Draft genome and description of *Mixta mediterraneensis* strain Marseille-Q2057^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 30-year-old healthy woman using the culturomic method, we isolated the new bacterial strain Marseille-Q2057^T (= CSUR-Q2057). 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 *Erwiniaceae*, phylum *Proteobacteria*. We describe here its main phenotypic characteristics, genome sequence and annotation of *Mixta mediterraneensis* strain Marseille-Q2057^T, a new member of the *Mixta* genus, that we propose as type strain.

Keywords: Bacteria, culturomics, genome, *Mixta mediterraneensis*, sp. nov., species, taxonogenomics
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Introduction

The genus *Mixta* was created in 2018 to resolve certain approximations in the taxonomy of the *Erwiniaceae* family, in the light of recent advances in combined genomic and phylogeny approaches [1]. *Mixta mediterraneensis* strain Marseille-Q2057^T was isolated using the culturomics approach, based on the use of a large panel of culture conditions to describe the microbial composition of a sample by high-throughput culture [2–4] 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 species [2,5].

Materials and methods

Strain isolation and phenotypic tests

Mixta mediterraneensis strain Marseille-Q2057^T was initially isolated by direct seeding of 50 µL of sample on an *Acinetobacter*-specific medium [6] incubated in aerobiosis at 31°C, MALDI-TOF MS protein analysis was carried out using a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [7]. Spectra from strain Marseille-Q2057T 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 Sud-Est IV under number ID-RCB: 2019-A01508-49. Different growth temperatures (30°C, 37°C, 45°C and 56°C), atmospheric conditions—anaerobic, aerobic and microaerophilic (CampyGEN, Oxoid, Basingstoke, UK) and pH (5, 6.5, 7.5, 8.5) were tested. API ZYM, API 20E and API 50 CH strips (BioMérieux, Marcy L'Étoile, 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 to evaluate bacterial structure on a TM4000 microscope approximately 60 cm in height and 33 cm in width. The standard disc method was applied for antimicrobial susceptibility testing according to the French Microbiology Society. Motility test was performed using the semi-solid TCC media as described by Tittsler and Sandholzer [8].

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Genome sequencing

Genomic DNA (gDNA) of *M. mediterraneensis* strain Marseille-Q2057^T was extracted in two steps: a mechanical treatment was first performed with glass beads acid-washed (G4649-500g; Sigma, St Louis, MO, USA) using a FastPrep-24™ 5G Grinder (mpBio, Irvine, 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. The gDNA of *M. mediterraneensis* strain Marseille-Q2057^T was quantified by a Qubit assay with the 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 respectively with 21 other genomic projects prepared with the Nextera XT DNA sample prep kit (Illumina). To prepare the paired end library, dilution was performed to require 1ng of each genome as input to prepare the paired end library. 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 then normalized on specific beads according to the Nextera XT protocol (Illumina). 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 4.5 Gb was obtained from a 462 K/mm^2 cluster density with a cluster passing quality control filters of 93.9%. Within this run, the index representation for *M. mediterraneensis* strain Marseille-Q2057^T was determined to index 3.06%. The 9 045 583 paired end reads were filtered according to the read qualities. To improve the quality of the assembly, an Oxford Nanopore approach was performed on 1D gDNA sequencing for the MinIon device using an SQK-LSK109 kit. The library was constructed from 1-µg gDNA without fragmentation and end repair. Adapters were ligated to both ends of gDNA. After purification on AMPure XP beads (Beckman Coulter), the library was quantified by a Qubit assay with the high sensitivity kit (Life Technologies, Carlsbad, CA, USA). A total of 1376 active pores were detected for the sequencing and the workflow WIMP was chosen for bioinformatic analysis in live. After 2 hours as run time and end life of the flowcell, 325,41K reads as raw data were generated.

Genome annotation and genome comparison

Genome annotation was obtain through the NCBI Prokaryotic Genome Annotation Pipeline [7]. The genome sequence data were uploaded to the Type (Strain) Genome Server (TYGS), a free bioinformatics platform available under https://tygs.dsmz.de, for a whole genome-based taxonomic analysis [9]. 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 via the MASH algorithm, a fast approximation of intergenomic relatedness [10], and, the ten type strains with the smallest MASH distances 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 [11] and each sequence was subsequently BLASTed [12] 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 [13]. These distances were finally used to determine the ten closest type strain genomes for each of the user genomes. All pairwise comparisons among the set of genomes were conducted using GBDP and accurate intergenomic distances 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-Q2057 with closely related species was estimated using OrthoANI software with default parameters [14], the nine closest species were determined on a DNA–DNA hybridization basis. Antibiotic-resistance genes and presence of pathogenesis-related proteins was investigated using the ABRicate tools v1.0.1 against ARG-ANNOT [15], EcOH [16], NCBI Bacterial Antimicrobial Resistance reference Gene Database [17], PlasmidFinder [18], Resfinder [19], CARD [20] and VFDB [21] using the Online Galaxy platform [22].

