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

L. Hadjadj¹, A. A. Bentorki², C. Michelle¹, K. Amoura³, A. Djahoudi³ and J.-M. Rolain¹

1) Unité de recherche sur les maladies infectieuses et tropicales émergentes (URMITE), UMR CNRS, IHU Méditerranée Infection, Faculté de Médecine et de Pharmacie, Aix-Marseille-Université, Marseille, France, 2) Laboratoire de Microbiologie, CHU Dorban and 3) Laboratoire de Microbiologie, Faculté de Médecine, Université Badji Mokhtar, Annaba, Algeria

Abstract

Strain MG13⁷ 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.

New Microbes and New Infections © 2015 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases.

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

Original Submission: 7 August 2015; Revised Submission: 5 October 2015; Accepted: 7 October 2015

Article published online: 23 October 2015

Introduction

*Mannheimia massilioguelmaensis* sp. nov. strain MG13⁷ (= 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 MG13⁷ 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
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 \textit{M. massilioguelmaensis} strain MG13\textsuperscript{T} (GenBank accession no. LN795822) exhibited an identity of 96.00\% with \textit{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 \cite{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\textsubscript{2}. Growth was observed under aerobic (with and without CO\textsubscript{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\textsuperscript{20} Cryo device (FEI) at an operating voltage of 200 kV. The rods had a length ranging from 1.1 to 1.9 \textmu m (mean 1.5 \textmu m), a width ranging from 0.4 to 0.6 \textmu m (mean 0.5 \textmu m) and a diameter ranging from 0.4 to 0.8 \textmu m (mean 0.6 \textmu m) (Fig. 2).

Differential phenotypic characteristics using API 50CH and API Zym system (bioMérieux) between \textit{M. massilioguelmaensis} sp. nov. strain MG13\textsuperscript{T} and other \textit{Mannheimia} species \cite{4} are detailed in Table 1.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Gram staining of \textit{Mannheimia massilioguelmaensis} strain MG13\textsuperscript{T}.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Transmission electron microscopy of \textit{Mannheimia massilioguelmaensis} strain MG13\textsuperscript{T} using Technai G\textsuperscript{20} Cryo device (FEI) at operating voltage of 200 kV. Scale bar = 500 nm.}
\end{figure}

Susceptibility testing was performed by the Etest strip (bioMérieux) method. Minimum inhibitory concentration was expressed in \textmu g/mL. \textit{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 \cite{9} using a Microflex spectrometer (Bruker). Twelve distinct deposits were done for strain MG13\textsuperscript{T} from 12 isolated colonies. The 12 MG13\textsuperscript{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 \textit{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\textsuperscript{T} against the Bruker database, thus suggesting that our isolate was
a new species. We incremented our database with the spectrum from strain MG13T (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 RNAmer [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 MG13T (CDQL00000000) with those of *Microbacterium granulomatis* strain DSM1956 (JHZD00000000), *Manheimia haemolytica* strain USDA-ARS-USMARC-183 (CP004752) and *Mannheimia varigena* strain USDA-ARS-USMARC-1312 (CP006944).

Digital DDH estimation of strain MG13T 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 MG13T.

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

| 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 | NA | NA | NA | NA | NA |
| Gram stain                         | –      | –      | –      | –      | –      | –      |
| 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     |
| Nitrile reductase                  | +      | +      | +      | +      | +      | +      |
| Urease                             | –      | –      | –      | –      | –      | –      |
| β-Galactosidase                    | –      | –      | –      | –      | –      | –      |
| Indole                             | –      | NA     | NA     | NA     | NA     | NA     |
| Esterase                           | w      | NA     | NA     | NA     | NA     | NA     |
| Esterase isopropionate             | –      | NA     | NA     | NA     | NA     | NA     |
| Leucine aminolide                   | –      | NA     | NA     | NA     | NA     | NA     |
| Cystine aminolide                   | –      | NA     | NA     | NA     | NA     | NA     |
| Valine aminolide                    | –      | NA     | NA     | NA     | NA     | NA     |
| Utilization of:                    |        |        |        |        |        |        |
| Mannitol                           | –      | NA     | NA     | NA     | NA     | NA     |
| Trehalose                          | –      | –      | –      | –      | –      | –      |
| L-Arabinose                        | –      | –      | –      | –      | –      | –      |
| D-Sorbitol                         | –      | –      | –      | –      | –      | –      |
| D-Xylose                           | –      | –      | –      | –      | –      | –      |
| D-Ribose                           | +      | NA     | NA     | NA     | NA     | NA     |
| D-Glucose                          | +      | NA     | NA     | NA     | NA     | NA     |
| D-Phenylacetic acid                | w      | –      | –      | –      | –      | –      |
| D-Fructose                         | +      | NA     | NA     | NA     | NA     | NA     |
| L-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   | 41.7   |        |
| Habitat                            | Human  | Bovine, ovine | Bovine | Bovine, leporine, deer | Bovine | Bovine, porcine |

*Maanheimia massilioguelmaensis* (M. ma) strain MG13T, *Mannheimia haemolytica* (M. ha) strain NCTC 980T, *Mannheimia glucosida* (M. gl) strain P925T, *Mannheimia granulomatis* (M. gr) strain ATCC 49244, *Mannheimia ruminalis* (M. ru) strain HPAS91, *Mannheimia varigena* (M. va) strain 177T.

