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ORIGINAL ARTICLE

Inediibacterium massiliense gen. nov., sp. nov., a new bacterial species isolated from the gut microbiota of a severely malnourished infant

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Abstract A novel strain, Mt12^T (=CSUR P1907 = DSM 100590), was isolated from the fecal sample of a 7-month-old girl from Senegal afflicted with severe acute malnutrition. This bacterium is a strictly anaerobic, spore-forming Gram-stain positive bacillus. The major cellular fatty acid was identified as tetradecanoic acid. Its 16S rRNA gene sequence exhibited 94.9% similarity with that of *Crassaminicella profunda* strain Ra1766H^T, currently the closest species with a validly published name. The draft genome of strain Mt12^T is 3,497,275-bp long with a 30.45% of G+C content. 3397 genes were predicted, including 3268 protein-coding genes and 129 RNAs, including eight 16S rRNAs. Genomic comparison with closely related species with an available genome showed a lower quantitative genomic content. The phylogenetic analysis alongside the dDDH values under 30% and phenotypic characteristics suggest that strain Mt12^T represents a new genus within the family *Clostridiaceae*, for which the name *Inediibacterium massiliense* gen. nov., sp. nov. is proposed.

Keywords *Inediibacterium massiliense* · Gut microbiota · Taxonogenomics · Culturomics · Genome

Abbreviations

AGIOS	Average of genomic identity of orthologous gene sequences
bp	Base pairs
COG	Clusters of orthologous groups
CSUR	Collection de souches de l'Unité des Rickettsies
DDH	DNA–DNA hybridization
DSM	Deutsche Sammlung von Mikroorganismen

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FAME	Fatty acid methyl ester
GC/MS	Gas chromatography/mass spectrometry
kb	Kilobases
MALDI-TOF MS	Matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry
ORF	Open reading frame
TE buffer	Tris-EDTA buffer
SDS	Sodium dodecyl sulfate
URMITE	Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergent

Introduction

The gut microbiota is a very complex and diverse ecosystem involved in many aspects of human health. It consists essentially of bacteria but also of viruses, archaea, fungi and protozoans (Lagier et al. 2012b) with an estimated ten times more bacterial cells (10^{14}) than human cells (10^{13}) and 150 times more bacterial genes in the body (Sankar et al. 2015). The gut microbiota is heavily implicated in digestion, mainly through the metabolism of non-digestible carbohydrates, in antimicrobial protection, and in immunomodulation in the gut. It also contributes to the integrity and maintenance of the intestinal barrier (Jandhyala et al. 2015). Colonisation of the gastrointestinal tract starts at birth and its composition reaches a mature and stable state between the ages of 2–3 years old (Palmer et al. 2007). The gut microbiota composition is dependent on various factors such as age, diet, environment, genetics and gut wall structure (Graf et al. 2015; Sankar et al. 2015). The major bacterial phyla present include the *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* (Jandhyala et al. 2015).

Dysbiosis of the gut microbiota is instrumental in several diseases such as inflammatory bowel disease, obesity, type 2 diabetes (Sankar et al. 2015) and severe acute malnutrition (Million et al. 2016; Tidjani Alou et al. 2016). In order to investigate the implication of the gut microbiota in severe acute malnutrition, a large-scale study was conducted using "culturomics", a high throughput culture method which consists in varying physico-chemical parameters of culture to explore the gut microbiota as thoroughly as possible (Lagier et al. 2012a). In the process, we isolated a strain, strain Mt12^T, representing a new genus, a member of the family *Clostridiaceae*. The family *Clostridiaceae* is currently comprised of at least 30 genera which contain strictly anaerobic species, most of which are Gram-stain positive and spore-forming (Parte 2014). To describe this new genus, we used a taxonogenomics approach to characterise strain Mt12^T (Fournier et al. 2015). Taxonogenomics mixes next generation sequencing, phylogenetic and phenotypic characteristics including a MALDI-TOF mass spectrometry protein profile to describe new bacterial species. We herein describe strain Mt12^T (=CSUR P1907^T = DSM 100590^T) as the type strain of the type species of *Inediibacterium massiliense* gen. nov., sp. nov.

Materials and methods

Sample information

A stool sample was collected from a 7-month old girl from Senegal who suffered from Kwashiorkor, a severe form of acute malnutrition. She had a height-for-age z-score of −0.17 and presented with a nutritional edema. The patient was not receiving antibiotics at the time of stool collection. The specimen was preserved at +4 °C for 7 days until it was transferred to our laboratory in Marseille where it was stored at −80 °C until further use. This child's parents gave their oral informed consent to participation in this culturomics study of the gut microbiota of malnourished children. The study was approved by the Institut Fédératif de Recherche 48 (Faculty of Medicine, Marseille, France) under Agreement No. 09-022.

Strain identification by MALDI-TOF MS and 16S rRNA sequencing

The stool sample was cultured using 18 culture conditions as previously described (Lagier et al. 2015). Colonies were purified through subculture and identified by MALDI-TOF MS using a Microflex spectrometer and a MTP 96 MALDI-TOF target plate (Bruker Daltonics, Leipzig, Germany), as described previously (Seng et al. 2009, 2013). The spectra obtained for each colony were matched against the MALDI Biotyper software version 3.0 (Bruker) and

URMITE databases using standard pattern matching (with default parameter settings). Identification scores used were as follows: a score over 1.9 allowed identification at the species level while a score under 1.9 did not allow any identification. In the latter case, the colony was identified by sequencing its 16S rRNA using the fD1 and rP2 primers, as previously described (Drancourt et al. 2000). Several studies have determined that similarity level thresholds of at least 98.7 and 95% are necessary to define a new species and a new genus respectively without performing DNA–DNA hybridization (Stackebrandt and Ebers 2006; Kim et al. 2014; Yarza et al. 2014). Upon identification, 12 MALDI-TOF MS spectra for strain Mt12^T were included in the URMITE database.

