Antonie van Leeuwenhoek (2020) 113:997–1008
https://doi.org/10.1007/s10482-020-01414-5

ORIGINAL PAPER

Draft genome and description of *Negativicoccus massiliensis* strain Marseille-P2082, a new species isolated from the gut microbiota of an obese patient

Amadou Hamidou Togo · Awa Diop · Mamadou Lamine Tall · Matthieu Million · Saber Khelaifia · Marie Maraninchi · Didier Raoult · Pierre-Edouard Fournier · Grégory Dubourg

Received: 20 June 2019 / Accepted: 7 April 2020 / Published online: 17 April 2020

Abstract Strain Marseille-P2082, an anaerobic, non-motile, asporogenous, Gram-negative, coccoid bacterium was isolated from the faeces of a 33 year-old obese French woman before bariatric surgery. The isolate exhibits 98.65% 16S rRNA gene nucleotide sequence similarity with *Negativicoccus succinicivorans* strain ADV 07/08/06-B-1388^T, its current closest phylogenetic neighbour with standing in nomenclature. However, the dDDH relatedness between the new isolate and *N. succinicivorans* type strain ADV 07/08/06-B-1388^T is 52.5 ± 2.7%. Strain Marseille-P2082 has a genome of 1,360,589 bp with a 51.1% G+C content. Its major fatty acids were identified as $C_{18:1n9}$, $C_{18:0}$ and $C_{16:0}$. Based on its phenotypic, genomic and phylogenetic characteristics, strain Marseille-P2082^T [= CSURP2082 (Collection de Souches de l'Unité des Rickettsies) = DSM 100853] is proposed as the type strain of the novel species *Negativicoccus massiliensis* sp. nov. The 16S rRNA gene sequence and whole-genome shotgun sequence have been deposited in EMBL-EBI under accession numbers LN876651 and LT700188, respectively.

Keywords Culturomics · Taxonomy · *Negativicoccus massiliensis* · Human gut microbiota · Obesity · Bariatric surgery

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10482-020-01414-5) contains supplementary material, which is available to authorized users.

A. H. Togo · M. L. Tall · M. Million · S. Khelaifia · D. Raoult · G. Dubourg (✉)
IRD198, APHM, MEPHI, IHU-Méditerranée Infection, Aix Marseille Univ, 19-21 Boulevard Jean Moulin, 13385 Marseille cedex 05, France
e-mail: gregory.dubourg@univ-amu.fr

A. H. Togo · M. L. Tall · M. Million · S. Khelaifia · D. Raoult · P.-E. Fournier · G. Dubourg
IHU Méditerranée Infection, Marseille, France

A. Diop · P.-E. Fournier
IRD, SSA, VITROME, IHU-Méditerranée Infection, Aix Marseille Univ, Marseille, France

M. Maraninchi
NORT "Nutrition, Obesity and Risk of Thrombosis", INSERM1062, INRA1260, Aix Marseille Université, Marseille, France

Introduction

Obesity is a major public health problem that increases the risk of several diseases such as metabolic diseases (type II diabetes) and cardiovascular diseases. According to Ng et al. (2014), the world rate of obesity has increased between 1980 and 2013. Obesity results from an imbalance between feed quantity intake and energy expenditure. This process involves both genetic and environmental factors (Frayling et al. 2007; Cecil et al. 2008). More recently, obesity has been associated with an imbalance of the gut

microbiota composition (Ley et al. 2006; Turnbaugh et al. 2006; Million et al. 2012).

Several methods of treating obesity have been proposed, mainly dietary and lifestyle measures, medical and surgical treatments. Bariatric surgery is one of the most effective treatments for obesity. It is also associated with an increase in microbial diversity (Zhang et al. 2009; Kong et al. 2013).

For the purpose of assessing gut microbiota dynamics from obese people before and after bariatric surgery, we studied the gut microbiota of stool samples from obese subjects before and after bariatric surgery by culturomics (Lagier et al. 2015). During this study, we isolated a new anaerobic, Gram-negative coccus strain Marseille-P2082^T, from a stool sample of a 33 year-old French woman living in Marseille after bariatric surgery. This new bacterium has been previously reported as a new species announcement without a thorough description and was provisionally named "*Negativicoccus massiliensis*" strain AT7 (Togo et al. 2016c).

