Genome sequence and description of *Mannheimia massilioguelmaensis* sp. nov.

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Abstract

Strain MG13^T sp. nov. is the type strain of *Mannheimia massilioguelmaensis*, a new species within the genus *Mannheimia*. This strain was isolated from the exudate of a skin lesion of an Algerian man. *Mannheimia massilioguelmaensis* is a Gram-negative, facultative anaerobic rod, member of the family *Pasteurellaceae*. Here we describe this organism, together with the complete genome sequence and annotation. The 2 186 813 bp long genome contains 2048 protein-coding and 55 RNA genes, including eight rRNA genes.

Keywords: Genome, human infection, infectious disease, *Mannheimia massilioguelmaensis*, taxonogenomics
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Introduction

Mannheimia massilioguelmaensis sp. nov. strain MG13^T (= CSUR P1431 = DSM 29915) is the type strain of *M. massilioguelmaensis* sp. nov. This bacterium is a Gram-negative, facultatively anaerobic, nonhaemolytic, indole-negative rod-shaped bacillus. It was isolated from the exudate of a skin lesion of an Algerian patient.

We recently proposed that genomic and proteomic data, which do not suffer from the lack of reproducibility and interlaboratory comparability that the reference standard DNA-DNA hybridization (DDH) and G+C content determination does [1], be included in the official description of new bacterial species [2,3].

The genus *Mannheimia* (Angen *et al.*, 1999) formerly *Pasteurella,* was created in 1999 [4] and currently comprises six species, including *M. haemolytica, M. granulomatis, M. glucosida, M. ruminalis, M. varigena* and *M. caviae*. *Mannheimia* species are Gram-negative, non-spore-forming, nonmotile, facultative anaerobic rod-shaped bacilli. Some species of *Mannheimia* are commonly isolated in the gastrointestinal or upper respiratory tract of animals but are not associated with disease [4]. Others are pathogenic, such as *Mannheimia haemolytica*, which is one of the most important respiratory pathogens of domestic ruminants and causes serious outbreaks of acute pneumonia in neonatal, weaned and growing lambs, calves and goats [5]. Infections are rare in humans but can be fatal when they do occur [6,7].

Here we present a summary classification and a set of features for *M. massilioguelmaensis* sp. nov. strain MG13^T together with the description of the complete genome sequence and annotation. These characteristics support the circumscription of the species *M. massilioguelmaensis.*

Organism information

A pus sample was collected from a 90-year-old Algerian patient in Guelma, northeastern Algeria, with a cutaneous abscess of

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the left forearm. At the time of sample collection, he had been hospitalized for fever and multiple abscesses in his left arm. One week before hospitalization, the patient had a first abscess in his left index finger after to pare one nail; this evolved into multiple abscesses of and a lymphangitis path in the left forearm. The patient signed informed consent, and agreement of the local ethics committee of the IFR48 (Marseille, France) was obtained (agreement 07-30). The bacterium was isolated in pure culture in September 2014.

When blasted to National Center for Biotechnology Information (NCBI) database, the 16S rRNA gene sequence of *M. massilioguelmaensis* strain MG13^T (GenBank accession no. LN795822) exhibited an identity of 96.00% with *Mannheimia haemolytica*. This value was the highest similarity observed but was lower than 97.8% 16S rRNA gene sequence threshold recommended by Stackebrandt and Ebers [8] to delineate a new species without carrying out DNA-DNA hybridization.

Different growth temperatures (25, 30, 37 and 45°C) were tested. Growth occurred between 25°C and 37°C, but optimal growth was observed at 37°C, 24 hours after inoculation. Colonies were smooth, greyish and approximately 1 mm in diameter on 5% sheep's blood–enriched agar (bioMérieux). Growth of the strain was tested in anaerobic and microaerophilic atmospheres using GasPak EZ Anaerobe Pouch (Becton Dickinson) and CampyGen Compact (Oxoid) systems, respectively, and in aerobic atmosphere, with or without 5% CO_2. Growth was observed under aerobic (with and without CO_2), microaerophilic and anaerobic conditions. Gram staining showed short Gram-negative rods unable to form spores (Fig. 1). A motility test was negative. The size of cells were determined by negative staining transmission electron microscopy on a Technai G^{20} Cryo device (FEI) at an operating voltage of 200 kV. The rods had a length ranging from 1.1 to 1.9 µm (mean 1.5 µm), a width ranging from 0.4 to 0.6 µm (mean 0.5 µm) and a diameter ranging from 0.4 to 0.8 µm (mean 0.6 µm) (Fig. 2).

