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Taxono-genomics and description of *Haloimpatiens massiliensis* sp. nov., a new bacterium isolated from the gut of a healthy infant

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Abstract

A polyphasic taxono-genomic strategy was used to describe a new bacterium, strain Marseille-PI935; isolated from the gut of a healthy infant. 16S rRNA sequencing showed that the isolate belongs to the genus *Haloimpatiens* in the family *Clostridiaceae*. Phenotypic analysis and whole-genome sequence analyses confirm the status of the new species. We propose the creation of the new species *Haloimpatiens massiliensis* strain Marseille-PI935^T (= CSURPI935^T; = DSM100591^T).

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Introduction

Culturomics is a concept of developing different culture conditions in order to enlarge our knowledge of the human microbiota through the discovery of previously uncultured bacteria [1–4]. Once isolated, bacterial strains are characterized using the taxono-genomic approach that combines matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), phylogenetic analysis, main phenotypic description and genome sequencing [5,6].

Isolation and growth conditions

In 2014, we isolated an unidentified bacterial strain from a stool sample collected from a 7-month-old healthy girl from Niger with a weight-for-height z score of 0.15. The child's

parents gave an informed consent to participating in the study. Screening was made by MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [7]. The obtained spectrum (Fig. 1) was imported into the MALDI Biotyper 3.0 software (Bruker Daltonics) and analysed against the reference spectra of bacteria included in two databases (Bruker and the constantly updated URMS databases (<https://www.mediterranee-infection.com/urms-data-base/>)). The study was validated by the ethics committee of the Institut Fédératif de Recherche 48 under number 09-022. The initial growth was obtained after 72 h of culture in 5% sheep-blood-enriched Columbia agar (bioMérieux, Marcy l'Étoile, France) in strict anaerobic conditions at 37°C medium.

Phenotypic characteristics

Colonies were irregular, large and greyish with a mean diameter of 8 mm. Bacterial cells were Gram-positive, rod-shaped, with a mean length of 2.99 µm and a mean width of 0.67 µm (Fig. 2). Strain Marseille-PI935 showed catalase-negative and oxidase-negative activities. API 50CH and ZYM were performed under strict anaerobic conditions at 37°C (Table 1).