Here, we describe the phenotypic characteristics of strain Marseille-P2082^T (formerly AT7) together with the description of its complete genome sequencing and annotation.

Material and methods

Sample collection, strain Marseille-P2082^T isolation and identification

A stool sample was collected in November 2011 from a 33 year-old obese French woman with a body mass index of 38.6 kg/m^2. Written and informed consent was obtained from the patient at the Nutrition, Metabolic disease and Endocrinology service, in la Timone Hospital, Marseille, France. The study and the assent procedure were approved by the local ethics committee of IFR 48, under number 09-022. The stool sample was stored at −80 °C after collection and studied by a microbial culturomics approach.

The strain was isolated under anaerobic conditions. To isolate this new strain, 1 g of stool sample was injected into an anaerobic blood culture vial (BACTEC Lytic/10 vials of anaerobic culture/F), enriched with 4 ml (5%) of rumen juice sterilised by filtration and 4 ml (5%) of sheep blood, then incubated at 37 °C. A one-month follow-up was conducted as described elsewhere (Togo et al. 2017). Emerged colonies were cultivated under the same conditions for isolation and then identified by MALDI-TOF as described elsewhere (Seng et al. 2009).

Negativicoccus succinicivorans strain ADV 07/08/06-B-1388^T (Marchandin et al. 2010) was generously provided by Professor Hélène Marchandin (HydroSciences Montpellier, CNRS, IRD, University of Montpellier, Department of Microbiology, Nîmes University Hospital, France) and cultured under comparable conditions.

Phylogenetic analysis

The 16S rRNA gene sequencing of the strain Marseille-P2082 was performed as previously reported (Drancourt et al. 2000) using the fD1-rP2 primers, a GeneAmp PCR System 2720 thermal cycler (Applied Bio systems, Bedford, MA, USA) and an ABI Prism 3130-XL capillary sequencer (Applied Biosciences, Saint Aubin, France).

For taxonomic assignment, CodonCode Aligner (101 Victoria Street Centerville, MA 02632, USA) was used to correct sequences and BLASTn searches was performed in the NCBI (National Centre for Biotechnology Information) web server at http://blast.ncbi.nlm.nih.gov.gate1.inist.fr/Blast.cgi using the 16S ribosomal RNA sequences (Bacteria and Archaea) database and excluding all sequences from uncultured species. Only reference sequences from the type strains of the closely related validly named species were considered. Sequences alignment was performed by a multiple sequence alignment program, ClustalW (Thompson et al. 2002). The Maximum Likelihood method based on the Kimura 2-parameter model was used to infer the tree. Rapid bootstrapping in conjunction with the autoMRE bootstopping criterion (Pattengale et al. 2010) and subsequent search for the best tree was used; for Maximum Parsimony analysis, 1000 bootstrapping replicates were used in conjunction with tree-bisection-and-reconnection branch swapping and ten random sequence addition replicates. Reliability of the nodes was estimated as to the percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a consensus tree. The sequences were checked for a compositional bias using the X^2 test as implemented in PAUP* (Swofford 2002).

Phenotypic and chemical characteristic analysis

Colony morphology and pigmentation were observed after cultivation of the strain on Columbia agar (bioMérieux, Marcy l'Etoile, France) at 37 °C for 48 h. Phenotypic characteristics of the strain was determined as previously described (Togo et al. 2016a, 2017). API strips: (API® 20A, API® Rapid ID 32A, API® ZYM, API® and API®50 CH) were used according to the manufacturer's instruction (bioMérieux). Growth temperature range from 25, 28, 37, 45 and 55 °C was tested in aerobic, anaerobic and microaerophilic conditions. The growth of strain Marseille-P2082 was attempted at various pH values (6, 6.5, 7 and 8.5). The salt tolerance of the strain was also tested using various NaCl concentrations (5, 10, 50, 75 and 100 g/l) on Schaedler agar enriched with 5% Sheep Blood (bioMérieux) in an anaerobic atmosphere at 37 °C. Anaerobic conditions were generated by incubating the culture in an anaerobic jar using the GENbag anaer system (bioMérieux). Cellular fatty acid methyl ester analysis was performed by Gas Chromatography/Mass Spectrometry (GC/MS) as described elsewhere (Togo et al. 2017).

