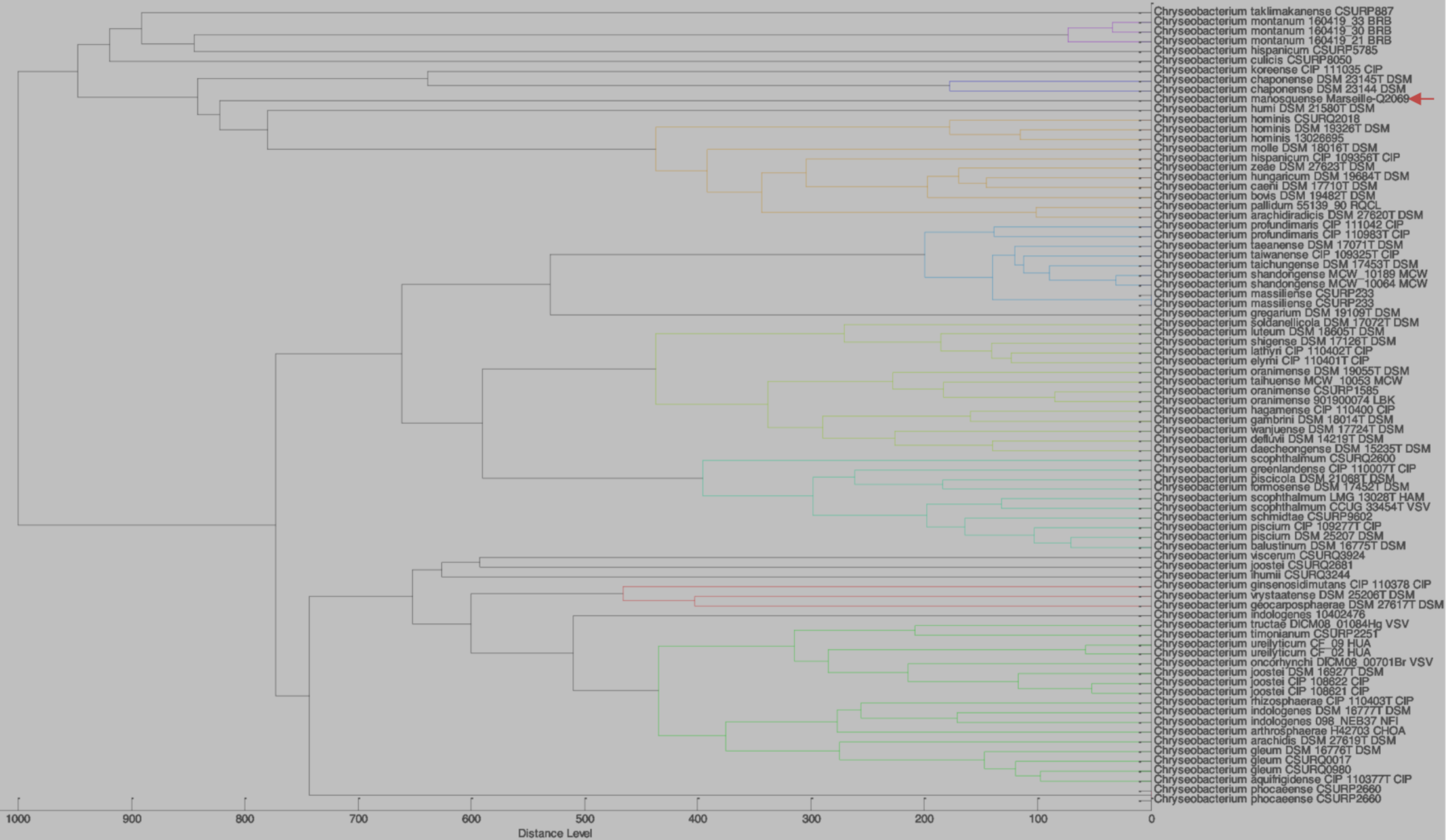
297

Species name	<i>manosquense</i>
Genus name	<i>Chryseobacterium</i>
Specific epithet	<i>Chryseobacterium</i>
Species Status	sp.nov
Species etymology	Gr. adj. <i>chruseos</i> , golden; L. neut. n. bacterium, a small rod; N.L. neut. n. <i>Chryseobacterium</i> , a yellow rod. <i>Manosquense</i> , translitt. L. adj., 'of <i>Manosque</i> ,' referring 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	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 conditions used for standard cultivation	R2A (Reasoner's 2A agar) 31°C in aerobiosis
Gram stain	Negative
Cell shape	Rods
Cell size	1.3 x 0.5 um
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 aerobe
Oxidase	+
Catalase	+