Growth conditions

Optimal growth conditions for strain Mt12^T were determined by testing five growth temperatures (25, 28, 37, 45 and 56 °C) in an aerobic atmosphere with or without 5% CO_2, and under anaerobic and microaerophilic conditions using the GENbag Anaer and GENbag microaer systems, respectively (BioMerieux, Marcy l'Etoile, France). Different pH values (6, 6.5, 7 and 8.5) and NaCl concentrations (0.5, 1, 5, 7.5 and 10%) were also tested.

Morphological, biochemical and antibiotic susceptibility tests

Phenotypic characteristics such as motility, sporulation, catalase and oxidase activities were tested as previously described (Lagier et al. 2015). Cell wall structure was determined using both Gram staining and the non-staining KOH method (Buck 1982; Lagier et al. 2015). Biochemical analysis of strain Mt12^T was carried out using API 50CH, API 20A, API ZYM strips (BioMérieux) in an anaerobic atmosphere. Antibiotic susceptibility was tested using the disk diffusion method (Matuschek et al. 2014) as per EUCAST 2015 recommendations.

Transmission electron microscopy was performed as follows: cells were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer for at least 1 h at 4 °C. A drop of cell suspension was deposited for approximately 5 min on glow-discharged formvar carbon film on 400 mesh nickel grids (FCF400-Ni, EMS). The grids were dried on blotting paper and cells were negatively stained for 10 s with 1% ammonium molybdate solution in filtered water at RT. Electron micrographs were acquired with a Tecnai G20 Cryo (FEI) transmission electron microscope operated at 200 keV.

Cellular fatty acid methyl ester (FAME) analysis was performed by GC/MS. Two samples were prepared with approximately 30 mg of bacterial biomass per tube harvested from several culture plates. Fatty acid methyl esters were prepared as described by Sasser (2006). GC/MS analyses were carried out as described before (Dione et al. 2016). Briefly, fatty acid methyl esters were separated using an Elite 5-MS column and monitored by mass spectrometry (Clarus 500—SQ 8 S, Perkin Elmer, Courtaboeuf, France). A spectral database search was performed using MS Search 2.0 operated with the Standard Reference Database 1A (NIST, Gaithersburg, USA) and the FAMEs mass spectral database (Wiley, Chichester, UK).

Genomic DNA preparation and genome sequencing

After lysis pretreatements by a lysozyme incubation at 37 °C for 2 h followed by a proteinase K action respectively, DNA was extracted using a EZ1 biorobot (Qiagen) with an EZ1 DNA tissues kit. The elution volume was 50 µl. Genomic DNA (gDNA) was quantified by a Qubit assay with the high sensitivity kit (Life technologies, Carlsbad, CA, USA) to 123.7 ng/µl.

The genomic DNA (gDNA) of strain Mt12^T was sequenced using a MiSeq sequencer (Illumina Inc, San Diego, CA, USA) and the mate pair strategy (http://support.illumina.com/content/dam/illumina-marketing/documents/products/appnotes/appnote-nextera-mate-pair-bacteria.pdf). The gDNA was barcoded in order to be mixed with 11 other projects using the Nextera Mate Pair sample prep kit (Illumina). The mate pair library was prepared with 1.5 µg of gDNA using the Nextera mate pair Illumina guide. The gDNA sample was simultaneously fragmented and tagged with a mate pair junction adapter. The fragmentation pattern was validated on an Agilent 2100 BioAnalyzer (Agilent Technologies Inc, Santa Clara, CA, USA) with a DNA 7500 labchip. The DNA fragments ranged in size from 1.5 kb up to 11 kb, with an optimal size at 4.031 kb. No size selection was performed and

385.5 ng of tagmented fragments were circularised. The circularised DNA was mechanically sheared to small fragments with an optimal size of ~1070 bp on the Covaris device S2 in T6 tubes (Covaris, Woburn, MA, USA). The library profile was visualised on a High Sensitivity Bioanalyzer LabChip (Agilent Technologies Inc, Santa Clara, CA, USA) and the final concentration of the library was measured as 2.40 nmol/l. The libraries were normalised at 2 nM and pooled. After a denaturation step and dilution at 15 pM, the pool of libraries was loaded onto the reagent cartridge and then onto the instrument along with the flow cell. The automated cluster generation and sequencing run were performed in a single 2 × 301-bp run. Total information of 7.3 Gb was obtained from a 511 K/mm^2 cluster density with a cluster passing quality control filters of 97.0% (12,079,000 passing filter paired reads). Within this run, the index representation for strain Mt12^T was determined as 9.51%. The 1,149,152 paired reads were trimmed then assembled to 14 scaffolds.