Genome sequencing and assembly

Genomic DNA (gDNA) of strain Marseille-P2082 and *N. succinicivorans* strain ADV 07/08/06-B-1388^T was sequenced using the MiSeq Technology (Illumina Inc, San Diego, CA, USA) with the Mate Pair strategy and assembled as previously described (Togo et al. 2016b).

Genome annotation and comparison

For the genome annotation of strain Marseille-P2082, Open Reading Frames (ORFs) were predicted using Prodigal (Hyatt et al. 2010) and the predicted protein sequences were searched against the GenBank and Clusters of Orthologous Groups (COGs) databases by BLASTP as previously described (Togo et al. 2017). The draft genome of *N. succinicivorans* strain ADV 07/08/06-B-1388^T was obtained in this study. For comparison, genomes were automatically retrieved for closely related species in the 16S rRNA tree: *Dialister micraerophilus* strain DSM 19965 (NZ_AFBB00000000), *Dialister pneumosintes* strain F0677 (NZ_CP017037), *Dialister succinatiphilus* strain YIT 11850 (NZ_ADLT00000000), *Dialister invisus* strain DSM 15470 (NZ_ACIM00000000) and *Selenomonas bovis* strain DSM 23594 (NZ_ARLB01000000) were selected. For each selected genome, complete genome sequence, proteomes sequences and Orfeomes sequences were retrieved from the FTP of NCBI. As a part of the taxonogenomics description, all proteomes were analysed with proteinOrtho (Lechner et al. 2011). Then for each pair of genomes, a similarity score was computed. This score is the mean value of nucleotide similarity between all pairs of orthologues between the two genomes studied (Ramasamy et al. 2014). An annotation of the entire proteome was performed to define the distribution of functional classes of predicted genes according to the clusters of orthologous groups of proteins. The genome of strain Marseille-P2082 was locally aligned 2-by-2 using BLSAT algorithm (Kent 2002; Auch et al. 2010) against each of the selected previously cited genomes and digital DNA-DNA hybridization (dDDH) values were estimated from a generalised model (Meier-Kolthoff et al. 2013), and the Orthologous Average Nucleotide Identity (OrthoANI) were calculated using Orthologous Average Nucleotide Identity Tool (OAT) software (Lee et al. 2016).

Results and discussion

MALDI-TOF analysis

The reference spectrum generated from isolated colonies was unable to be matched with any of those in the Bruker database (Fig. 1). A gel view was performed in order to detect the spectral differences of strain Marseille-P2082 and closely related species (Fig. 2).

Phylogenetic analysis

The 16S rRNA phylogenetic analysis showed that strain Marseille-P2082^T exhibits a 98. 65% sequence similarity with *N. succinicivorans* (Marchandin et al. 2010), which is classified in the family *Veillonellaceae* (Rogosa 1971). A Maximum Likelihood phylogenetic tree (Fig. 3) based on 16S rRNA gene sequences showed that strain Marseille-P2082 is closely related to *N. succinicivorans*. The 16S rRNA sequence of