Differential phenotypic characteristics using API 50CH and API Zym system (bioMérieux) between *M. massilioguelmaensis* sp. nov. strain MG13^T and other *Mannheimia* species [4] are detailed in Table 1.

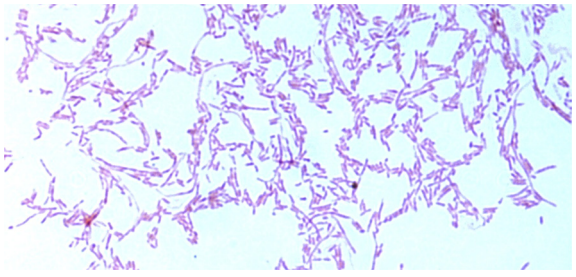

FIG. 1. Gram staining of *Mannheimia massilioguelmaensis* strain MG13^T.

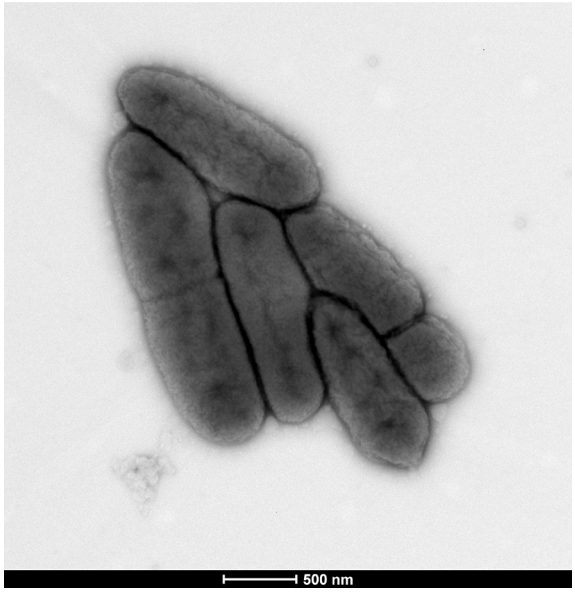

FIG. 2. Transmission electron microscopy of *Mannheimia massilioguelmaensis* strain MG13^T using Technai G^{20} Cryo device (FEI) at operating voltage of 200 kV. Scale bar = 500 nm.

Susceptibility testing was performed by the Etest strip (bioMérieux) method. Minimum inhibitory concentration was expressed in µg/mL. *M. massilioguelmaensis* was susceptible to amoxicillin (0.19), amoxicillin–clavulanate (0.125), gentamicin (0.094), amikacin (1), imipenem (0.75), trimethoprim–sulfamethoxazole (0.064), ciprofloxacin (0.012), ceftriaxone (1.5) and cholistine (0.19) but resistant to vancomycin (>256).

Extended features descriptions

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) protein analysis was performed as previously described [9] using a Microflex spectrometer (Bruker). Twelve distinct deposits were done for strain MG13^T from 12 isolated colonies. The 12 MG13^T spectra were imported into the MALDI BioTyper 2.0 software (Bruker) and analysed by standard pattern matching (with default parameter settings) against the main spectra of 4108 bacteria, including seven spectra from four *Mannheimia* species, used as reference data, in the BioTyper database. A score enabled the identification (or not) from the tested species: a score of >2 with a validated species enabled the identification at the species level; a score of >1.7 but <2 enabled the identification at the genus level; and a score of <1.7 did not enable any identification. No significant MALDI-TOF score was obtained for strain MG13^T against the Bruker database, thus suggesting that our isolate was

TABLE 1. Differential characteristics of *Mannheimia* species

Characteristic	*M. ma*	*M. ha*	*M. gl*	*M. gr*	*M. ru*	*M. va*
Cell diameter (μm)	0.6	NA	NA	NA	NA	NA
Oxygen requirement	Facultative anaerobic	Facultative anaerobic	Facultative anaerobic	Facultative anaerobic	Facultative anaerobic	Facultative anaerobic
Gram strain	−	−	−	−	−	−
Motility	−	−	−	−	−	−
Endospore formation	−	NA	NA	NA	NA	NA
Production of:						
Alkaline phosphatase	+	+	+	+	+	+
Acid phosphatase	w	NA	NA	NA	NA	NA
Catalase	−	NA	NA	NA	NA	NA
Oxidase	−	NA	NA	NA	NA	NA
Nitrate reductase	+	+	+	+	+	+
Urease	−	−	−	−	−	−
β-Galactosidase	−	v	+	v	+	v
Indole	−	NA	NA	NA	NA	NA
Esterase	w	NA	NA	NA	NA	NA
Esterase lipase	w	NA	NA	NA	NA	NA
Leucine arylamidase	w	NA	NA	NA	NA	NA
Cystine arylamidase	−	NA	NA	NA	NA	NA
Valine arylamidase	−	NA	NA	NA	NA	NA
Utilization of:						
Mannitol	−	NA	NA	NA	NA	NA
Trehalose	−	−	−	−	−	−
L-Arabinose	−	−	v	−	−	+
D-Sorbitol	−	+	+	+	v	−
D-Xylose	−	+	+	v	v	+
D-Ribose	+	NA	NA	NA	NA	NA
D-Glucose	+	NA	NA	NA	NA	NA
D-Mannose	w	−	−	−	−	−
D-Fructose	+	NA	NA	NA	NA	NA
Glycerol	+	NA	NA	NA	NA	NA
N-Acetylglucosamine	+	NA	NA	NA	NA	NA
G+C content (mol%)	36.2	43.6	41.6	39.2	NA	41.7
Habitat	Human	Bovine, ovine	Ovine	Bovine, leporine, deer	Ovine	Bovine, porcine