Results

Strain identification and classification

Mixta mediterraneensis strain Marseille-Q2057^T was isolated from the hand skin swab of a 30 -year-old healthy woman. *Mixta mediterraneensis* strain Marseille-Q2057^T was not 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/) (Fig. 1); it analysed within the closest members of *Erwiniaceae* on the IHU databases available spectra

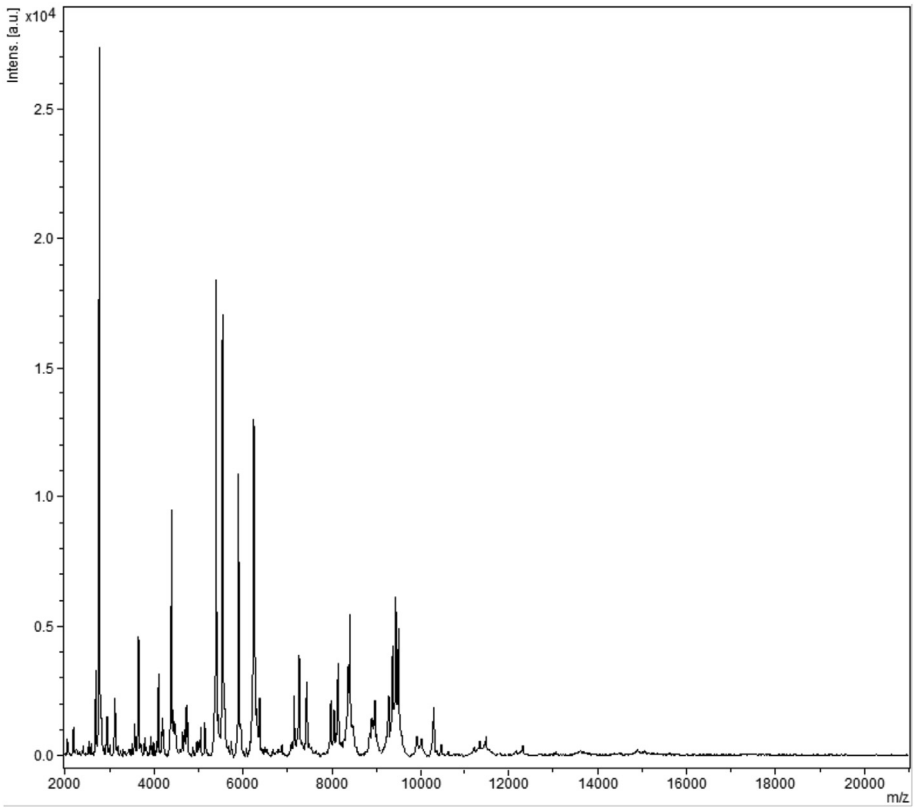

FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies of strain Marseille-Q2057^T were compared and a reference spectrum was generated.