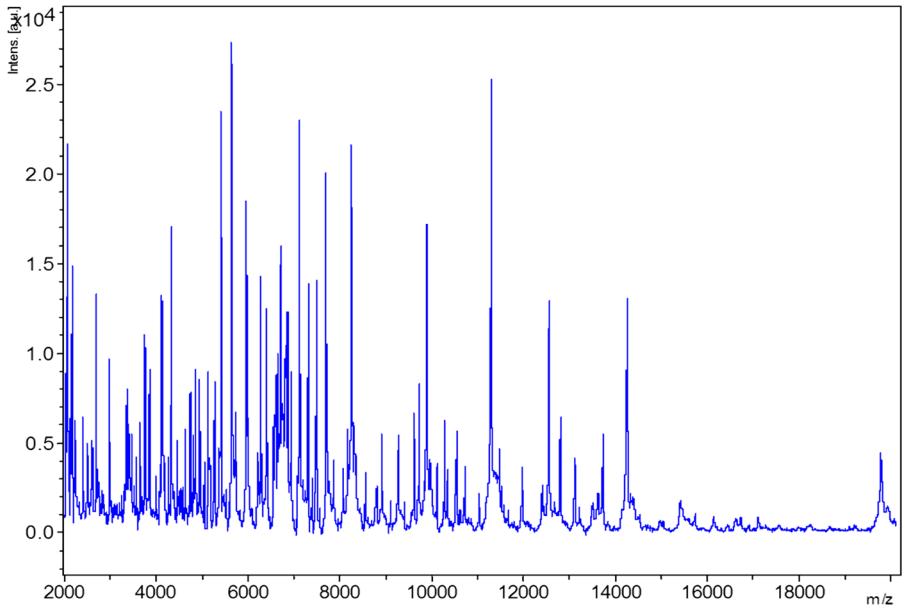

Fig. 1 Reference mass spectrum from strain Marseille-P2082^T. Spectra from 12 individual colonies were compared and a reference spectrum was generated

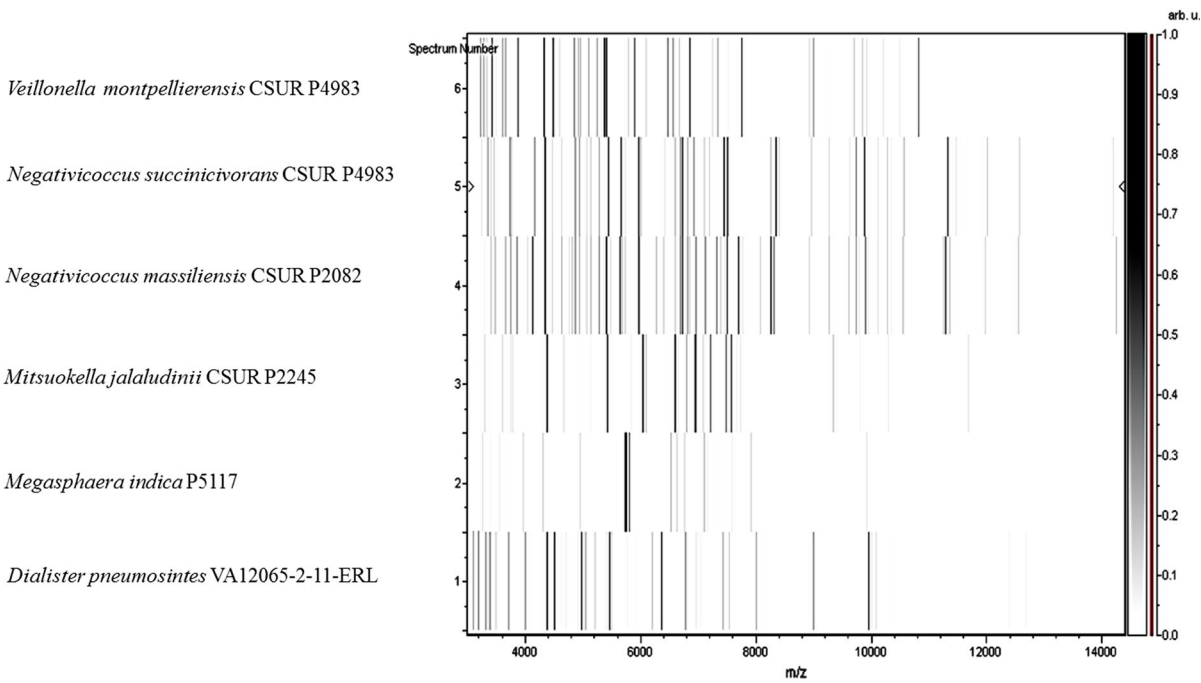

Fig. 2 Gel view comparing strain Marseille-P2082^T to other closely related species. The gel view displays the raw spectra of loaded spectrum files arranged in a pseudo-gel like look. The x-axis records the m/z value. The left y-axis displays the running spectrum number originating from subsequent spectra loading. The peak intensity is expressed by a Grey scale scheme code. The colour bar and the right y-axis indicate the relation between the colour a peak is displayed with and the peak intensity in arbitrary units. Displayed species are indicated on the left