Mannheimia massilioguelmaensis (*M. ma*) strain MG13^T, *Mannheimia haemolytica* (*M. ha*) strain NCTC 980^T, *Mannheimia glucosida* (*M. gl*) strain P925^T, *Mannheimia granulomatis* (*M. gr*) strain ATCC 49244^T, *Mannheimia ruminalis* (*M. ru*) strain HPA92^T, *Mannheimia varigena* (*M. va*) strain 177^T.
+, positive result; −, negative result; v, variable result; w, weakly positive result; NA, data not available.

a new species. We incremented our database with the spectrum from strain MG13^T (Fig. 3).

Genome sequencing information

This strain was the 23rd genome of a *Mannheimia* species and the first genome of *Mannheimia massilioguelmaensis* sp. nov. (CDQL00000000).

After DNA extraction by the phenol–chloroform method, genomic DNA of *Mannheimia massilioguelmaensis* was sequenced on the MiSeq Technology (Illumina) with the mate-pair strategy.

For genome annotation, open reading frames (ORFs) were predicted using Prodigal [10] with default parameters, but the predicted ORFs were excluded if they spanned a sequencing gap. The predicted bacterial protein sequences were searched against the Clusters of Orthologous Groups (COGs) database and the GenBank database [11] using BLASTP. The tRNAScanSE tool [12] was used to find tRNA genes, whereas ribosomal RNAs were found by using RNAmmer [13] and BLASTn against the GenBank database. Transmembrane helices and lipoprotein signal peptides were predicted using the Phobius Web server [14]. ORFans were identified if their BLASTP *E* value was lower than 1e-03 for alignment length greater than 80 aa. If alignment lengths were smaller than 80 aa, we used an *E* value of 1e-05. Such parameter thresholds have already been used in previous works to define ORFans [2,3]. Finally, we used the online Genome-to-Genome Distance Calculator (GGDC; http://ggdc.dsmz.de) to estimate of the overall similarity among the compared genomes and to replace the wet-lab DDH by a digital DDH [15,16]. GGDC 2.0 BLAST+ was chosen as the alignment method, and the recommended formula 2 was taken into account to interpret the results.

We compared the genome sequences of *M. massilioguelmaensis* strain MG13^T (CDQL00000000) with those of *Microbacterium granulomatis* strain DSM1956 (JHZD00000000), *Mannheimia haemolytica* strain USDA-ARS-USMARC-183 (CP004752) and *Mannheimia varigena* strain USDA-ARS-USMARC-1312 (CP006944).

Digital DDH estimation of strain MG13^T against the compared genomes ranged between 13.60 to 13.90. These values were very low and below the cutoff of 70%, thus again confirming the new species status of strain MG13^T.

The genome is 2 186 813 bp long with 36.21% G+C content (Fig. 4 and Table 2). It is composed of eight scaffolds (composed