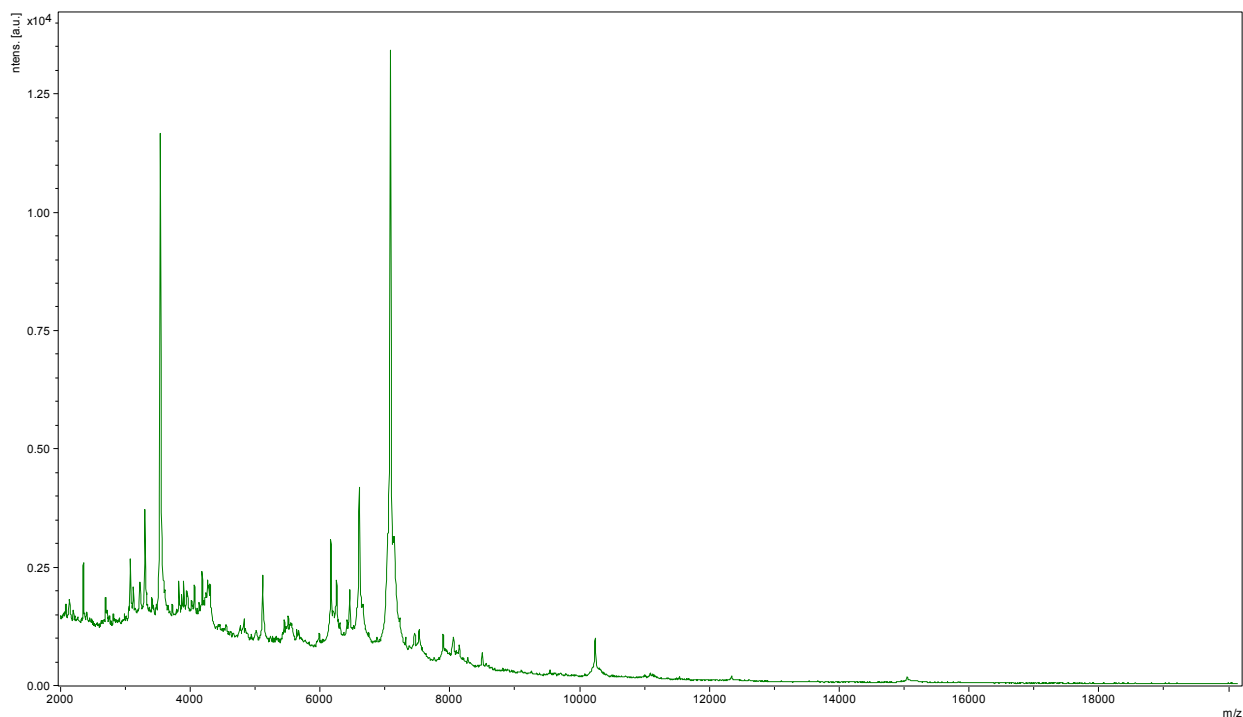


FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

Strain identification

The 16S rRNA gene was sequenced to classify this bacterium. Amplification was done using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequencing used the Big Dye® Terminator v1.1 Cycle Sequencing Kit and ABI Prism

3130xl Genetic Analyzer capillary sequencer (ThermoFisher, Saint-Aubin, France), as previously described [8]. The 16S rRNA nucleotide sequence was assembled and corrected using the CODONCODE ALIGNER software (<http://www.codoncode.com>). In 2017, strain Marseille-P1935 was considered as a representative of a new bacterial species within a new genus for which the name *Khelaifiella massiliensis* was proposed [9]. However, this name was not validly published and, in the present study, we obtained a 99.12% 16S rRNA nucleotide identity between strain Marseille-P1935 and *Haloimpatiens lingqiaonensis* strain JCM-19210^T (GenBank accession number KC869664), the phylogenetically closest species with standing in nomenclature (Fig. 3) [10]. We consequently deduced that strain Marseille-P1935 was most likely a member of the *Haloimpatiens* genus. However, in a previous work, we had demonstrated that 16S rRNA-based nucleotide similarity is often unable to classify adequately bacterial isolates at the species level [11]. Therefore, genomic analyses were performed to verify the status of strain Marseille-P1935 within the genus *Haloimpatiens*.

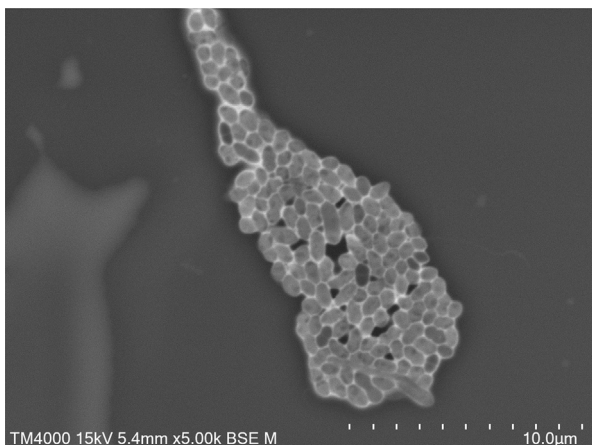


FIG. 2. Scanning electron micrograph of *Haloimpatiens massiliensis* sp. nov. using a TM4000 microscope (Hitachi). Scale bar and acquisition settings are shown on the micrograph.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit

TABLE 1. Biochemical tests of *Haloimpatiens massiliensis* sp. nov. (API 50 CH (a) and API ZYM (b))