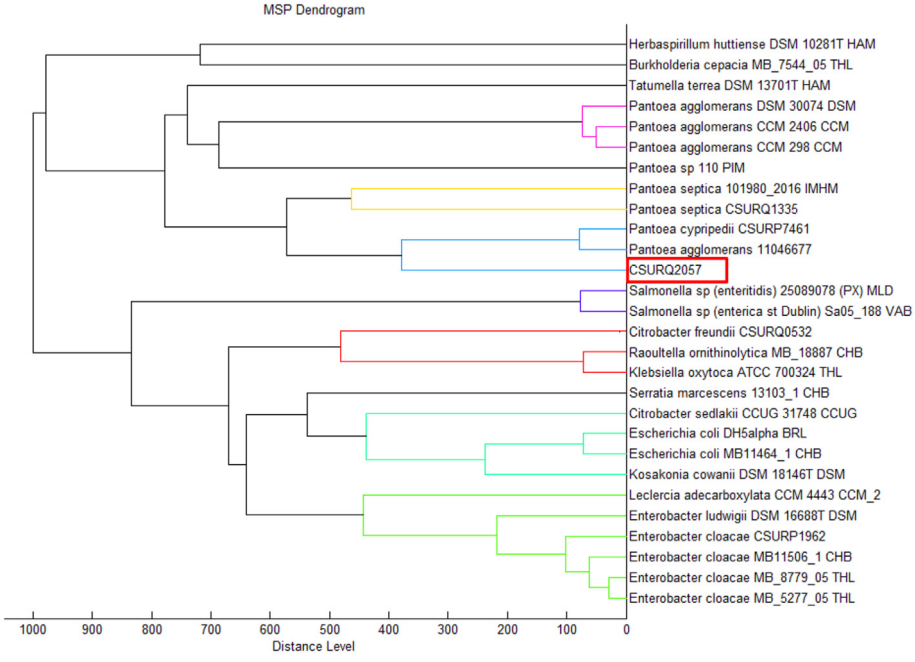

FIG. 2. MALDI-TOF MS dendrogram highlighting the position of *Mixta mediterraneensis* sp. nov. within *Erwiniaceae* family most closely related species.

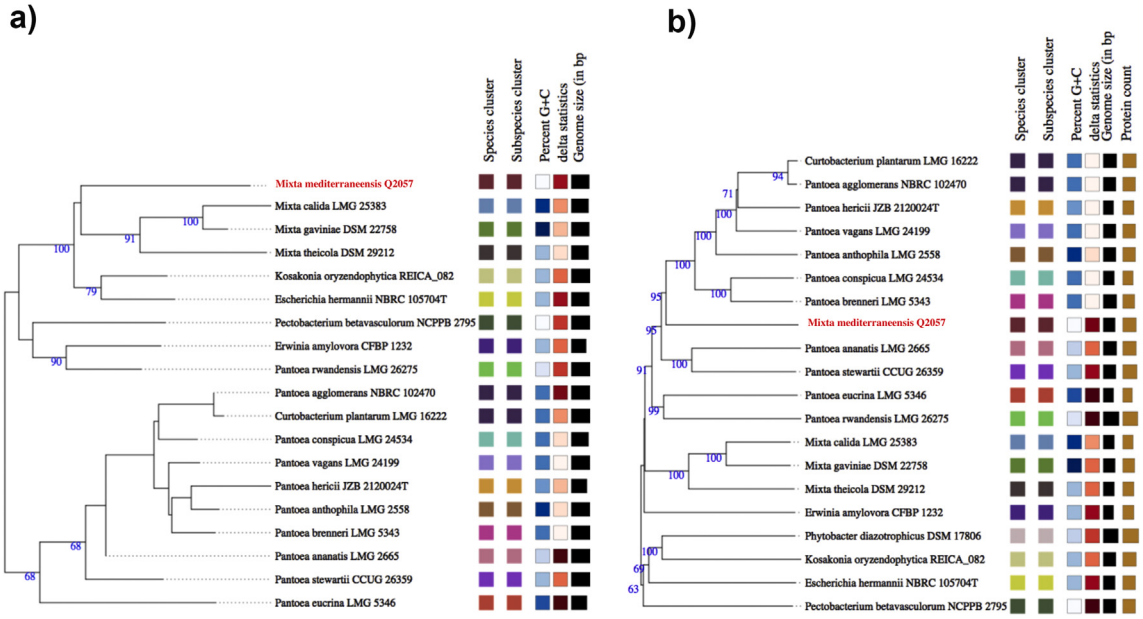

FIG. 3. (a) 16s rRNA-based phylogenetic tree, and (b) whole-genome-based phylogenetic tree highlighting the position of *Mixta mediterraneensis* sp. nov., strain Marseille-Q2057^T relative to other closely related bacterial taxa. Trees were generated with FastME 2.1.6.1 [24] from Genome BLAST Distance 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 [25].

and did not belong to any known cluster (Fig. 2). Moreover, strain Marseille-Q2057^T exhibited 97.66% 16S rRNA sequence similarity with *Mixta gaviniae* strain DSM 22758 (extracted from the genome accessible CP026377.1), the phylogenetically closest bacterium with standing in nomenclature (Fig. 3a) Furthermore, digital DNA–DNA hybridization revealed a maximum identity similarity of only 23.6% (Fig. 3b and Table 1) and an OrthoANI parameter provided a value of 80.76% (Fig. 4) between the novel organism and *Pantoea conspicua* LMG 24534 (GCA_002095315). Taken altogether these results confirm the status of this strain as a new member of the *Mixta* genus for which the name *Mixta mediterraneensis* strain Marseille-Q2057^T is proposed.

