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

T. Takakura[1,2], H. Anani[4], A. Fadlane[2], A. Fontanini[2], D. Raoult[2,3] and J. Y. Bou Khalil[2]

1) *Hitachi High-Technologies Corporation, Analytical & Medical Solution Business Group, Ibaraki-ken, Japan,* 2) *Institut Hospitalo-Universitaire Méditerranée-Infection, Marseille, France,* 3) *Aix-Marseille Université, Institut de Recherche pour le Developpement (IRD), UMR Microbes Evolution Phylogeny and Infections (MEPHI), Marseille, France and* 4) *Aix Marseille Univ, IRD, AP-HM, SSA, VITROME, IHU-Méditerranée Infection, Marseille, France*

Abstract

Enterobacter timonensis strain mt20^T (= CSUR P2201^T, = DSM101775^T) is a new species isolated from a fresh human stool specimen.

Keywords: Culturomics, *Enterobacter timonensis*, Human gut, New species, taxonogenomics
Original Submission: 17 May 2019; **Revised Submission:** 17 June 2019; **Accepted:** 26 June 2019
Article published online: 3 July 2019

Corresponding author: J.Y. Bou Khalil, MEPHI, Institut Hospitalo-Universitaire Méditerranée Infection, 19–21 Boulevard Jean Moulin 13005 Marseille Cedex 05, France.
E-mail: boukhaliljacques@gmail.com

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 mt20^T. 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 mt20^T 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 fD1 and rP2 (Eurogentec,

https://doi.org/10.1016/j.nmni.2019.100586

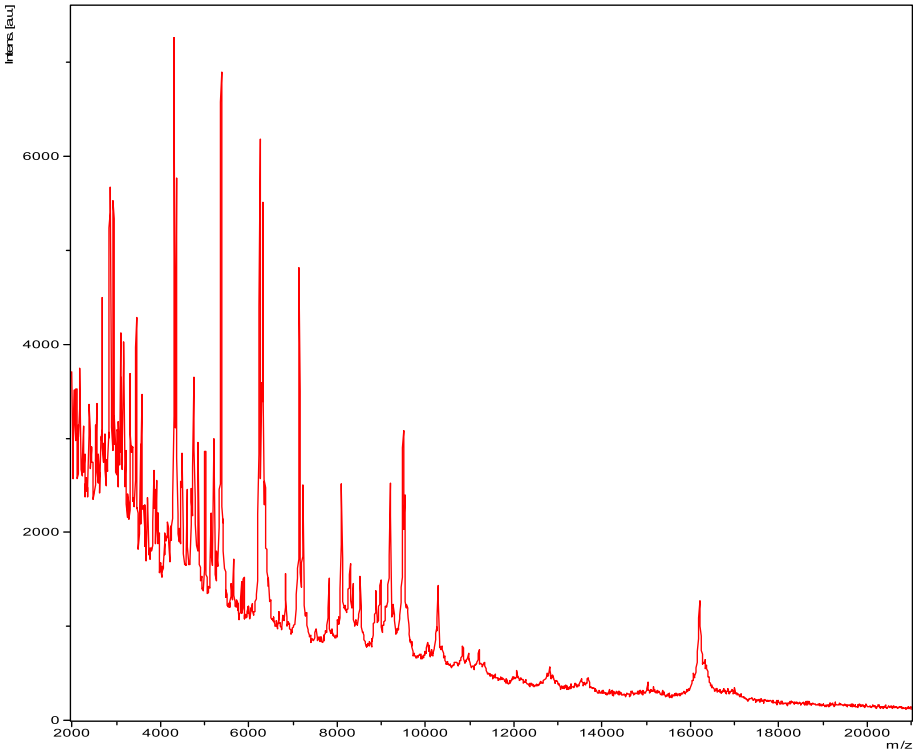


FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

Angers, France) and sequencing used the Big Dye® Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer capillary3500xLGenetic Analyzer capillary sequencer (Thermofisher, 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.

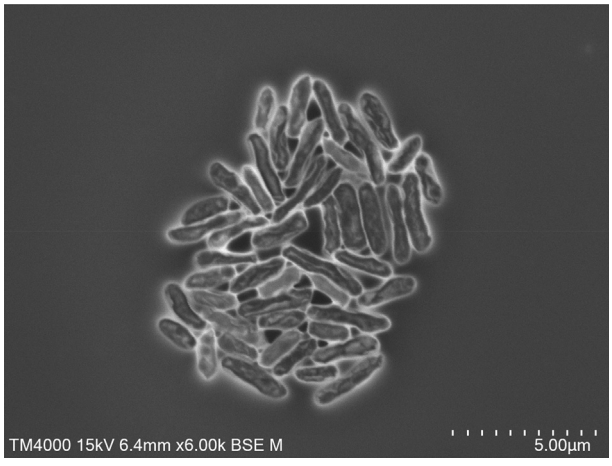
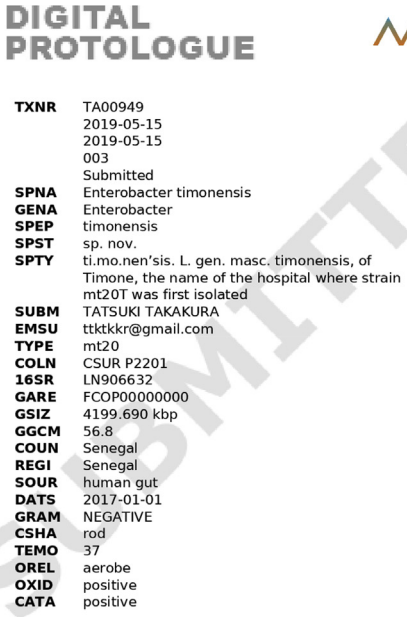