Test	Results (+/-)
API 50 CH	
(a) Bacterium: <i>Haloimpatiens massiliensis</i> sp. nov	
Control	-
Glycerol	-
Erythrol	-
D-arabinose	-
L-arabinose	-
D-ribose	-
D-xylose	-
L-xylose	-
D-adonitol	-
Methyl-β-D-xylopyranoside	-
D-galactose	+
D-glucose	+
D-fructose	+
D-mannose	+
L-sorbose	+
L-rhamnose	+
Dulcitol	+
Inositol	+
D-mannitol	+
D-sorbitol	+
Methyl-α-D-mannopyranoside	+
Methyl-α-D-glucopyranoside	+
N-acetylglucosamine	+
Amygdaline	+
Arbutine	+
Esculine	+
Salicine	+
D-cellobiose	+
D-maltose	+
D-lactose	+
D-melibiose	+
D-saccharose	+
D-trehalose	+
Inuline	+
D-melezitose	+
D-raffinose	+
Amidon	+
Glycogene	+
Xylitol	+
Gentibiose	+
D-turanose	+
D-lyxose	+
D-tagatose	+
D-fucose	+
L-fucose	+
D-arabitol	+
L-arabitol	+
Potassium gluconate	+
Potassium 2-ketogluconate	+
Potassium 5-ketogluconate	+
API ZYM	
(b) Bacterium: <i>Haloimpatiens massiliensis</i> sp. nov	
Control	-
Alkaline phosphatase	+
Esterase (C 4)	+
Esterase lipase (C 8)	-
Lipase (C 14)	-
Leucine arylamidase	+
Valine arylamidase	-
Cystine arylamidase	-
Trypsine	-
α-chymotrypsine	+
Acid phosphatase	+
Naphthalo-AS-BI-phosphohydrolase	+
α-galactosidase	-
β-galactosidase	-
β-glucuronidase	-
α-glucosidase	-
β-glucosidase	-
N-acetyl-β-glucosaminidase	-
α-mannosidase	-
α-fucosidase	+

and then sequenced on the MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera Mate Pair sample prep

kit (Illumina), as previously described [12]. The assembly was performed with a pipeline incorporating different softwares (VELVET [13], SPADES [14] and SOAP DENOVO [15]), on trimmed data (MiSEQ and TRIMMOMATIC [16–19] 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 N). The genome of strain Marseille-PI935 was 4 214 685 bp long with a 29.8 mol% G + C content and 28 contigs. The degree of genomic similarity of strain Marseille-PI935 with closely related species was estimated using the ORTHOANI software [20–22]. Strain Marseille-PI935 exhibited an 87.18% ORTHOANI value with *H. lingqiaonensis* (Fig. 4). An ANI value < 95% between two strains suggests that they belong to distinct species. Using the Genome-to-Genome Distance Calculation software [23–25], the digital DNA-DNA hybridization (dDDH) of strain Marseille-PI935 with *H. lingqiaonensis* was 34.9% (Table 2). This value was lower than the 70% threshold used for delineating prokaryotic species. Finally, the phylogenetic tree based on the genome-to-genome distance showed that strain Marseille-PI935 and *H. lingqiaonensis* belonged to distinct species (Fig. 5). Therefore, these various analyses suggested that strain Marseille-PI935 could be classified as a member of a new species within the genus *Haloimpatiens*, in the family Clostridiaceae and the phylum Firmicutes.

Conclusion

Strain Marseille-PI935^T exhibiting ORTHOANI and dDDH values lower than 95% and 70%, respectively, with its phylogenetically closest species with standing in nomenclature, is consequently proposed as the type strain of the new species *Haloimpatiens massiliensis* sp. nov.

Description of *Haloimpatiens massiliensis* sp. nov

Haloimpatiens massiliensis (mas.si.li.en'sis; L. masc. adj. massiliensis for Massilia, the Roman name of Marseille, where strain Marseille-PI935^T was first isolated). Bacterial cells are Gram-positive, rod-shaped, with a mean length of 2.99 μm and a mean width of 0.67 μm. Colonies grown on 5% sheep-blood-enriched Columbia agar (bioMérieux) are irregular, large and greyish after 72 hours of incubation in a strict anaerobic atmosphere, with a mean diameter of 8 mm. Growth occurs at 37°C. Cells grow anaerobically only.