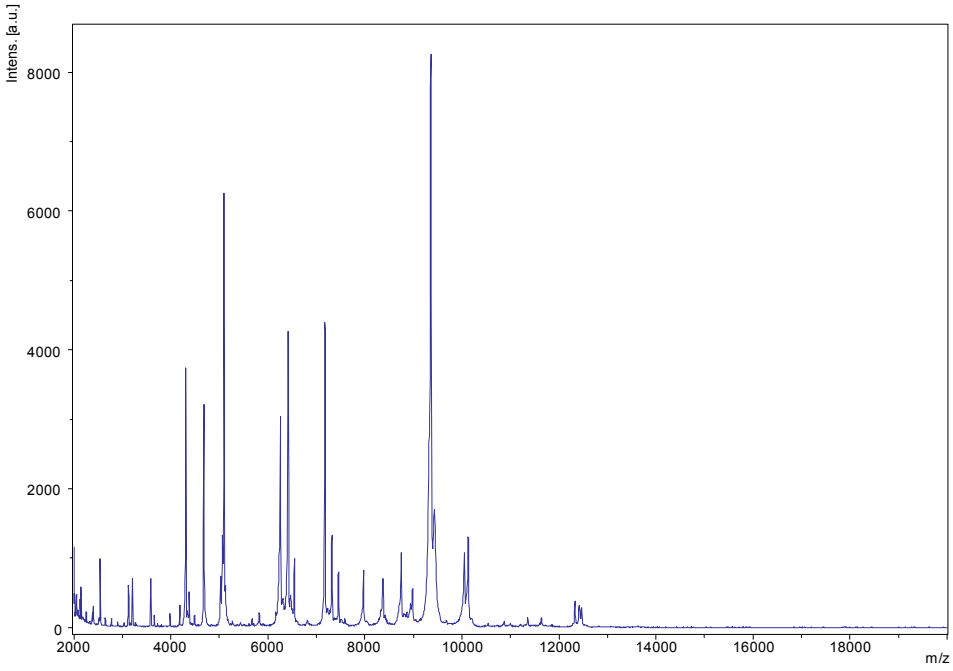

FIG. 3. Reference mass spectrum from *Mannheimia massilioguelmaensis* strain MG13^T. Spectra from 12 individual colonies were compared and reference spectrum generated.

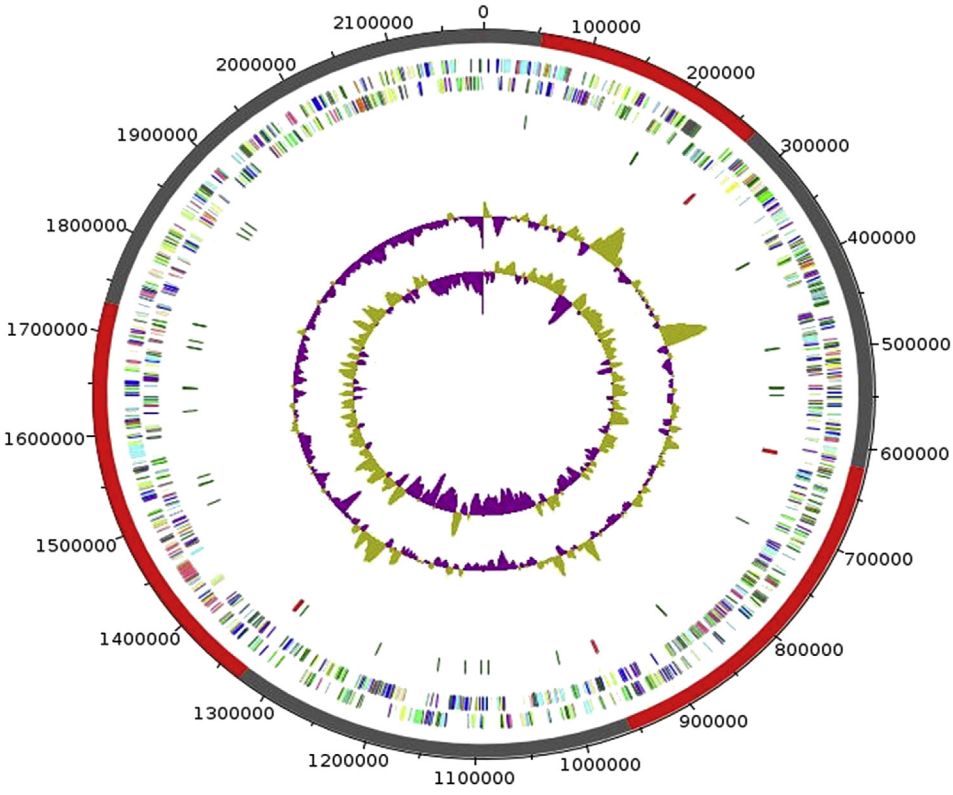

FIG. 4. Graphical circular map of chromosome. From outside to center: genes on forward strand (colored by COGs categories), genes on reverse strand (colored by COGs categories), RNA genes (tRNAs green, rRNAs red), G+C content, G+C skew.

