Enterobacter timonensis sp. nov., a new bacterium isolated from a fresh human stool specimen

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Abstract

Enterobacter timonensis strain mt20T (= CSUR P2201T, = DSM101775T) is a new species isolated from a fresh human stool specimen. © 2019 The Author(s). Published by Elsevier Ltd.

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Introduction

Culturomics is a concept developing different culture conditions in order to enlarge our knowledge of the human microbiota through the discovery of previously uncultured bacteria [1–4]. Once an isolate is obtained, we use 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 to describe it [5,6].

Isolation and growth conditions

In 2016, we isolated from the stool specimen of a 38-month-old healthy girl from Senegal, the bacterial strain mt20T. Screening was performed by MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [7]. The obtained spectra (Fig. 1) were imported into MALDI BIOTYPER 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in two databases (Bruker and the constantly updated MEPHI databases [http://backup.mediterranee-infection.com/article.php?larub=280&titre=urms-database]). The study was validated by the ethics committee of the Institut Fédératif de Recherche IFR48 under number 09-022. Initial growth was obtained after 72 h of culture in a liquid medium containing 37 g of Difco Marine Broth (Becton Dickinson, Le Pont de Claix, France) per litre of sterile water at 37°C and on Columbia agar enriched with 5% sheep blood in strict aerobic conditions at 37°C.

Phenotypic characteristics

Colonies were brown and circular with a mean diameter of 8 mm. Bacterial cells were Gram-negative, bacillus-shaped with a mean diameter of 0.8 μm and a mean length of 2.5 μm (Fig. 2). Strain mt20T showed catalase-positive and oxidase-positive activities. API ZYM was performed under strict aerobic conditions at 37°C as described in Table 1. Main characteristics of the strain are summarized in Fig. 3.

Strain identification

The 16S rRNA gene was sequenced to classify this bacterium. Amplification used the primer pair F1D1 and rP2 (Eurogentec,
Angers, France) and sequencing used the Big Dye® Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer capillary 3500xl Genetic Analyzer capillary sequencer (Thermo Fisher, Saint-Aubin, France), as previously described [8]. The 16S rRNA nucleotide sequences were assembled and corrected using CODONCODE ALIGNER software (http://www.codoncode.com). Strain mt20\(^T\) exhibited a 98.74% sequence identity with Enterobacter cloacae strain ATCC 23373\(^T\) (GenBank Accession number NR_118011.1), the phylogenetically closest species with standing in nomenclature (Fig. 4). Based on the phylogenetic tree, we found that strain mt20\(^T\) do not reach the threshold of 16S required to identify a new species. For this reason, we investigated the genome sequence of this bacterial species and found that it is, nevertheless, a new species.

TABLE 1. Biochemical tests of Enterobacter timonensis (API ZYM)

| Test                                      | Results (+/-) |
|-------------------------------------------|---------------|
| Control                                   | -             |
| Alkaline phosphatase                      | +             |
| Esterase (C4)                             | +             |
| Esterase lipase (C8)                      | -             |
| Lipase (C14)                              | -             |
| Leucine arylamidase                       | +             |
| Valine arylamidase                        | -             |
| Cystine arylamidase                       | -             |
| Trypsin                                   | -             |
| α-chymotrypsin                            | -             |
| Acid phosphatase                          | +             |
| Naphthol-AS-Bi-phosphohydrolase           | +             |
| α-galactosidase                           | +             |
| β-galactosidase                           | -             |
| β-glucuronidase                           | -             |
| α-glucosidase                             | +             |
| β-glucosidase                             | -             |
| N-acetyl-β-glucosaminidase                | +             |
| N-acetyl-α-mannosidase                    | +             |
| α-fucosidase                              | -             |

FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

FIG. 2. Scanning electron micrograph of Enterobacter timonensis using TM4000Plus microscope from Hitachi. Scale bar and acquisition settings are shown on the original micrograph.
Genetic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit and then sequenced on the MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit (Illumina), as previously described [9]. The assembly was performed with a pipeline incorporating different softwares (VELOCITY [10], SPADES [11] and SOAP DENOVO [12]), on trimmed data (MiSeq and TRIMMOMATIC [13] softwares) or untrimmed data (only MiSeq software). GapCloser was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value < 25% of the mean depth were removed. The best assembly was selected by using different criteria (number of scaffolds, N50, number of
The genome of strain mt20T is 4,199,690 bp long with a 56.8 mol% G+C content and 13 contigs. The degree of genomic similarity of mt20T with closely related species was estimated using the OrthoANI software [14]. Values among closely related species (Fig. 5) ranged from 79.29%, between Enterobacter soli and Enterobacter kobei, to 91.59%, between Enterobacter asburiae and Enterobacter bugandensis. When the isolate was compared with these closely related species, values ranged from 82.03% with Enterobacter soli to 83.18% with Enterobacter asburiae. Based on the whole genome sequence,
we consequently classify this strain as a member of a new species within the genus *Enterobacter*, family *Enterobacteriaceae*, phylum *Proteobacteria*.

**Conclusion**

After genome sequencing, strain mt20T exhibited an average of nucleotide identity value < 95% with its phylogenetically closest species with standing in nomenclature, and is consequently proposed as the type strain of the new species *Enterobacter timonensis* sp. nov.

**Nucleotide sequence accession number**

The 16S rRNA gene and genome sequences were deposited in GenBank under Accession numbers LN906632 and FCOP00000000, respectively.

**Deposit in culture collections**

Strain mt20T was deposited in the strain collection under number (= CSUR P2201T, = DSM101775T).

**Conflict of interest**

None to declare.

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