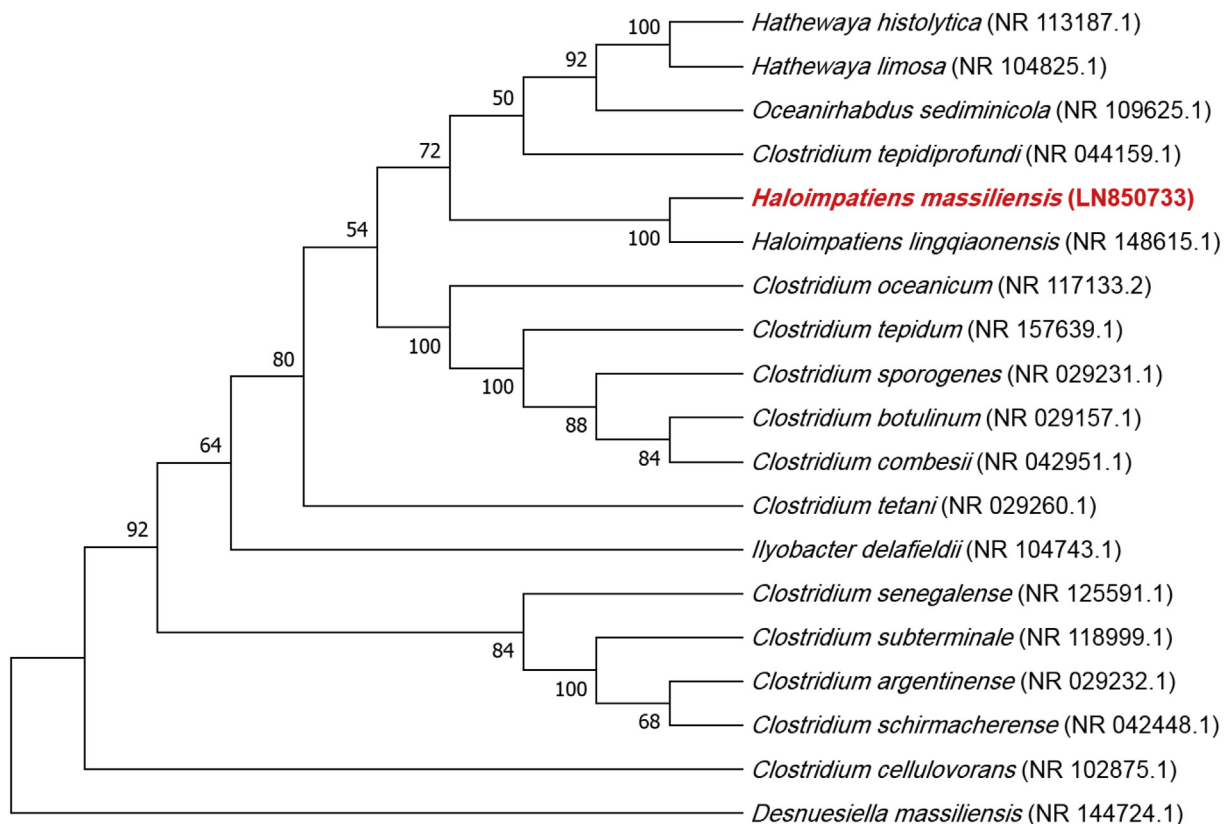


FIG. 3. Phylogenetic tree showing the position of *Haloimpatiens massiliensis* sp. nov. strain Marseille-PI935^T relative to other phylogenetically close species. The respective GenBank accession numbers for 16S rRNA genes are indicated in parentheses. Sequences were aligned using MUSCLE v3.8.3.1 with default parameters and phylogenetic inferences were obtained using the maximum likelihood method within the 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.

Strain Marseille-PI935^T exhibits catalase-negative and oxidase-negative activities. Using an API ZYM strip, positive reactions are observed for esterase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, alkaline and acid phosphatases, α -fucosidase and α -chymotrypsin but negative reactions are obtained for esterase lipase, lipase, valine arylamidase, cystine arylamidase, trypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase and α -mannosidase activities. Using an API 50 CH strip, strain Marseille-PI935 is able to metabolize D-galactose, D-maltose, D-tagatose, D-glucose, D-fructose, D-mannose, methyl- α D-glucopyranoside, *N*-acetylglucosamine, D-lactose, D-saccharose, D-trehalose, D-turanose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl- α D-mannopyranoside,

amygdalin, arbutin, esculin, salicin, D-cellobiose, D-melibiose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 5-ketogluconate and potassium 2-ketogluconate. However, negative reactions are obtained for glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, Kd-xylose, L-xylose, D-adonitol and methyl- β D-xylopyranoside. The genome is 4 214 685-bp long and its G + C content is 29.8%.

The type strain, Marseille-PI935^T, isolated from a stool sample collected from a 7-month-old healthy girl from Niger with a weight-for-height z score of 0.15, was deposited in the CSUR and DSMZ collections under accession numbers CSUR PI935 and DSM I00591, respectively.



Heatmap generated with OrthoANI values calculated from the OAT software. Please cite Lee et al. 2015.