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

Subject strain	dDDH (%) with *Mixta mediterraneensis*	95% CI	G+C content difference (in %)
Pantoea conspicua LMG 24534	23.6	21.3–26.0	3.8
Pantoea brenneri LMG 5343	23.6	21.3–26.0	3.99
Pantoea vagans LMG 24199	23.2	20.9–25.6	3.58
[Curtobacterium] plantarum LMG 16222	23	20.7–25.4	3.3
Pantoea agglomerans NBRC 102470	23	20.7–25.4	3.36
Pantoea eucalypti LMG 24197	22.8	20.5–25.2	2.51
Pantoea stewartii CCUG 26359	22.7	20.4–25.2	1.83
Pantoea anthophila LMG 2558	22.6	20.4–25.1	5.01
Pantoea ananatis LMG 2665	21.9	19.6–24.3	1.64
Pectobacterium betavasculorum NCPPB 2795	21.1	18.9–23.5	0.6
Pantoea rwandensis LMG 26275	20.8	18.6–23.3	0.85
Mixta theicola DSM 29212	20.7	18.5–23.1	2.23
Pantoea eucrina LMG 5346	20.7	18.4–23.1	4.5
Mixta gaviniae DSM 22758	20.6	18.4–23.1	6.28
Mixta calida LMG 25383	20.5	18.3–22.9	5.22
Phytobacter diazotrophicus DSM 17806	20.5	18.3–23.0	1.29
Erwinia amylovora CFBP 1232	20.1	17.9–22.5	1.81
Escherichia hermannii NBRC 105704T	20.1	17.9–22.5	2.32
Kosakonia oryzendophytica REICA 082	19.9	17.7–22.3	1.97

Abbreviation: dDDH, digital DNA–DNA hybridization.

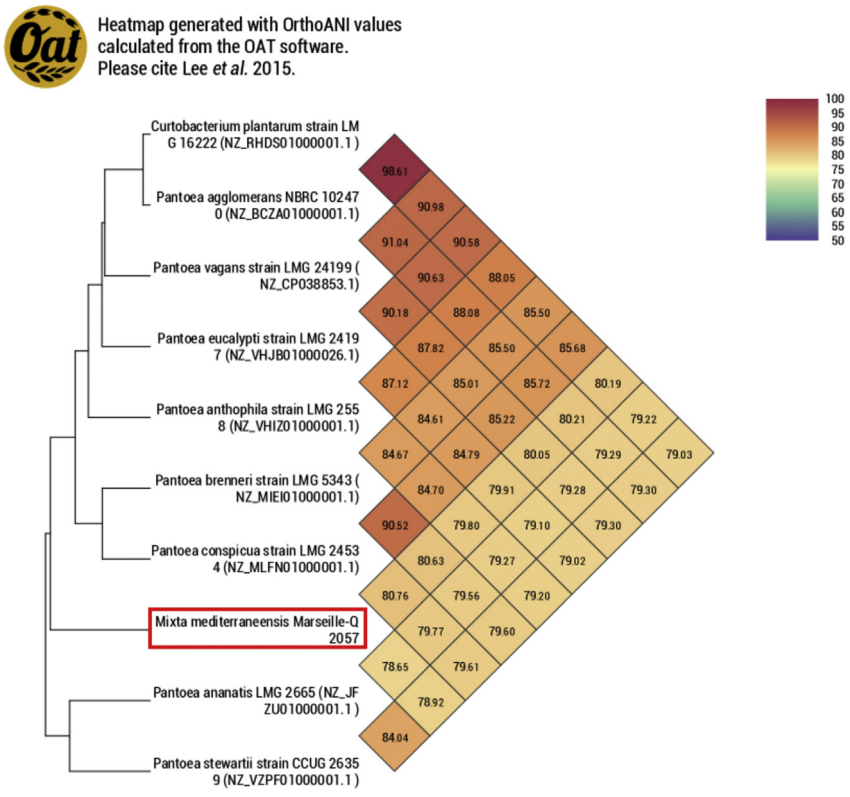

FIG. 4. Heatmap generated with ORTHOANI values calculated using the OAT software between Mixta mediterraneensis sp. nov., strain Marseille-Q2057T and other closely related species with standing in nomenclature.