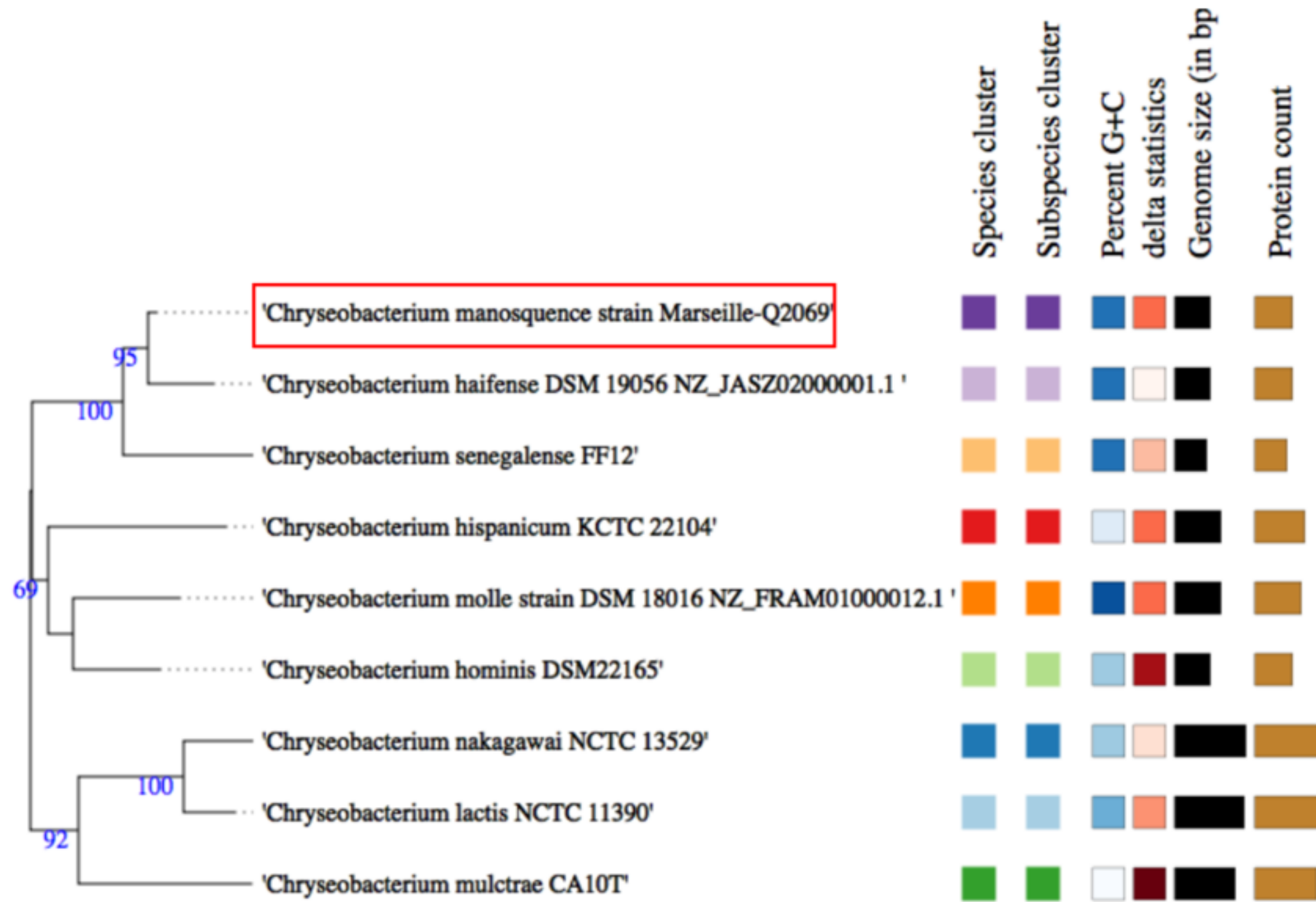
298



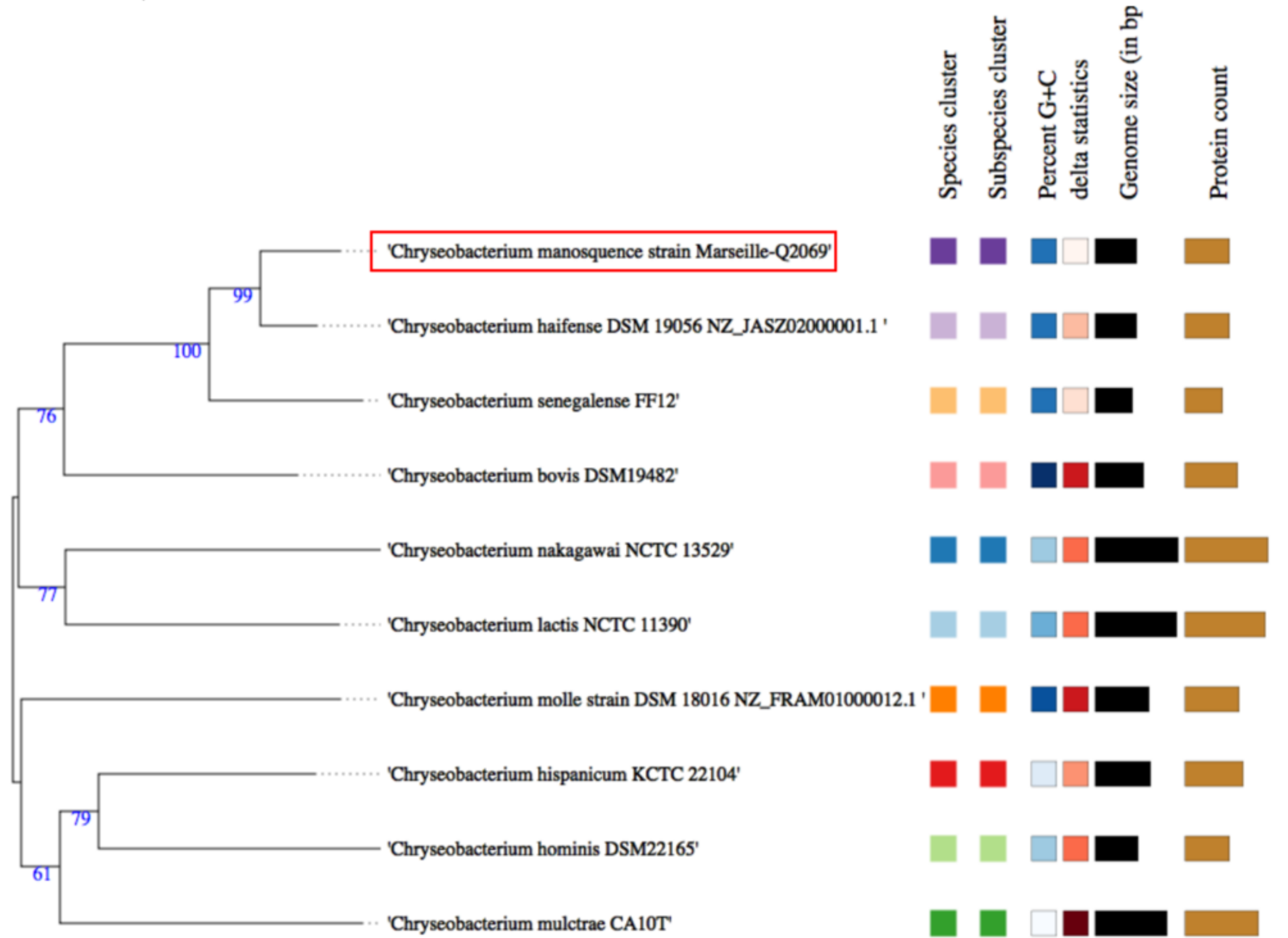
MSP Dendrogram



A)



B)



TM4000 15kV 6.8mm x10.0k BSE M

5.00μm