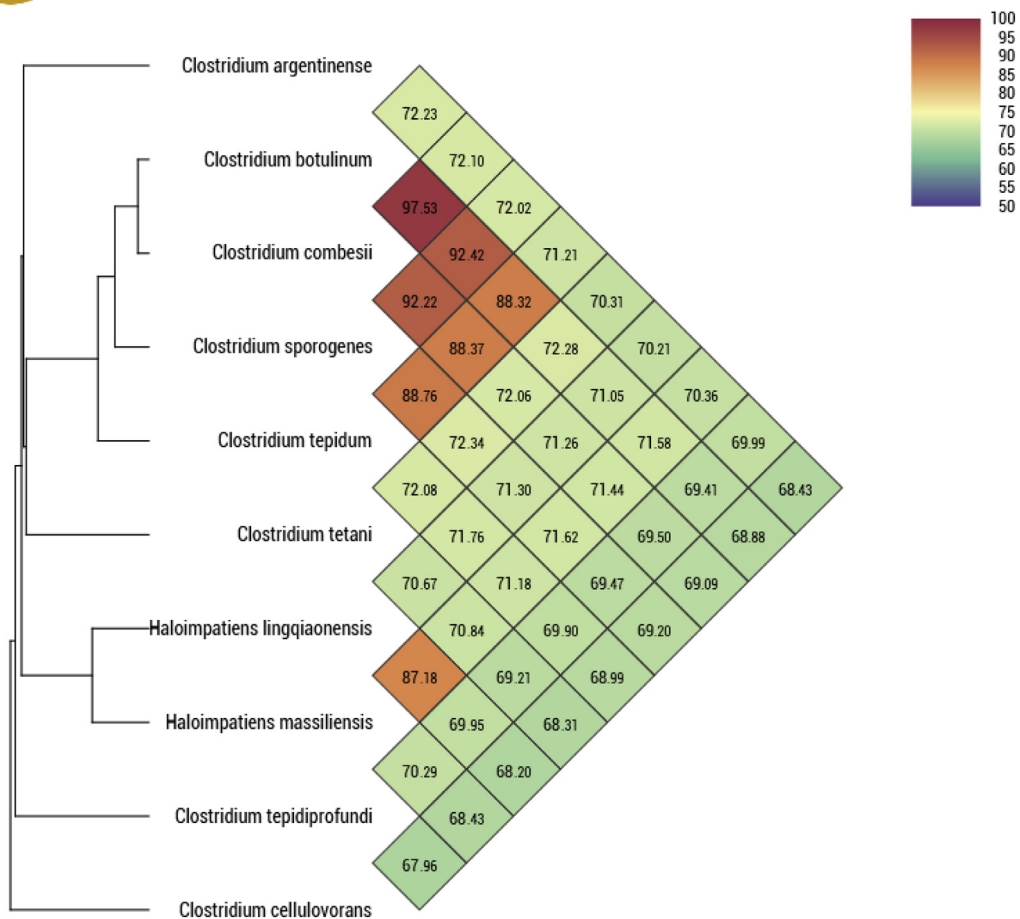


FIG. 4. Heatmap generated with ORTHOANI values calculated using the OAT software between *Haloimpatiens massiliensis* sp. nov. and other closely related species with standing in nomenclature.

TABLE 2. dDDH values obtained by comparison of all studied genomes

	1	2	3	4	5	6	7	8	9	10
<i>C. argentinense</i>	100	28.2	22.2	27	27	19.4	24.2	21.6	20.8	21.5
<i>C. botulinum</i>		100	24.1	77.7	47.3	21.7	35.4	20.7	20.9	22.4
<i>C. cellulovorans</i>			100	22.9	26.5	22.8	25.7	20.8	21.6	24.2
<i>C. combesii</i>				100	46.5	19.8	35.3	20.3	20.4	21.4
<i>C. sporogenes</i>					100	21.8	36	21.7	21.2	21.8
<i>C. tepidiprofundum</i>						100	21	19.8	19.3	21.8
<i>C. tepidum</i>							100	19.5	21.5	21.2
<i>C. tetani</i>								100	19.3	21.2
<i>H. lingqiaonensis</i>									100	34.9
<i>H. massiliensis</i>										100

1, *Clostridium argentinense*; 2, *Clostridium botulinum*; 3, *Clostridium cellulovorans*; 4, *Clostridium combesii*; 5, *Clostridium sporogenes*; 6, *Clostridium tepidiprofundum*; 7, *Clostridium tepidum*; 8, *Clostridium tetani*; 9, *Haloimpatiens lingqiaonensis*; 10, *Haloimpatiens massiliensis*.