TABLE 2. Differential characteristics of *Mixta mediterraneensis* strain Marseille-Q2057 and its most closely related species with standing in nomenclature

Properties	Mixta mediterraneensis Marseille-Q2057	Pantoea conspicua LMG 24534	Pantoea brenneri LMG 5343	Pantoea vagans LMG 24199	[Curtobacterium] plantarum LMG 16222	Pantoea agglomerans NBRC 102470
Cell size	0.8 × 3.8 µm	0.9 × 1.5–3.0 µm	0.9 × 1.5–3.0 µm	0.9 × 1.5–3.0 µm	0.3–0.5 × 0.6–3.0 µm	NA
Oxygen requirement	facultative	facultative	facultative	facultative	+	facultative
Gram stain	—	—	—	—	+	—
Motility	—	+	+	+	+	+
Endospore formation	NA	—	—	—	—	—
Optimum temperature for growth (°C)	31°C	28°C–30°C	28°C–30°C	NA	28°C–30°C	30°C
Production of:						
Alkaline phosphatase	+	NA	NA	NA	NA	NA
Catalase	+	NA	NA	NA	NA	NA
Oxidase	—	—	—	—	NA	—
α-Glucosidase	—	NA	NA	NA	NA	NA
β-Galactosidase	+	NA	NA	+	NA	+
Acid from:						
N-Acetylglucosamine	+	+	+	+	+	+
L-arabinose	—	+	+	+	+	+
D-ribose	—	+	+	+	+	+
D-mannose	+	+	+	+	+	+
D-mannitol	—	+	+	+	+	+
D-glucose	+	+	+	+	+	NA
D-fructose	+	+	+	+	+	+
D-maltose	—	+	+	+	+	+
D-lactose	—	+	+	+/–	—	—
G+C content (mol%)	51.76	55.7	55.4	55.4	55.1	55.1
Habitat	Healthy human skin	Human blood sample	Human blood sample	Plants, humans, food products	Leaves of various plants	Plant surfaces, seeds, water, humans (wounds, blood, urine, internal organs) and animals

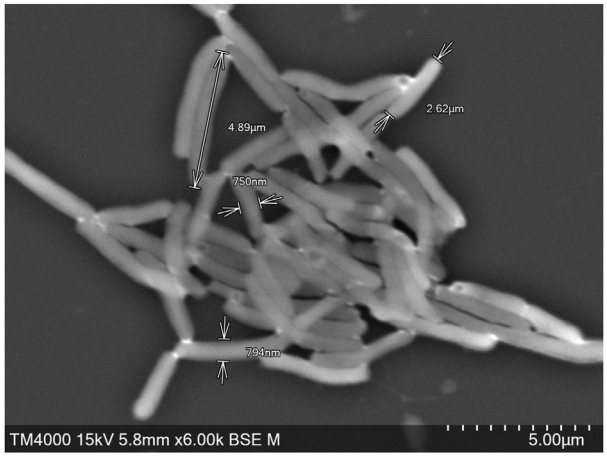

FIG. 5. Scanning electron microscopy of Mixta mediterraneensis sp. nov., strain Marseille-Q2057T using a Tabletop microscope TM 4000 plus (Hitachi, Tokyo, Japan). The scale bar represents 5 µm.

Phenotypic characteristics

Growth of *M. mediterraneensis* strain Marseille-Q2057^T was initially isolated by direct seeding of 50 µL of sample on *Acinetobacter*-specific medium [6] incubated in aerobiosis at 31°C. Colonies from strain Marseille-Q2057^T showed a beige pigmentation and no hemolysis. Bacterial cells were Gram-negative, motile bacilli with a length of about 3.8 µm and a width of about 0.8 µm determined by electronic scanning microscopy (Fig. 5). Strain Marseille-Q2057^T is aerobic, anaerobic and microaerophilic. Optimum pH of this bacterium is comprised between pH 5 and pH 7.5. The sporulation test (20 minutes at 80°C) was negative. Using API strips, positive reactions were shown for alkaline phosphatase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, sodium pyruvate, carbon substrate, D-glucose, D-fructose, D-mannose, *N*-acetyl glucosamine, esculin, D-trehalose. All other reactions tested were negative. In addition, this bacterium was catalase positive and oxidase negative. These results are summarized in Table 2).