FIG. 2. Scanning electron micrograph of *Enterobacter timonensis* using TM4000Plus microscope from Hitachi. Scale bar and acquisition settings are shown on the original micrograph.

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

Bacteria: *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	+
Naphthalo-AS-BI-phosphohydrolase	+
α-galactosidase	+
β-galactosidase	+
β-glucuronidase	–
α-glucosidase	+
β-glucosidase	+
N-acetyl-β-glucosaminidase	+
α-mannosidase	–
α-fucosidase	–


DIGITAL PROTOLOGUE

TXNR	TA00949
	2019-05-15
	2019-05-15
	003
	Submitted
SPNA	Enterobacter timonensis
GENA	Enterobacter
SPEP	timonensis
SPST	sp. nov.
SPTY	ti.mo.nen'sis. L. gen. masc. timonensis, of Timone, the name of the hospital where strain mt20T was first isolated
SUBM	TATSUKI TAKAKURA
EMSU	ttktkkr@gmail.com
TYPE	mt20
COLN	CSUR P2201
16SR	LN906632
GARE	FCOP00000000
GSIZ	4199.690 kbp
GGCM	56.8
COUN	Senegal
REGI	Senegal
SOUR	human gut
DATS	2017-01-01
GRAM	NEGATIVE
CSHA	rod
TEMO	37
OREL	aerobe
OXID	positive
CATA	positive

FIG. 3. Description of *Enterobacter timonensis* according to the digitalized protologue TA00949 on the www.imedea.uib.es/dprotologue website.

Genome sequencing

Genomic 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 (Velvet [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

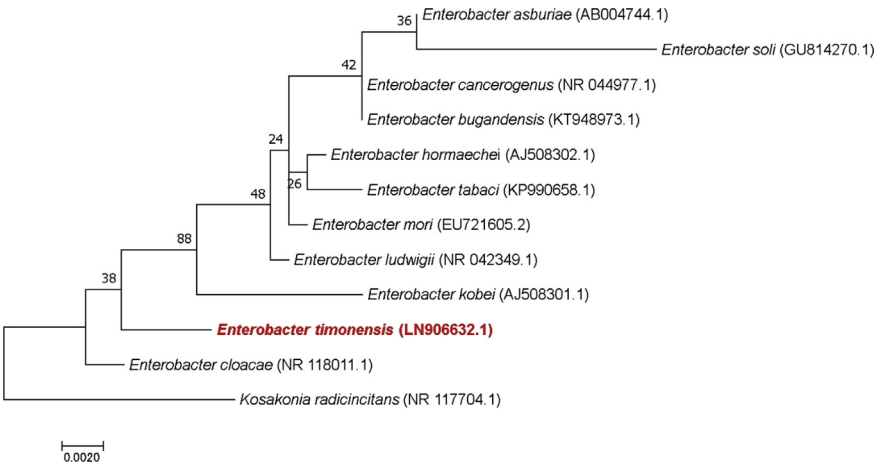


FIG. 4. Phylogenetic tree showing the position of '*Enterobacter timonensis*' strain mt20^T relative to other phylogenetically close neighbours. The respective GenBank Accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences were aligned using MUSCLE v3.8.31 with default parameters and phylogenetic inferences were obtained using the maximum likelihood method within MEGA 7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. The scale bar indicates a 0.2% nucleotide sequence divergence.

N). The genome of strain mt20^T is 4 199 690 bp long with a 56.8 mol% G+C content and 13 contigs. The degree of genomic similarity of mt20^T 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,

