Draft genome and description of *Mixta mediterraneensis* strain Marseille-Q2057T 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-Q2057T (= 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 *Erwiniaeeae*, phylum *Proteobacteria*. We describe here its main phenotypic characteristics, genome sequence and annotation of *Mixta mediterraneensis* strain Marseille-Q2057T, a new member of the *Mixta* genus, that we propose as type strain.

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Introduction

The genus *Mixta* was created in 2018 to resolve certain approximations in the taxonomy of the *Erwiniaeeae* family, in the light of recent advances in combined genomic and phylogeny approaches [1]. *Mixta mediterraneensis* strain Marseille-Q2057T 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-Q2057T 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 BIO Typer 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].
Genome sequencing

Genomic DNA (gDNA) of *M. mediterraneensis* strain Marseille-Q2057\(^7\) 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\(^7\) 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 1 ng 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\(^7\) 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 Minlon 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 obtained 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 RNAHER [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\(^7\) was isolated from the hand skin swab of a 30 -year-old healthy woman. *Mixta mediterraneensis* strain Marseille-Q2057\(^7\) was not identified by our systematic MALDI-TOF MS screening, suggesting that the corresponding species was not in the database (https://www.mediterrane-infection.com/acces-ressources/base-de-donnees/urms-data-base/) (Fig. 1); it analysed within the closest members of *Erwinia*aece on the IHU databases available spectra

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FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies of strain Marseille-Q2057T 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.
and did not belong to any known cluster (Fig. 2). Moreover, strain Marseille-Q2057T 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-Q2057T 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 bremeri* 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 mucidae* LMG 24197     | 22.8                                   | 20.5 – 25.2   | 2.51                          |
| *Pantoea stewartii* CCUG 26359  | 22.7                                   | 20.4 – 25.2   | 1.83                          |
| *Pantoea ananatis* LMG 2665     | 21.9                                   | 19.6 – 24.3   | 1.64                          |
| *Pectobacterium* facilitale LMG 16900 | 21.1       | 18.9 – 23.5   | 0.6                           |
| *Pantoea ananatis* LMG 2558     | 22.6                                   | 20.4 – 25.1   | 5.01                          |
| *Pantoea ravenhali* LMG 2665    | 21.9                                   | 19.6 – 24.3   | 1.64                          |
| *Mixta theicola* DSM 29212      | 20.7                                   | 18.5 – 23.1   | 2.23                          |
| *Pantoea stewartii* CCUG 26359  | 20.7                                   | 18.4 – 23.1   | 4.5                           |
| *Mixta mediterraneensis* DSM 22758 | 20.6       | 18.3 – 22.9   | 5.22                          |
| *Phyllobacter diazotrophicus* DSM 17806 | 20.5 | 18.3 – 22.9   | 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 | Pantoea conspicua | Pantoea brenneri | Pantoea vagans | [Curtobacterium] plantarum | Pantoea agglomerans |
|---------------------------------|------------------------|-------------------|-------------------|----------------|----------------------------|-------------------|
|                                 | Marseille-Q2057 | LMG 24534 | LMG 5343 | LMG 24199 | LMG 16222 | NBRC 102470 |
| Cell size (μm)                  | 0.8 × 3.8            | 0.9 × 1.5–3.0    | 0.9 × 1.5–3.0    | 0.9 × 1.5–3.0 | 0.3–0.5 × 0.6–3.0 | NA                |
| Oxygen requirement              | facultative          | facultative      | facultative      | facultative   | + facultative             | + facultative     |
| Gram stain                      | —                      | —                | +                 | —              | —                         | —                |
| Motility                        | —                      | —                | +                 | +              | +                         | +                |
| Endospore formation             | NA                    | NA               | NA               | NA             | NA                        | 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                         | —                      | —                | —                | —              | —                         | —                |
| α-Glucosidase                   | —                      | NA               | NA               | NA             | NA                        | NA               |
| β-Galactosidase                 | +                      | NA               | NA               | NA             | NA                        | NA               |
| Acid from:                      |                       |                  |                   |               |                           |                   |
| N-Acetylglucosamine             | +                      | +                | +                | +              | +                         | +                |
| L-arabinose                     | —                      | —                | —                | +              | +                         | +                |
| d-Ribose                        | —                      | —                | —                | +              | +                         | +                |
| d-Mannose                       | +                      | +                | +                | +              | +                         | +                |
| d-Manitol                       | —                      | —                | —                | +              | +                         | +                |
| d-Glucose                       | +                      | +                | +                | +              | +                         | +                |
| 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 |