Genome properties

The genome of strain Marseille-Q2057 was 4 532 310 bp long with a 51.76% G+C content. The genome assembly of this strain was achieved on 34 contigs. Of the 4537 predicted genes, 4067 were protein-coding genes and 108 were RNAs (7 16S rRNA, 8 additional 5S rRNAs, 7 additional 23S rRNAs, 77 tRNAs and 9 ncRNAs) (Fig. 6). The distribution of genes into clusters of orthologous groups (COGs) functional categories for strain Marseille-Q2057^T and other closely related bacterial taxa is detailed in Table 3. Analysis of the COGs categories shows that the mobilome, amino acid transport and metabolism elements of the strain Marseille-Q2057 appear to be the more numerous putative functions (by COGs) (412 in category X, 343 in category E, respectively). Through this analysis, we can see that the repartition of all COG categories is similar across

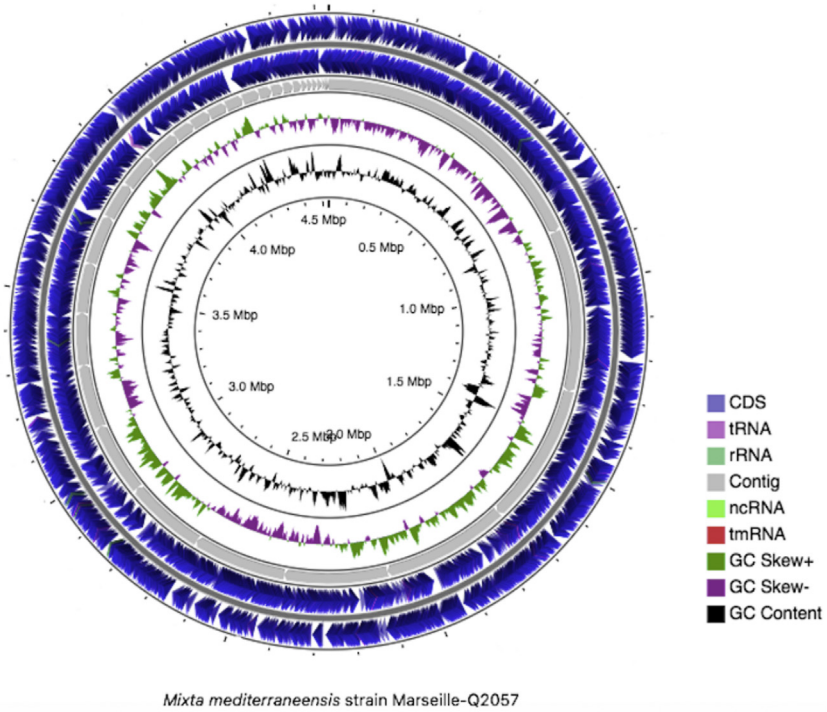

FIG. 6. Graphical circular map of the genome from *Mixta mediterraneensis* strain Marseille-Q2057^T obtained by CGView Server online tool [26].

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

	Pantoea agglomerans NBRC 102470 (NZ_BCZA01000001.1)	*Pantoea vagans* strain LMG 24199 (NZ_CP038853.1)	*Pantoea brenneri* strain LMG 5343 (NZ_MIEI01000001.1)	*Pantoea conspicua* strain LMG 24534 (NZ_MLFN01000001.1)	*[Curtobacterium] plantarum* strain LMG 16222 (NZ_RHDS01000001.1)	*Pantoea stewartii* strain CCUG 26359 (NZ_VZPF01000001.1)	*Mixta mediterraneensis* strain Q2057
Information storage and processing							
[J] Translation, ribosomal structure and biogenesis	265	259	284	264	268	264	250
[A] RNA processing and modification	1	1	1	1	1	1	1
[K] Transcription	378	376	402	330	403	351	277
[L] Replication, recombination and repair	138	134	177	155	154	201	224
[B] Chromatin structure and dynamics	0	0	0	0	0	0	0
Cellular processes and signalling							
[D] Cell cycle control, cell division, chromosome partitioning	48	48	55	53	50	51	53
[Y] Nuclear structure	0	0	0	0	0	0	0
[V] Defence mechanisms	104	105	114	89	106	81	85
[T] Signal transduction mechanisms	241	241	256	226	251	248	181
[M] Cell wall/membrane/envelope biogenesis	272	287	302	273	286	270	280
[N] Cell motility	106	106	103	96	108	136	101
[Z] Cytoskeleton	0	0	0	0	0	0	0
[W] Extracellular structures	20	19	21	19	21	19	27
[U] Intracellular trafficking, secretion and vesicular transport	71	86	80	55	87	85	72
[O] Post-translational modification, protein turnover, chaperones	152	151	171	144	161	150	145
[X] Mobilome: prophages, transposons	37	40	64	37	64	198	412
Metabolism							
[C] Energy production and conversion	194	202	239	200	202	184	180
[G] Carbohydrate transport and metabolism	411	405	478	420	423	402	342
[E] Amino acid transport and metabolism	411	442	453	423	426	392	343
[F] Nucleotide transport and metabolism	110	108	113	107	108	106	97
[H] Coenzyme transport and metabolism	203	213	224	195	209	188	182
[I] Lipid transport and metabolism	136	144	167	135	140	128	136
[P] Inorganic ion transport and metabolism	266	282	307	273	277	243	214
[Q] Secondary metabolites biosynthesis, transport and catabolism	85	97	102	76	93	64	64
Poorly characterized							
[R] General function prediction only	383	394	432	359	411	334	266
[S] Function unknown	228	233	238	219	236	214	219
Hypothetical protein	614	632	794	653	814	1298	980