Genome annotation and analysis

Open reading frames (ORFs) were predicted using Prodigal (Hyatt et al. 2010) with default parameters but the predicted ORFs were excluded if they spanned a sequencing gap region. The predicted bacterial protein sequences were searched against the GenBank (Benson et al. 2012) and the clusters of orthologous groups (COG) databases using BLASTP (E-value 1e−03, coverage 0.7 and identity percent 30%). If no hit was found, it was searched against the NR database using BLASTP with an E-value of 1e^{-03}, a coverage of 0.7 and an identity percentage of 30%, and if the sequence length was smaller than 80 amino acids (aa), we used an E-value of 1e^{-05}. The tRNAScanSE tool (Lowe and Eddy 1997) was used to find tRNA genes, while ribosomal RNAs were found using RNAmmer (Lagesen et al. 2007). Signal peptides and the number of transmembrane helices were predicted using Phobius (Käll et al. 2004). Mobile genetic elements were predicted using PHAST (Zhou et al. 2011) and RAST (Aziz et al. 2008). ORFans were identified if all the BLASTP searches performed did not give positive results (*E*-value lower than 1e^{-03} for ORFs with sequence size larger than 80 aa or *E*-value lower than 1e^{-05} for ORFs with sequence length smaller 80 aa). Such parameter thresholds have already been used in previous studies to define ORFans. Artemis (Rutherford et al. 2000) and DNA Plotter (Carver et al. 2009) were used for data management and the visualisation of genomic features, respectively. The Mauve alignment tool (version 2.3.1) was used for multiple genomic sequence alignment (Darling et al. 2004).

Comparator species for genomic comparison were identified in the 16S RNA tree using the Phylopattern software (Gouret et al. 2009). The genome of strain Mt12^T was compared to those of *Alkaliphilus metalliredigens* strain QYMF, *Clostridium aceticum* strain DSM 1496, *Alkaliphilus transvaalensis* strain SAGM1 and *Alkaliphilus oremlandii* strain OhILAs.

For each selected genome, the complete genome sequence, proteome genome sequence and Orfeome genome sequence were retrieved from the FTP of NCBI. An annotation of the entire proteome was performed to define the distribution of functional classes of predicted genes according to the COGs of proteins (using the same method as for the genome annotation). Annotation and comparison processes were performed in the Multi-Agent software system DAGOBAH (Gouret et al. 2011) which includes Figenix (Gouret et al. 2005) libraries which provide pipeline analysis.

To evaluate the genomic similarity between studied genomes, we determined two parameters, digital DDH (dDDH) which exhibits a high correlation with DDH (Auch et al. 2010; Meier-Kolthoff et al. 2013) and

Table 1 Classification and general features of *Inediibacterium massiliense* strain Mt12^T

Property	Term
Current classification	Domain: *Bacteria*
	Phylum: *Firmicutes*
	Class: *Clostridia*
	Order: *Clostridiales*
	Family: *Clostridiaceae*
	Genus: *Inediibacterium*
	Species: *Inediibacterium massiliense*
	Type strain: Mt12
Gram stain	Positive
Cell shape	Rod
Motility	Motile
Sporulation	Sporulating
Temperature range	Mesophilic
Optimum temperature	37 °C

AGIOS, which was designed to be independent from DDH (Ramasamy et al. 2014). The AGIOS score is the mean value of nucleotide similarity between all couples of orthologous proteins between the two studied genomes (Ramasamy et al. 2014).

Results and discussion

Strain identification and phylogenetic analyses

Strain Mt12^T was first isolated after a 10-day pre-incubation of a stool sample in an anaerobic blood culture bottle supplemented with 0.2µ filter-sterilised rumen and sheep blood and seeding on 5% sheep blood-enriched Colombia agar in anaerobic atmospheric conditions at 37 °C. Strain Mt12^T (Table 1) could not be identified using MALDI-TOF MS and, therefore, the 16S rRNA was sequenced. The resulting sequence (Genbank Accession No. LN850734) revealed a 94.9% similarity level with the 16S rRNA of *Crassaminicella profunda* strain Ra1766H^T, currently the closest species with a validly published name (Fig. 1). Based on the current recommended thresholds (Stackebrandt and Ebers 2006; Kim et al. 2014; Yarza et al. 2014), strain Mt12^T may, therefore, be a representative strain of a new genus within the family *Clostridiaceae*, as is *C. profunda* (Lakhal et al. 2015). The other phylogenetically closely related species are phylogenetically located within order