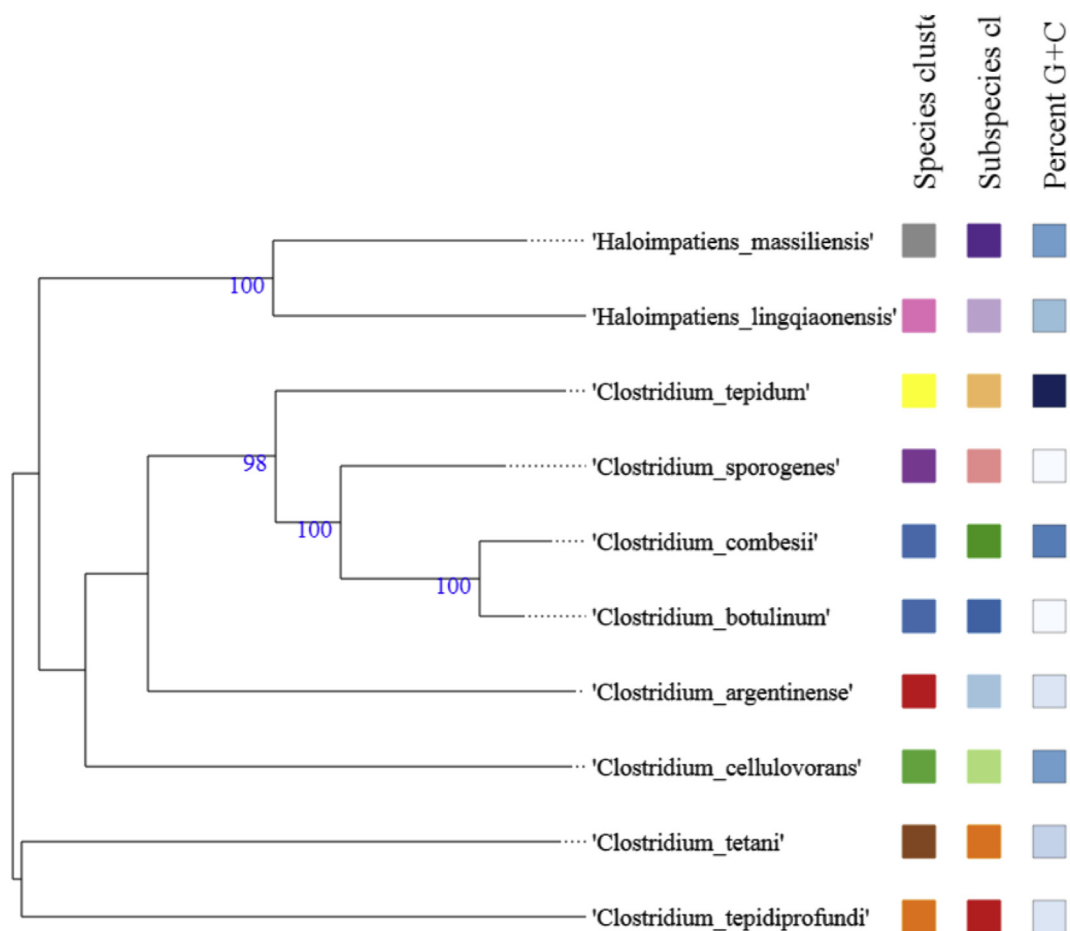


FIG. 5. Phylogenetic tree showing the position of *Haloimpatiens massiliensis* sp. nov. strain Marseille-P1935^T relative to other phylogenetically close species on the basis of the genome-to-genome distance. Tree inferred with FASTME 2.1.6.1 (23) from genome BLAST distance phylogeny (GBDP) distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d_5 . The numbers above branches are GBDP pseudo-bootstrap support values > 60% from 100 replications, with an average branch support of 65.6%. The tree was rooted at the midpoint [24].

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession number LN850733 and FXWX00000000.1, respectively.

Deposit in culture collections

Strain Marseille-P1935^T was deposited in two different strain collections under number (= CSURP1935; = DSM100591).

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Conflict of interest

None to declare.

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