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Phenotypic characteristics

Growth of *M. mediterraneensis* strain Marseille-Q2057 was initially isolated by direct seeding of 50 μL of sample on *Aci-netobacter*-specific medium [6] incubated in aerobiosis at 31°C. Colonies from strain Marseille-Q2057 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 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, α-glucose, α-fructose, α-mannose, N-acetyl glucosamine, esculin, α-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 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...
| 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** | **Pantoea vagans** | **Pantoea brenneri** | **Pantoea conspicua** | **[Curtobacterium] plantarum** | **Pantoea stewartii** | **Mixta mediterraneensis** |
| NBRC 102470 (NZ_BCZA01000001.1) | strain LMG 24199 (NZ_CP038853.1) | strain LMG 5343 (NZ_MIEI01000001.1) | strain LMG 26534 (NZ_MLFN01000001.1) | strain CCLUG 36359 (NZ_VZPF01000001.1) | strain Q2057 |
| Information storage and processing | | | | | | |
| [J] Translation, ribosomal structure and biogenesis | 265 | 259 | 284 | 264 | 268 | 264 | 250 |
| [A] rRNA 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 |
| Cellular processes and signaling | | | | | | |
| [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 |
| [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 |
| [J] Lipid transport and metabolism | 136 | 144 | 167 | 135 | 140 | 128 | 136 |
| [P] Inorganic ion transport and metabolism | 266 | 282 | 307 | 273 | 277 | 243 | 214 |
| Poorly characterized | 85 | 97 | 102 | 76 | 93 | 64 | 64 |
| [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 | 633 | 814 | 1298 | 980 |
these species (Fig. 7 and Table 3). The in silico resistome of the strain Marseille-Q2057T 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**

| Species name | mediterraneensis |
|--------------|------------------|
| Genus name   | Mixta            |
| Specific epithet | Mixta, the mixed one, referring to the mixed lifestyles of species in the genus. |
| Species status | sp.nov          |
| Species etymology | Mixta N.L. fem. n. Mixta, the mixed one, referring to the mixed lifestyles of species in the genus. Me.diter.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 | MWX77953 |
| Genome accession number | JACFX000000000.1 |
| 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 | Non-sporulating |
| Sporulation | Circular |
| Temperature range | 21°C–56°C |
| Temperature optimum | 31°C |
| Relationship to O2 | Facultative aerobe |
| O2 for strain testing | Strictly aerobe |
| Oxidase | 1+ |
| Catalase | 1+ |
phenotypic properties of a putative new taxon [23], we have characterized a new bacterial species representing a new species within the family Erwiniaeae found on human hand skin. It was named as *M. mediterraneensis* strain Marseille-Q2057<sup>7</sup>. Members of *Erwiniaeae* 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).

*Mixta* N.L. fem. n. *Mixta*, the mixed one, referring to the mixed lifestyles of species in the genus. *Mediter.rane.en*’s, L. masc. adj., *mediterraneensis*, ‘of Mediterranean,’ 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<sup>7</sup>, was deposited in CSUR collections under accession CSUR-Q2057. The 16S strain Marseille-Q2057<sup>T</sup>, was deposited in culture collections and sequences database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. Antimicrob Agents Chemother 2019. 63: e00483-19. https://doi.org/10.1128/AAC.00483-19.

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