+ positive result; −, negative result; v, variable result; w, weakly positive result; NA, data not available.
FIG. 3. Reference mass spectrum from *Mannheimia massilioguilaumensis* strain MG13T. 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.

New Microbes and New Infections © 2015 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases, NMNI, 8, 131–136
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)
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.

| Gene category               | Value               |
|----------------------------|---------------------|
| Total genes                | 2103                |
| Protein coding genes       | 2048                |
| RNA genes                  | 55                  |
| Pseudo genes               | 8                   |
| Genes in internal clusters | 590                 |
| Genes with function prediction | 1692           |
| Genes assigned to Cogs     | 1712                |
| Genes with Pfam domains    | 1985                |
| Genes with signal peptides | 327                 |
| Genes with tRNA            | 431                 |

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

| Code | Value | % of total | Description                                      |
|------|-------|------------|--------------------------------------------------|
| J    | 160   | 7.81       | Translation, ribosomal structure and biogenesis  |
| A    | 3     | 0.05       | RNA processing and modification                 |
| K    | 98    | 4.74       | Transcription                                   |
| L    | 116   | 5.66       | Replication, recombination and repair            |
| B    | 0     | 0          | Chromatin structure and dynamics                 |
| D    | 23    | 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 mobility                                   |
| 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                                    |

**TABLE 2. Nucleotide content and gene count levels of genome**

| Attribute                  | Value               | % of total | Description |
|----------------------------|---------------------|------------|-------------|
| Genome size (bp)           | 2 186 813           | 100        |             |
| DNA coding (bp)            | 1 965 580           | 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 tRNA            | 431                 | 21         |             |

COGs, Clusters of Orthologous Groups database; CRISPR, clustered regularly interspaced short palindromic repeat.

*Total is based on either size of genome (in base pairs) or 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 MG13T.

**Taxonomic and nomenclatural proposals**

**Description of Mannheimia massilioguelmaensis**

*Mannheimia massilioguelmaensis* (ma.sil.io.guel.ma.en-sis. L. gen. masc. n. massilioguelmaensis, combination of Guelma, where strain MG13T 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 chloristine 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 MG13T (= CSUR P1431 = DSM 29915) was isolated from a cutaneous abscess of a patient in Guelma in northeastern Algeria.

**Conflict of interest**

None declared.

**Acknowledgement**

The authors thank the Xegen Company (http://www.xegen.fr/) for automating the genomic annotation process.
References

[1] Rossello-Mora R. DNA-DNA reassociation methods applied to microbial taxonomy and their critical evaluation. In: Stackebrandt E, editor. Molecular identification, systematics, and population structure of prokaryotes. Berlin: Springer; 2015. p. 23–50.

[2] Bendjama E, Loucif L, Diene SM, Michelle C, Gacemi-Kirane D, Rolain JM. Non-contiguous finished genome sequence and description of Paucisalibacillus algeriensis sp. nov. Stand Genomic Sci 2014;9:1352–65.

[3] Bendjama E, Loucif L, Diene SM, Michelle C, Gacemi-Kirane D, Rolain JM. Non-contiguous finished genome sequence and description of Bacillus massilioalgeriensis sp. nov. Stand Genomic Sci 2014;9:1046–61.

[4] Angen O, Mutters R, Caugant DA, Olsen JE, Bisgaard M. Taxonomic relationships of the [Pasteurella] haemolytica complex as evaluated by DNA-DNA hybridizations and 16S rRNA sequencing with proposal of Mannheimia haemolytica gen. nov., comb. nov., Mannheimia granulomatis comb. nov., Mannheimia glucosida sp. nov., Mannheimia ruminalis sp. nov. and Mannheimia vangena sp. nov. Int J Syst Bacteriol 1999;49(pt 1):67–86.

[5] Ackermann MR, Brogden KA. Response of the ruminant respiratory tract to Mannheimia (Pasteurella) haemolytica. Microbes Infect 2000;2:1079–88.

[6] Lau JS, Omaleki L, Turni C, Barber SR, Browning GF, Francis MJ, et al. Human wound infection with Mannheimia glucosida following lamb bite. J Clin Microbiol 2015;53:3374–6.

[7] Punpanich W, Srijuntongsi R. Pasteurella (Mannheimia) haemolytica sepsicaemia in an infant: a case report. J Infect Dev Ctries 2012;6:584–7.

[8] Stackebrandt E, Ebers J. Taxonomic parameters revisited: tarnished gold standards. Microbiol Today 2006;33:152–5.

[9] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 2009;49:543–51.

[10] Prodigal. http://prodigalornl.gov.

[11] Benson DA, Karsch-Mizrachi I, Clark K, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic Acids Res 2012;40(Database issue):D48–53.

[12] Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 1997;25:955–64.

[13] Lagesen K, Hallin P, Rodland EA, Staerfeldt HH, Rognes T, Ussery DW. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 2007;35:3100–8.

[14] Kall L, Krogh A, Sonnhammer EL. Advantages of combined trans-membrane topology and signal peptide prediction—the Phobius Web server. Nucleic Acids Res 2007;35(Web server issue):W429–32.

[15] Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 2013;14:60.

[16] Meier-Kolthoff JP, Klenk HP, Goker M. Taxonomic use of DNA G+C content and DNA-DNA hybridization in the genomic age. Int J Syst Evol Microbiol 2014;64(pt 2):352–6.