TABLE 2. Nucleotide content and gene count levels of genome

Attribute	Value	% of total[a]
Genome size (bp)	2 186 813	100
DNA coding (bp)	1 969 580	90.1
DNA G+C (bp)	791 841	36.2
DNA scaffolds	8	—
Total genes	2103	100
Protein coding genes	2048	97.4
RNA genes	55	2.6
Pseudo genes	8	—
Genes in internal clusters	590	—
Genes with function prediction	1692	82.6
Genes assigned to COGs	1712	83.6
Genes with Pfam domains	1985	94
Genes with signal peptides	327	16
Genes with transmembrane helices	431	21
CRISPR	4	—

COGs, Clusters of Orthologous Groups database; CRISPR, clustered regularly interspaced short palindromic repeat.
[a]Total is based on either size of genome (in base pairs) or total number of protein-coding genes in annotated genome.

of eight contigs). Of the 2103 predicted genes, 2048 were protein-coding genes and 55 were RNAs (three genes are 5S rRNA, two genes are 16S rRNA, three genes are 23S rRNA and 47 genes are TRNA genes). A total of 1692 genes (82.62%) were assigned as putative function (by cogs or by NR blast). Twenty-five genes were identified as ORFans (1.22%). The remaining genes were annotated as hypothetical proteins (144 genes, 7.03%). The distribution of genes into COGs functional categories is presented in Table 3.

TABLE 3. Number of genes associated with 25 general COGs functional categories

Code	Value	% of total[a]	Description
J	160	7.81	Translation, ribosomal structure and biogenesis
A	1	0.05	RNA processing and modification
K	98	7.78	Transcription
L	116	5.66	Replication, recombination and repair
B	0	0	Chromatin structure and dynamics
D	25	1.22	Cell cycle control, mitosis and meiosis
Y	0	0	Nuclear structure
V	20	0.98	Defense mechanisms
T	46	2.25	Signal transduction mechanisms
M	128	6.25	Cell wall/membrane biogenesis
N	6	0.29	Cell motility
Z	0	0	Cytoskeleton
W	0	0	Extracellular structures
U	44	2.15	Intracellular trafficking and secretion
O	94	4.59	Posttranslational modification, protein turnover, chaperones
C	131	6.40	Energy production and conversion
G	113	5.51	Carbohydrate transport and metabolism
E	176	8.59	Amino acid transport and metabolism
F	63	3.08	Nucleotide transport and metabolism
H	93	4.54	Coenzyme transport and metabolism
I	43	2.10	Lipid transport and metabolism
P	129	6.30	Inorganic ion transport and metabolism
Q	27	1.32	Secondary metabolites biosynthesis, transport and catabolism
R	210	10.25	General function prediction only
S	187	9.13	Function unknown
—	336	16.41	Not in COGs

COGs, Clusters of Orthologous Groups database.
[a]Total is based on total number of protein-coding genes in annotated genome.

Conclusions

On the basis of phenotypic, phylogenetic and genomic analysis (taxonogenomics), we formally propose the creation of *Mannheimia massilioguelmaensis* sp. nov. that contains the strain MG13^T.

Taxonomic and nomenclatural proposals

Description of *Mannheimia massilioguelmaensis* sp. nov.

Mannheimia massilioguelmaensis (ma.sil.io.guel.ma.en'sis. L. gen. masc. n. *massilioguelmaensis,* combination of Guelma, where strain MG13^T was isolated, and Massilia, the Latin name of Marseille, where the strain was sequenced).

Colonies were moderately opaque and approximately 1 mm in diameter on 5% sheep's blood–enriched agar. Cells are Gram-negative, nonhaemolytic, short, rod-shaped facultative anaerobic with a mean length of 1.5 µm, a mean width of 0.5 µm and a mean diameter of 0.6 µm. Growth occurred between 25°C and 37°C, but optimal growth was observed at 37°C.

Alkaline phosphatase and weak acid phosphatase, esterase (C4), esterase lipase (C8) and leucine arylamidase activities were present. The nitrate reduction was also positive, but catalase, oxidase, β-galactosidase and urease activities were negative. Positive reactions were obtained for D-glucose, D-ribose, D-fructose, glycerol and N-acetylglucosamine and a weak fermentation of D-mannose. *M. massilioguelmaensis* was susceptible to amoxicillin, amoxicillin–clavulanate, gentamicin, amikacin, imipenem, trimethoprim–sulfamethoxazole, ciprofloxacin, ceftriaxone and cholistine but resistant to vancomycin.

The G+C content of the genome is 36.21%. The 16S rRNA and genome sequences are deposited in GenBank under accession numbers LN795822 and CDQL00000000, respectively. The type strain MG13^T (= CSUR P1431 = DSM 29915) was isolated from a cutaneous abscess of a patient in Guelma in northeastern Algeria.

Conflict of interest

None declared.

Acknowledgement

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