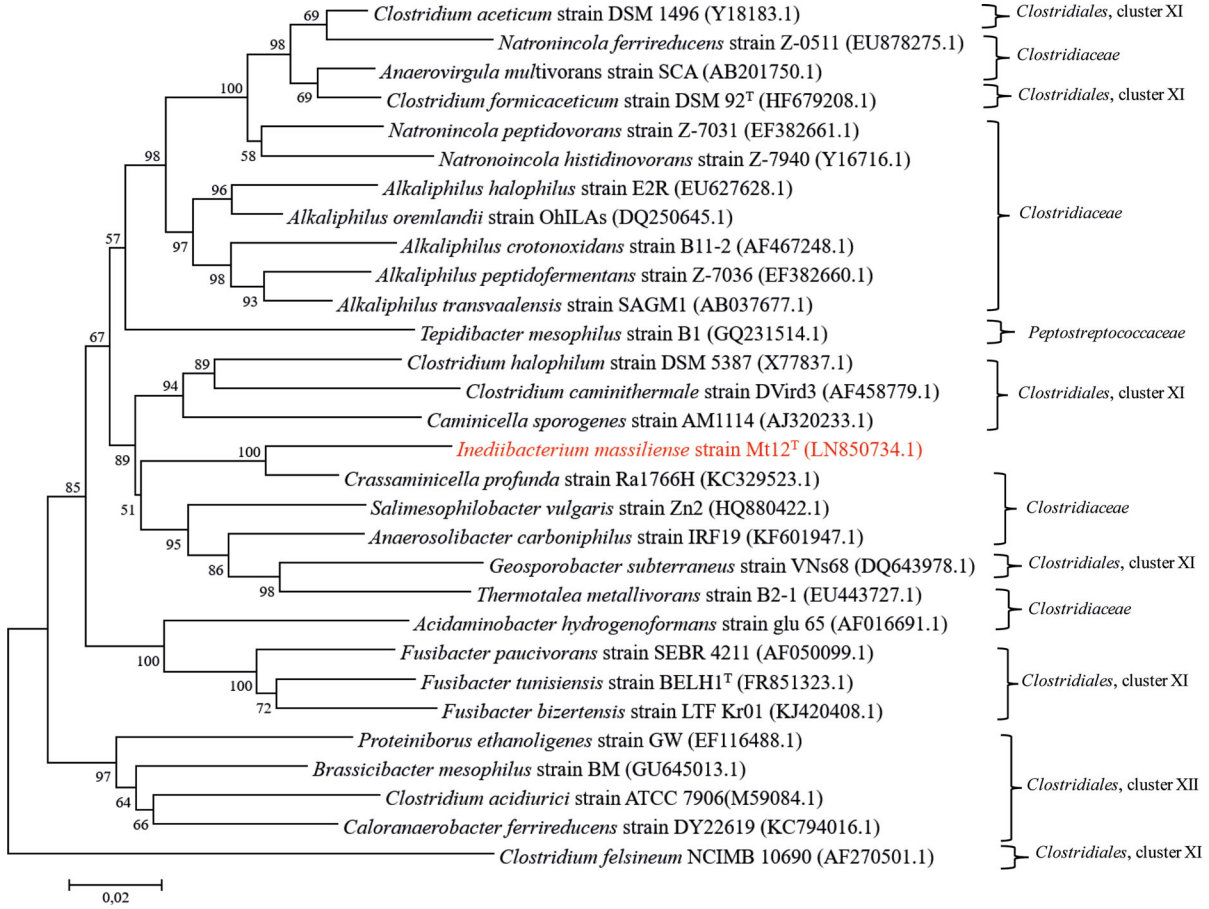

Fig. 1 Phylogenetic tree highlighting the position of *Inediibacterium massiliense* strain Mt12^T relative to other closely related strains. The respective GenBank accession numbers for 16S rRNA genes are indicated in *parenthesis*. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using the maximum-likelihood method within the MEGA6 software. *Numbers* at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. *Clostridium felsineum* strain NCIMB 10690 was used as an outgroup. The *scale bar* represents a 2% nucleotide sequence divergence

Clostridiales, cluster XI (Collins et al. 1994) (Fig. 1). In this respect, strain Mt12^T seems to be at the junction between the members of the family *Clostridiaceae* and cluster XI of the order *Clostridiales* (Fig. 1).

The reference protein spectra for strain Mt12^T (Fig. 2) were included in the URMITE database (http://www.mediterranee-infection.com/article.php?laref=256&titre=urms-database).

Phenotypic description

Growth of strain Mt12^T was observed between 25 and 37 °C in anaerobic conditions. No growth occurred in aerobic and microaerophilic conditions. The optimal growth yield was obtained after 48 h in anaerobic conditions. Growth was observed at all tested pH values (6, 6.5, 7 and 8.5) and only at the minimal concentration of NaCl (0.5%). Cells were observed to be motile and monotrichous. Gram staining revealed spore-forming Gram-negative rods (Supplementary Fig. 1). Nevertheless, the cell wall architecture was that of a Gram-positive bacterium as revealed by the KOH method. The discordance between the two tests is probably due to the damage to the cell wall caused by exposure to oxygen, as observed in several other obligate anaerobes (Johnson et al. 1995). Colonies were observed to be irregular and translucent with a white center and a mean diameter of 3 mm. Spores were terminal and deforming (Fig. 3). Negative staining visualized with electron microscopy revealed bacilli with a mean length and width of 6.9 and 0.5 µm, respectively (Fig. 3). These morphological characteristics are similar to those of members of genera within the family *Clostridiaceae* which are generally also strictly anaerobic, Gram-stain positive and spore-forming bacteria.