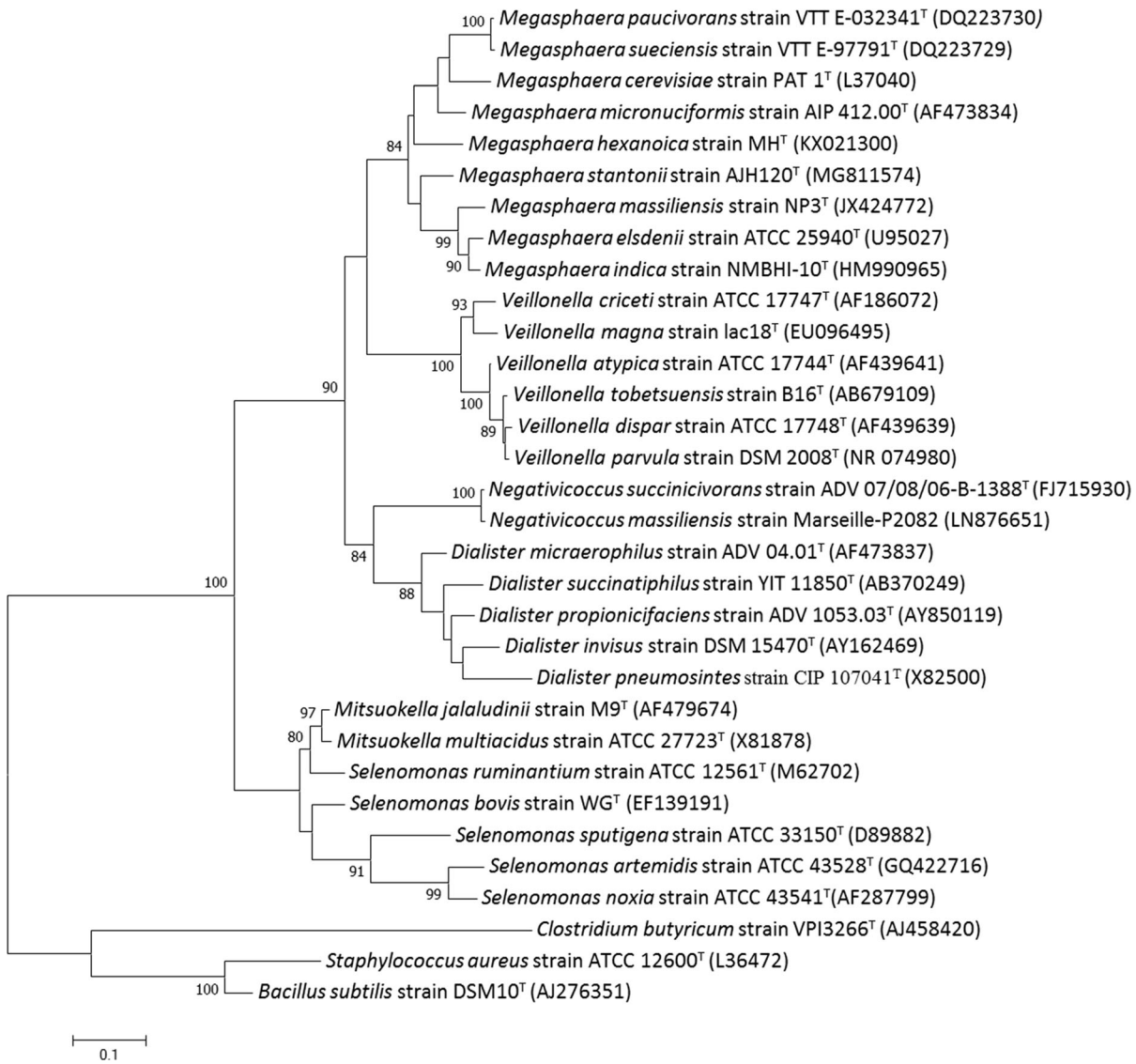

Fig. 3 Maximum Likelihood phylogenetic tree based on 16S rRNA sequence comparison highlighting the position of strain Marseille-P2082^T compared to other closely related species. The corresponding GenBank accession numbers for 16S rRNA genes of each strain are indicated in brackets. Sequences were aligned using ClustalW with default parameters and Phylogenies were inferred by the GGDC web server available at http://ggdc.dsmz.de/ using the DSMZ phylogenomics pipeline. The scale bar represents a 3% nucleotide sequence divergence

strain Marseille-P2082 has been deposited in EMBL-EBI under accession number LN876651.

