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.

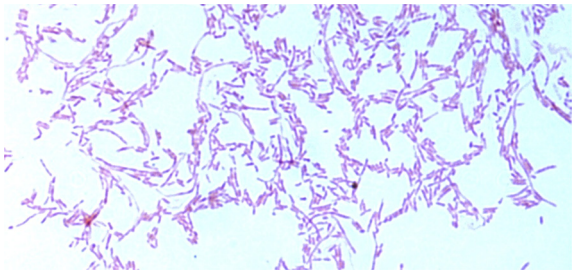

FIG. 1. Gram staining of *Mannheimia massilioguelmaensis* strain MG13^T.

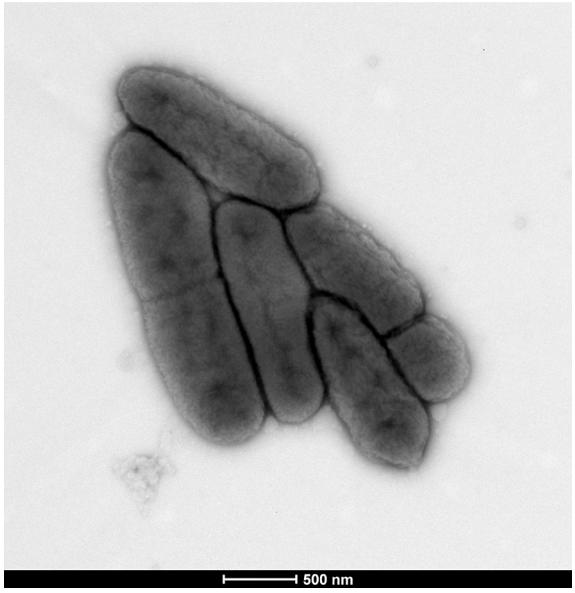

FIG. 2. Transmission electron microscopy of *Mannheimia massilioguelmaensis* strain MG13^T using Technai G^{20} Cryo device (FEI) at operating voltage of 200 kV. Scale bar = 500 nm.

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