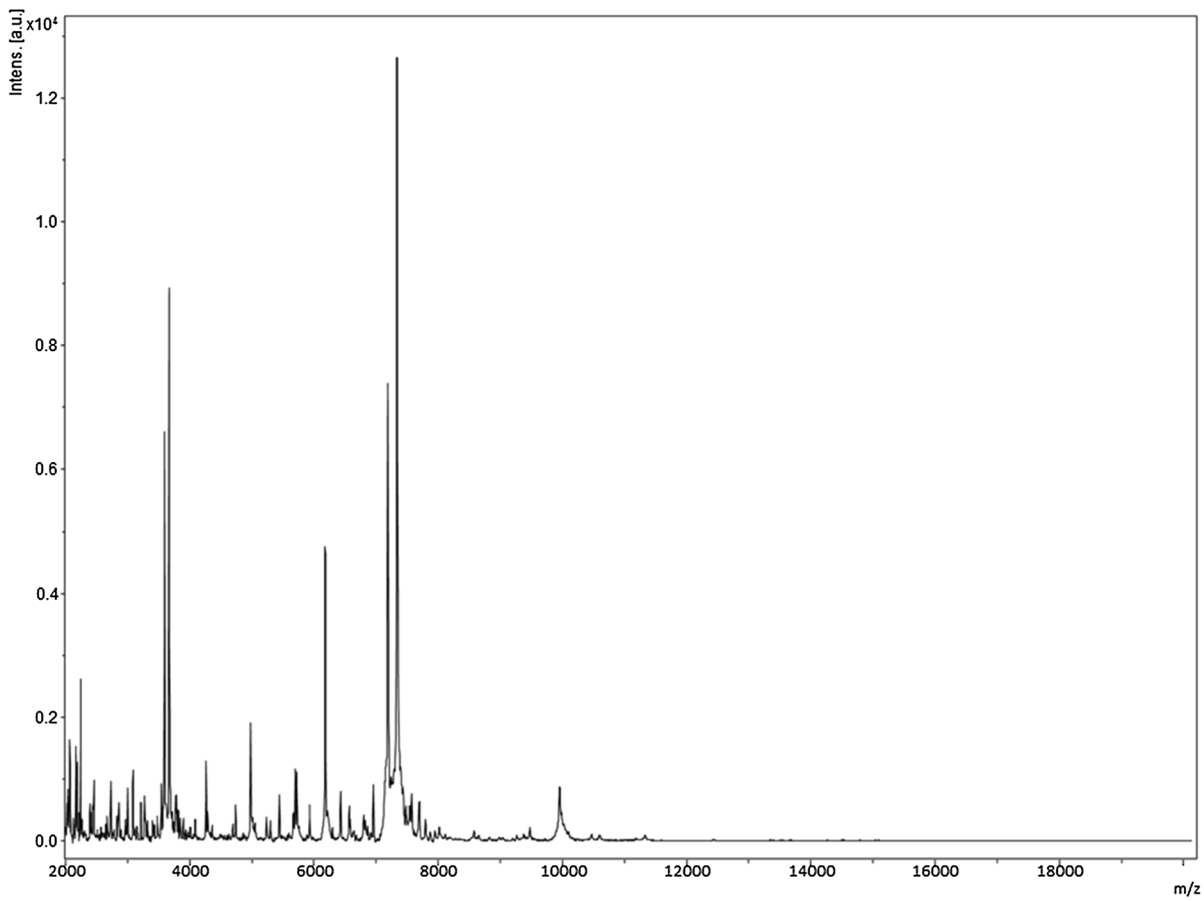

Fig. 2 Reference mass spectrum from *Inediibacterium massiliense* strain Mt12^T. Spectra from 12 individual colonies were compared and a reference spectrum was generated

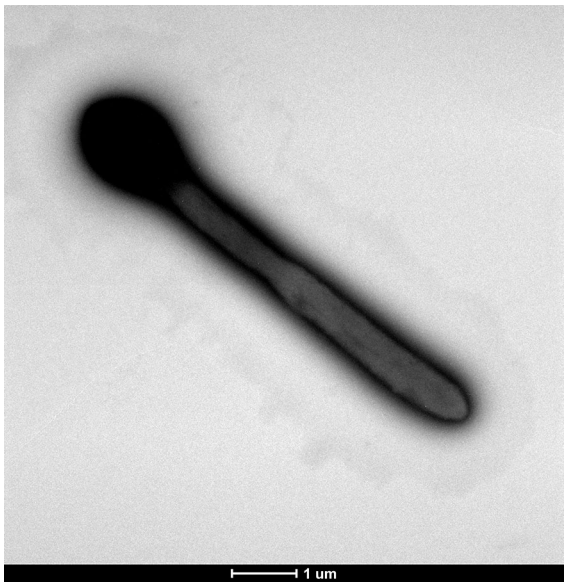

Fig. 3 Transmission electron microscopy of *Inediibacterium massiliense* strain Mt12^T using a Tecnai G20 transmission electron microscope (FEI Company) at operating voltage of 200 keV. The *scale bar* represents 500 nm

The major cellular fatty acids of strain Mt12^T were identified as $C_{14:0}$ (46%), $C_{16:1n7}$ (21%) and $C_{16:0}$ (18%). $C_{4:0}$, a short chain fatty acid, was also detected as shown in Table 2.

Catalase and oxidase activities were absent, consistent with the anaerobic metabolism of strain Mt12^T. Using API ZYM strips, other enzymatic activities were detected, such as esterase C4, esterase lipase C8, leucine arylamidase, valine arylamidase, naphtol-AS-BI-phosphohydrolase and β-galactosidase. No activity was detected for the following enzymes: alkaline phosphatase, lipase C14, cysteine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Using API 20A strips, indole production was present as well as β-glucosidase activity as revealed by the hydrolysis of ferric citrate esculin. Protease and urease activities were negative. Acid was found to be produced from D-glucose, D-lactose, D-sucrose, D-maltose, salicin, D-cellobiose, D-mannose, D-raffinose, D-sorbitol and D-trehalose. No acid production was observed from D-mannitol, D-xylose, L-arabinose, glycerol, D-melezitose and L-rhamnose. Only a few carbohydrates were found to be metabolised: D-ribose, D-melibiose, glycogen, D-tagatose and potassium 5-ketogluconate as revealed using API 50 CH strips. The other tested carbohydrates (glycerol, erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, D-adonitol, methyl-βD-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-αD-mannopyranoside, methyl-αD-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, esculin ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-sucrose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, xylitol, gentiobiose, D-turanose, D-lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, and potassium 2-ketogluconate) were not utilised. Differential characteristics between strain Mt12^T and close relatives are presented in Table 3. Strain Mt12^T differed from these species in terms of several phenotypic characteristics including salt tolerance and habitat. Strikingly, fructose was not metabolised in contrast to most of the compared species.