Phenotypic, biochemical and chemotaxonomic characterisation

Strain Marseille-P2082 was observed to be a Gram-stain negative, non-motile, coccoid bacterium. The strain exhibits no catalase nor oxidase activity. Growth was found to occur between 28 and 55 °C, but optimal growth was observed at 37 °C after 72 h of incubation in both anaerobic and microaerophilic atmospheres on 5% sheep blood Columbia agar (bioMérieux). No growth of the strain was observed in an aerobic atmosphere. No growth of the bacterium was observed using NaCl concentration of 10 g/l or more on 5% sheep blood Schaedler agar (bioMérieux). Strain Marseille-P2082 was found to grow at pH values

Table 1 Differential characteristics of strain Marseille-P2082

Properties	1	2	3	4	5	6	7
Motility	−	−	−	−	+	−	+
Spore formation	−	−	−	−	−	−	−
Microaerophilic growth	+	+	+	−	NR	−	−
Indole production	−	−	−	−	NR	−	−
Nitrate reductase	−	−	+	+	+	+	−
Acid production from							
Aesculin	+	−	−	+	−	+	+
Arabinose	−	−	−	v	−	+	+
Cellobiose	−	−	−	+	−	+	+
Fructose	+	−	−	+	+	+	−
Galactose	+	−	−	+	−	+	−
Glucose	+	−	−	+	+	+	+
Glycerol	+	−	−	+	−	−	−
Inositol	−	−	−	+	−	+	−
Lactose	v	−	−	+	−	+	+
Maltose	+	−	−	+	+	+	−
Mannitol	+	−	−	−	+	+	−
Mannose	+	−	−	+	−	+	+
Melibiose	−	−	−	+	−	+	+
Raffinose	v	−	−	−	w	+	+
Rhamnose	−	−	−	−	−	+	−
Ribose	−	−	−	+	−	+	−
Salicin	v	−	−	+	−	+	+
Sorbitol	−	−	−	+	−	−	−
Sucrose	+	−	−	+	+	+	+
Trehalose	+	−	−	+	−	+	+
Xylose	v	−	−	v	−	+	−
Xylitol	−	−	−	+	−	+	−
Isolation source	Human feces	human clinical samples	Human tongue biofilm	Cattle rumen	Human gingival crevice	Human and Pig feces	Yak rumen
G+C content (mol %)	**51.1**	**48.3**	38.1	58.1	57.3	58.1	59.2

(1) compared with the closely related species *N. succinicivorans* strain ADV (Data from Marchandin et al., 2010) (2); *Dialister micraerophilus* (3); *Dialister invisus* (4); *Dialister succinatiphilus* (5); *Dialister pneumosintes* (6); *Selenomonas bovis* (7)

In bold difference between strain Marseille-P2082 and *N. succinicivorans* strain ADV

+ = positive; − = negative; w = weakly positive; v = variable; NR = data not reported

ranging from 6 to 8.5 but the optimal pH was 7.5. Colonies were observed to be circular, translucent and very small, with a mean diameter less than 0.5 mm. No haemolysis was seen on Columbia agar (bioMérieux) after 72 h of incubation in anaerobic condition at 37 °C. Cells were found to have a diameter ranging from 0.2 to 0.5 µm by electron microscopy (Supplementary Fig. 1).

Using rapid ID 32A strips, arginine dihydrolase, arginine arylaminidase, histidine arylamidase, leucyl