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

these species (Fig. 7 and Table 3). The *in silico* resistome of the strain Marseille-Q2057^T and the search for virulence factors [21] of this strain showed on the 7 contig an 85.77% identity gene with *Crp* gene that could be implied in fluoroquinolone, macrolide and penam resistance (using CARD). Two IncFII plasmids were detected on the 18 and 19 contigs.

Discussion and conclusion

In the past 8 years, a culturomic approach has led to the discovery of more than 500 bacterial species [2]. Using the taxonogenomics concept, i.e. the combination of the genomic and

TABLE 4. Description of *Mixta mediterraneensis* sp. nov. strain Marseille-Q2057^T

Species name	*mediterraneensis*
Genus name	*Mixta*
Specific epithet	*Mixta*
Species status	sp.nov
Species etymology	*Mix'ta N.L. fem. n. Mixta*, the mixed one, referring to the mixed lifestyles of species in the genus. *Me.di.ter.ra.ne.en'sis*, L. masc. adj., *mediterraneensis*, 'of *Mediterraneum*,' the Latin name of the Mediterranean Sea by which Marseille is located and the bacteria isolated.
Authors	Manon Boxberger, Angéline Antezack, Sibylle Magnien, Nadim Cassir, Bernard La Scola
Designation of the type strain	Marseille-Q2057
Strain collection number	CSUR-Q2057
16S rRNA gene accession number	MW177953
Genome accession number	JACFXY000000000.1
Genome status	Draft
Genome size	4 532 310 -bp
GC%	51.76
Country of origin	Marseille, France
Date of isolation	2019
Source of isolation	Human healthy skin
Growth medium, incubation	*Acinetobacter*-specific medium [6]
Conditions used for standard cultivation	31°C in aerobiosis
Gram stain	Negative
Cell shape	Rods
Cell size	3.8 µm and a width of about 0.8 µm
Motility	Motile
Sporulation	Non-sporulating
Colony morphology	Circular
Temperature range	21°C–56°C
Temperature optimum	31°C
Relationship to O_2	Facultative aerobe
O_2 for strain testing	Strictly aerobe
Oxidase	—
Catalase	+

phenotypic properties of a putative new taxon [23], we have characterized a new bacterial species representing a new species within the family *Erwiniaceae* found on human hand skin. It was named as *M. mediterraneensis* strain Marseille-Q2057^T. Members of *Erwiniaceae* are commonly found associated with plants, so it is reasonable to think that our species, found on skin, is part of the transient cutaneous microbiota (see Table 4).

Mix'ta N.L. fem. n. Mixta, the mixed one, referring to the mixed lifestyles of species in the genus. *Me.di.ter.ra.ne.en'sis*, L. masc. adj., *mediterraneensis*, 'of *Mediterraneum*,' the Latin name of the Mediterranean Sea by which Marseille is located and the bacteria isolated.

Deposit in culture collections and sequences database

Mixta mediterraneensis strain Marseille-Q2057^T, was deposited in CSUR collections under accession CSUR-Q2057. The 16S rRNA and genome sequences are available under accession numbers MW177953 and JACFXY000000000.1, respectively.

Conflict of interest

The authors have no conflicts of interest to declare.

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