Table 2 Cellular fatty acid composition (%)

Fatty acids	Name	Mean relative %[a]
14:0	Tetradecanoic acid	46.4 ± 3.4
16:1n7	9-Hexadecenoic acid	20.5 ± 2.4
16:0	Hexadecanoic acid	18.1 ± 0.6
16:1n9	7-Hexadecenoic acid	6.9 ± 1.0
4:0	Butanoic acid	3.1 ± 0.6
18:1n9	9-Octadecenoic acid	1.8 ± 0.3
18:0	Octadecanoic acid	1.7 ± 0.3
18:2n6	9,12-Octadecadienoic acid	TR
14:1	Tetradecenoic acid	TR

TR trace amounts <1%

[a] Mean peak area percentage

Strain Mt12^T was found to be susceptible to rifampicin, gentamicin 500 µg, tobramycin, penicillin G, oxacillin, ceftriaxone, doxycycline, and ciprofloxacin but resistant to gentamicin 30 µg, imipenem, trimethoprim/sulfamethoxazole, teicoplanin, metronidazole, colistin, and erythromycin.

Genome properties

The draft genome of strain Mt12^T is 3,497,275-bp long with a 30.45% G+C content (Fig. 4; Table 4). It is composed of 14 scaffolds and 15 contigs. Of the 3397 predicted genes, 3268 are protein-coding genes and

Table 3 Differential characteristics of *Inediibacterium massiliense* strain Mt12^T DSM 100590, *Crassaminicella profunda* strain Ra1766H^T DSM 27501, *Salimesophilobacter vulgaris* Zn2^T DSM 24770, *Anaerosolibacter carboniphilus* strain IRF19^T JCM 19988, *Geosporobacter subterraneus* strain VNs68^T DSM 17957, *Thermotalea metallivorans* strain B2-1^T DSM 21119, *Caminicella sporogenes* strain AM1114^T DSM 14501, *Clostridium caminithermale* strain DVird3^T DSM 15212, *Clostridium halophilium* strain W6 DSM 5388, *Tepidibacter mesophilus* strain B1^T JCM 16806 (Fendrich et al. 1990; Alain et al. 2002; Brisbarre et al. 2003; Klouche et al. 2007; Ogg and Patel 2009; Tan et al. 2012; Zhang et al. 2013; Lakhal et al. 2015; Hong et al. 2015)

Properties	*Inediibacterium massiliense*	*Crassaminicella profunda*	*Salimesophilobacter vulgaris*	*Anaerosalibacter carboniphilus*	*Geosporobacter subterraneus*
Cell diameter (μm)	0.5–0.7	1.0–2.0	0.5–0.8	0.4–0.6	0.5
Gram stain	+	+	+	−	+
Salt tolerance	−	+	+	+	+
Motility	+	+	+	+	−
Endospore formation	+	+	−	−	+
Major cellula fatty acid	14:0	14:0	15:0	iso 15:0	na
Acid from					
L-Arabinose	−	−	−	na	na
Ribose	+	−	−	+	+
Mannose	+	+	−	+	−
Mannitol	−	−	−	+	−
Sucrose	−	−	−	na	na
D-Glucose	+	+	−	+	+
D-Fructose	−	−	−	+	+
D-Maltose	+	na	−	na	na
Habitat	Human gut	Sediments	Wastewater	Soil	Water

Properties	*Thermotalea metallivorans*	*Caminicella sporogenes*	*Clostridium caminithermale*	*Clostridium halophilium*	*Tepidibacter mesophilus*
Cell diameter (μm)	0.6–0.7	0.5–0.7	0.4–0.5	0.8–1.0	1.1–1.6
Gram stain	−	−	+	+	+
Salt tolerance	+	+	+	+	+
Motility	+	+	+	+	+
Endospore formation	−	+	+	+	+
Major cellula fatty acid	na	na	na	na	iso 15:0
Acid from					
L-Arabinose	+	na	−	na	−
Ribose	+	na	+	+	+
Mannose	+	na	+	+	−
Mannitol	−	na	−	+	−
Sucrose	+	na	−	na	−
D-Glucose	+	+	+	−	+
D-Fructose	+	−	+	+	+
D-Maltose	+	+	+	na	+
Habitat	Water	Chimney rocks	Chimney rocks	Sediments	Soil