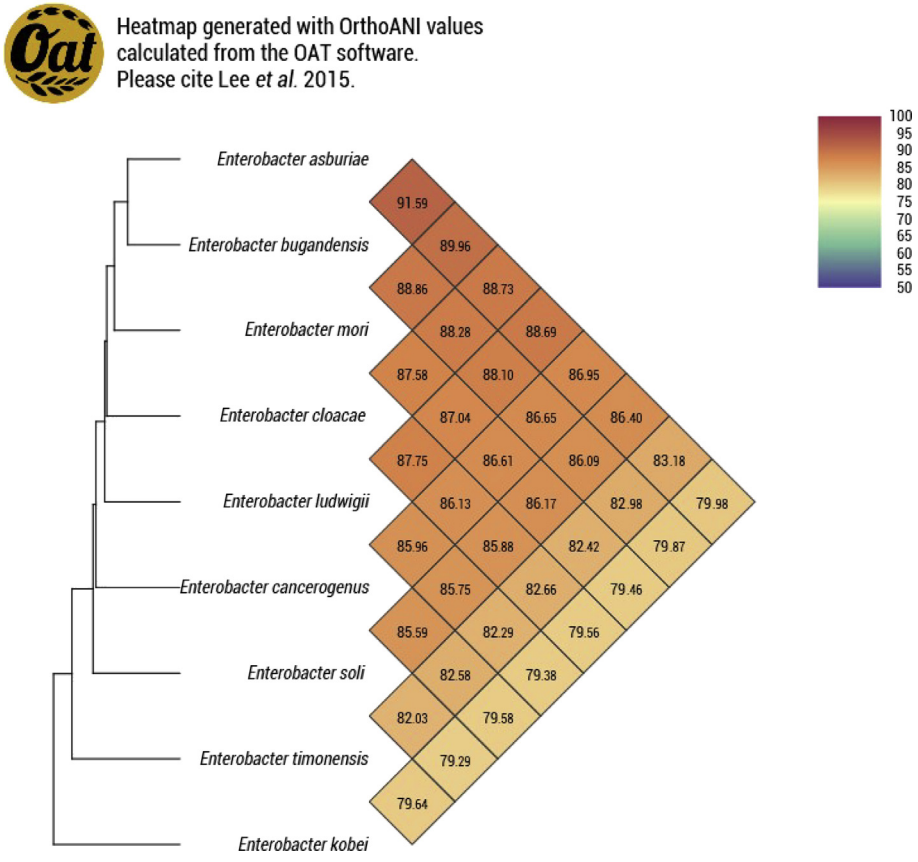


FIG. 5. Heatmap generated with ORTHOANI values calculated using the OAT software between *Enterobacter timonensis* and other closely related species with standing in nomenclature.

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 mt20^T 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 mt20^T was deposited in the strain collection under number (= CSUR P2201^T, = DSM101775^T).

Conflict of interest

None to declare.

Acknowledgements

We sincerely thank Sakazume Taku, Takashi Irie, Kyoko Imai, Shigeki Matsubara, Akiko Hisada, Yusuke Ominami, and all the Hitachi Team in Japan for the collaborative study we are conducting together between Hitachi High-Technologies Corporation and the Institut Hospitalo-Universitaire Méditerranée-Infection, and for the installation and servicing of the TM4000Plus microscope in our facility.

Funding sources

The study was supported by the Méditerranée Infection foundation, the National Research Agency under the programme 'Investissements d'avenir' (ANR-10-IAHU-03) and by Région Provence Alpes Côte d'Azur and European funding (FEDER PRIMI).

References

[1] Lagier JC, Armougom F, Million M, Hugon P, Pagnier I, Robert C, et al. Microbial culturomics: paradigm shift in the human gut microbiome study. Clin Microbiol Infect 2012;18:1185–93.
[2] Lagier JC, Hugon P, Khelaifia S, Fournier PE, La Scola B, Raoult D. The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. Clin Microbiol Rev 2015;28:237–64.
[3] Lagier JC, Khelaifia S, Alou MT, Ndongo S, Dione N, Hugon P, et al. Culture of previously uncultured members of the human gut microbiota by culturomics. Nat Microbiol 2016;1:16203.
[4] Lagier JC, Edouard S, Pagnier I, Mediannikov O, Drancourt M, Raoult D. Current and past strategies for bacterial culture in clinical microbiology. Clin Microbiol Rev 2015;28:208–36.
[5] Fournier PE, Lagier JC, Dubourg G, Raoult D. From culturomics to taxonomogenomics: a need to change the taxonomy of prokaryotes in clinical microbiology. Anaerobe 2015;36:73–8.
[6] Ramasamy D, Mishra AK, Lagier JC, Padhmanabhan R, Rossi M, Sentausa E, et al. A polyphasic strategy incorporating genomic data for the taxonomic description of novel bacterial species. Int J Syst Evol Microbiol 2014;64:384–91.
[7] 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.
[8] Morel AS, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta JP, et al. Complementarity between targeted real-time specific PCR and conventional broad-range 16S rDNA PCR in the syndrome-driven diagnosis of infectious diseases. Eur J Clin Microbiol Infect Dis 2015;34:561–70.
[9] Diop A, Khelaifia S, Armstrong N, Labas N, Fournier PE, Raoult D, Million M. Microbial culturomics unravels the halophilic microbiota repertoire of table salt: description of *Gracilibacillus massiliensis* sp. nov. Microb Ecol Health Dis 2016;27:32049.
[10] Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 2008;18:821–9.
[11] Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19:455–77.
[12] Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, et al. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. GigaScience 2012;1:18.
[13] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114–20.
[14] Lee I, Ouk Kim Y, Park SC, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–3.