na non available data, *w* weak reaction

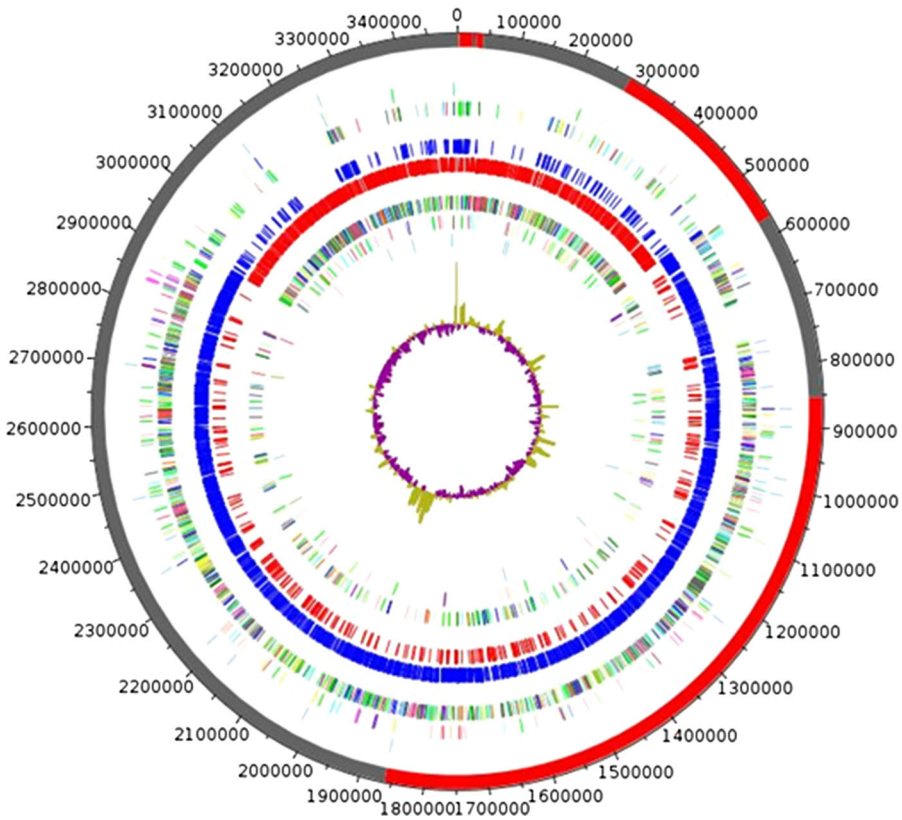

Fig. 4 Graphical circular map of the chromosome. *From outside to the center* Genes on the forward strain colored by COG categories (only gene assigned to COG), RNA genes (tRNAs *green*, rRNAs *red*), G+C content and G+C skew. *COGs* clusters of orthologous groups database

Table 4 Nucleotide content and gene count levels of the genome

Attribute	Genome (total)	
	Value	% of total[a]
Size (bp)	3,497,275	100
G+C content (%)	1,064,697	30.45
Coding region (bp)	3,070,894	87.80
Total genes	3397	100
RNA genes	129	3.79
Protein-coding genes	3268	96.20
Genes with function prediction	2322	70.65
Genes assigned to COGs	2069	71.44
Genes with peptide signals	346	10.18
CRISPR repeats	3	0.08
ORFans genes	272	8.0
Genes with transmembrane helices	831	24.46
Genes associated with PKS or NRPS	04	0.11
No of antibiotic resistance genes	0	0
No. of genes associated with Pfam-A domains	3042	89.54

[a] The total is based on either the size of the genome in base pairs or the total number of protein-coding genes in the annotated genome

129 are RNAs (10 5S rRNAs, 8 16S rRNAs, 4 23S rRNAs, 107 tRNAs). A total of 2400 genes (73.4%) were assigned a putative function (through comparison with the COGs or NR databases). A total of 272 genes were identified as ORFans (8.3%). The 448 remaining genes were annotated as hypothetical proteins (13.7%). The properties and statistics of the genome are summarised in Table 4 while the distribution of genes into COG functional categories is presented in Table 5.

Genome comparison

Genomic characteristics of strain Mt12^T were compared to those of closely related species with an available genome (Table 6). The genome size of strain Mt12^T (3.49 Mb) is larger than that of *A. oremlandii* (3.12 Mb) but smaller than those of *A. metalliredigens*, *C. aceticum* and *A. transvaalensis* (4.93, 4.2 and 4.02 respectively). The G+C content of strain Mt12^T (30.44%) is lower than those of all compared species (34.0–36.8%). Similarly, the total number of genes (3397) and the number of protein-coding genes (3268) of strain Mt12^T are smaller than that of all compared species except *A. oremlandii* (3016). The distribution of genes into COG categories is similar for all compared species except for the presence of 1 protein in the extracellular structures category which is only present in strain Mt12^T (Fig. 5). While we observed a lower genome size and quantitative content, the similarity in the distribution of proteins into COG categories shows a comparable qualitative content in the genome of all compared species.

Among species with standing in nomenclature, AGIOS values ranged from 68.30 between *A. transvaalensis* and *A. oremlandii* to 69.74 between *C. aceticum* and *A. metalliredigens*. When compared to strain Mt12^T, AGIOS values ranged from 66.56

Table 5 Number of genes associated with the 25 general COG functional categories

Code	Value	% of total[a]	Description
J	173	5.29	Translation
A	0	0.0	RNA processing and modification
K	219	6.70	Transcription
L	189	5.78	Replication, recombination and repair
B	1	0.03	Chromatin structure and dynamics
D	30	0.92	Cell cycle control, mitosis and meiosis
Y	0	0.0	Nuclear structure
V	74	2.26	Defense mechanisms
T	199	6.09	Signal transduction mechanisms
M	139	4.25	Cell wall/membrane biogenesis
N	87	2.66	Cell motility
Z	0	0.0	Cytoskeleton
W	1	0.03	Extracellular structures
U	40	1.22	Intracellular trafficking and secretion
O	77	2.36	Post-translational modification, protein turnover, chaperones
C	154	4.71	Energy production and conversion
G	98	3.00	Carbohydrate transport and metabolism
E	205	6.27	Amino acid transport and metabolism
F	60	1.84	Nucleotide transport and metabolism
H	101	3.09	Coenzyme transport and metabolism
I	54	1.65	Lipid transport and metabolism
P	145	4.44	Inorganic ion transport and metabolism
Q	41	1.25	Secondary metabolites biosynthesis, transport and catabolism
R	298	9.12	General function prediction only
S	228	6.98	Function unknown
–	253	7.44	Not in COGs

[a] The total is based on the total number of protein coding genes in the annotated genome

Table 6 Genome comparison of closely related species to *Inediibacterium massiliense* strain Mt12^T

Organisms	INSDC	Size (Mb)	G+C (%)	Protein coding genes	Total genes
Inediibacterium massiliense strain Mt12^T	CXYX00000000.1	3.49	30.44	3268	3397
Alkaliphilus metalliredigens strain QYMF	CP000724.1	4.93	36.8	4576	4801
Clostridium aceticum strain DSM 1496	CP009687.1	4.2	35.3	3705	3847
Alkaliphilus transvaalensis strain SAGM1	JHYF00000000.1	4.02	34.0	3604	3725
Alkaliphilus oremlandii strain OhILAs	CP000853.1	3.12	36.3	2878	3016

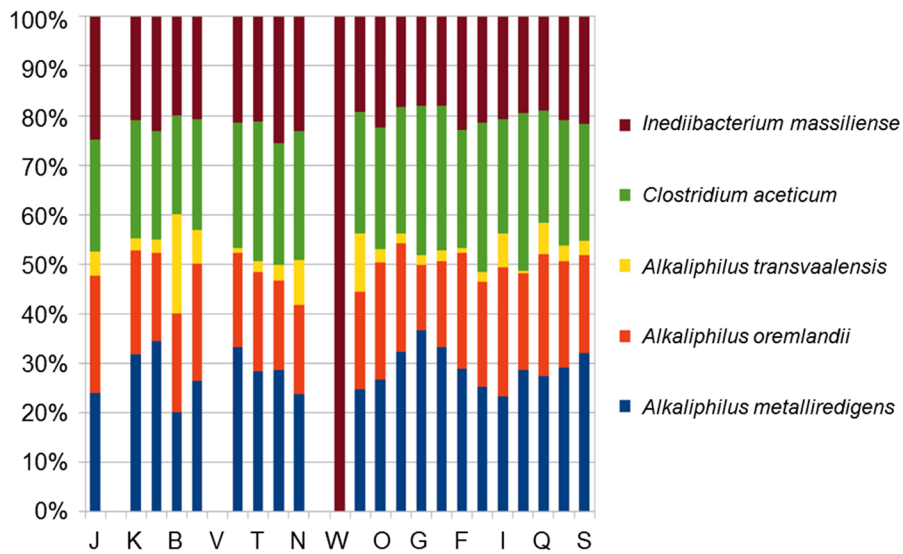

Fig. 5 Distribution of functional classes of predicted genes according to the clusters of orthologous groups of protein

Table 7 The numbers of orthologous proteins shared between genomes (upper right), average percentage similarity of nucleotides corresponding to orthologous proteins shared between genomes (lower left) and numbers of proteins per genome (bold)

	Alkaliphilus metalliredigens	*Alkaliphilus oremlandii*	*Alkaliphilus transvaalensis*	*Clostridium aceticum*	*Inediibacterium massiliense*
Alkaliphilus metalliredigens	**4823**	1395	1796	1512	1357
Alkaliphilus oremlandii	68.38	**2980**	1432	1342	1198
Alkaliphilus transvaalensis	68.82	68.30	**3752**	1664	1312
Clostridium aceticum	69.74	68.52	69.69	**3882**	1305
Inediibacterium massiliense	66.56	66.90	67.23	67.69	**3268**

with *A. metalliredigens* to 67.69 with *C. aceticum* (Table 7). Among species with standing in nomenclature, dDDH values ranged from 12.5% between *A. oremlandii* and *A. transvaalensis* to 26.8% between *A. metalliredigens* and *A. oremlandii*. dDDH values between strain Mt12^T and the compared species ranged from 16.2% with *A. oremlandii* to 29% with *A. metalliredigens* (Supplementary Table 1). These

low dDDH values, along with the AGIOS values, support the status of strain Mt12^T as representative of a putative new genus. However, additional comparative genomics, notably with *C. profunda*, are desirable as additional sequences become available.

Conclusion

Considering the specific phenotypic properties of strain Mt12^T, including its low matching MALDI-TOF MS score, the 94.9% 16S rRNA similarity level with *C.profunda*, and its genomic analysis, we hereby suggest the creation of a new genus within the family *Clostridiaceae* named *Inediibacterium*, with the type species *Inediibacterium massiliense*, type strain Mt12^T (=CSUR P1907 = DSM 100590).

Description of *Inediibacterium* gen. nov

Inediibacterium (In.e.di.i.bac.te'ri,um. L. fem. n. *inedia* fasting; N.L. neut. n. *bacterium* rod; N.L. neut. n. *Inediibacterium* rod associated with abstinence from food).

Strictly anaerobic, spore-forming, Gram-stain positive rod-shaped bacteria. Oxidase and catalase negative. Urease negative. β-glucosidase positive. Indole is produced. Optimal growth temperature is 37 °C and no salt tolerance is observed. The major cellular fatty acid is tetradecanoic acid. The G+C content of the type strain of the type species is 30.44%. The type species is *Inediibacterium massiliense.*

Description of *Inediibacterium massiliense* sp. nov

Inediibacterium massiliense (mas.si.li.en'se. L. masc. adj., *massiliense*, of Massilia, the Latin name of Marseille, where the type strain was first isolated).

Motile bacilli with a mean length of 6.9 μm and a mean diameter of 0.5 μm. Forms irregular translucent colonies with a white center and a mean diameter of 3 mm. The spore position is terminal, causing a swelling of the cell. Mesophilic. The draft genome of the type strain has a G+C content of 30.45%. The 16S rRNA and draft genome sequences are available in the EBI/EMBL database under Accession No. LN850734 and CXYX00000000, respectively. The type strain Mt12^T (=CSUR P1907 = DSM 100590) was isolated from the stool sample of a 7-month-old girl from Senegal with Kwashiorkor.

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Compliance with ethical standards See Methods

Conflict of interest The authors declare no conflict of interest.

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