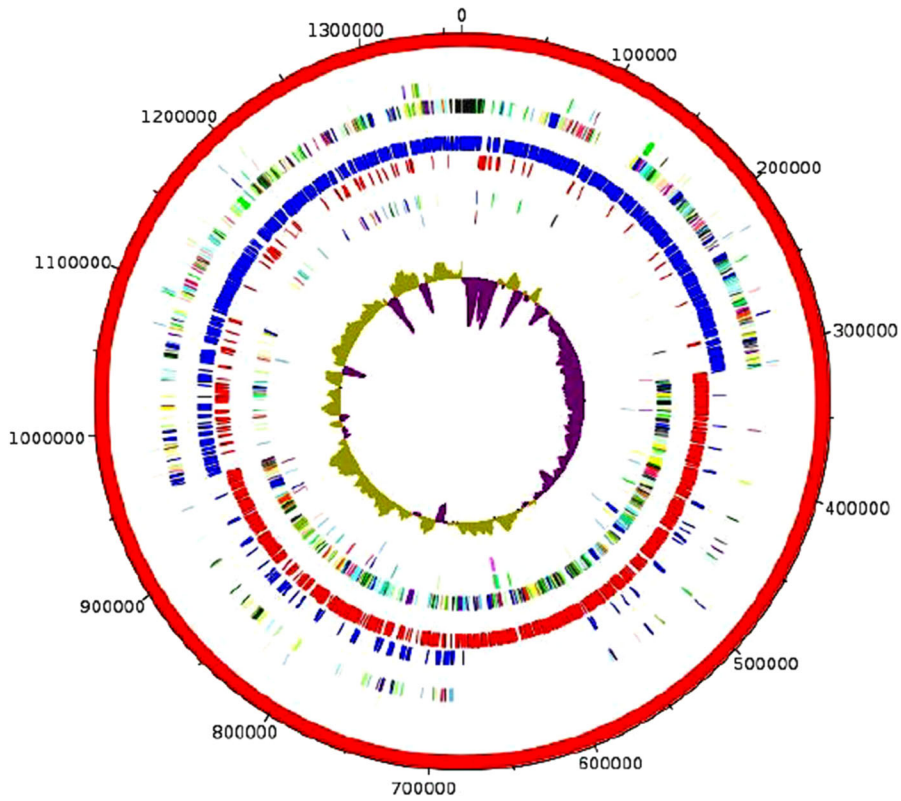

Fig. 4 Graphical circular map of the genome of strain Marseille-P2082^T. From outside to the centre: Contigs (red/grey), COG category of genes on the forward strand (three circles), genes on forward strand (blue circle), genes on the reverse strand (red circle), COG category on the reverse strand (three circles), GC content. (Color figure online)

glycine arylamidase, phenylalanine arylamidase and tyrosine arylamidase activities were found to be positive. Urease and indole are not produced, and nitrate is not reduced. Mannose and raffinose are not fermented. Alkaline phosphatase, alanine arylamidase, glycine arylamidase, glutamyl glutamic acid arylamidase, leucine arylamidase, proline arylamidase, pyroglutamic acid arylamidase, serine arylamidase, glutamic acid decarboxylase, n-acetyl-β-glucosaminidase, α-arabinose, α-galactosidase, β-galactosidase, β-galactosidase 6 phosphate, α-glucosidase, β-glucosidase, β-glucuronidase, and α-fucosidase activities were found to be negative.

Using API® Zym strips, positive reactions were observed for esterase, esterase lipase, valine arylamidase, acid phosphatase, and naphtol-AS-BI-phosphohydrolase, but alkaline phosphatase, lipase, leucine arylamidase, cystine arylamidase, N-acetyl-β-glucosaminidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fuctosidase activities were negative.

Using API® 20A strips, positive reactions were observed with D-glucose, D-lactose, D-maltose, D-mannose, D-melezitose, D-saccharose, and glycerol. Indole production, esculin hydrolyse, urease, D-cellulose, D-mannitol, D-raffinose, D-sorbitol D-trehalose, D-xylose, gelatine, salicin, L-arabinose and L-rhamnose were found to be negative.

Using API 50 CH strips, positive fermentation reactions were observed with aesculin, D-galactose, D-glucose, D-fructose, D-lactose, D-maltose, D-mannose, D-melezitose, D-ribose, D-saccharose, D-tagatose, D-turanose, glycerol, N-acethylglucosamine, potassium 5-cetogluconate and starch. Negative reactions were observed for fermentation of adonitol, amygdaline, arbutine, D-arabinose, D-arabitol, D-cellobiose, D-fucose, D-lyxose, D-mannitol, D-melibiose, D-raffinose, D-sorbitol, D-trehalose, D-xylose, dulcitol, erythritol, gentiobiose, glycogen, inositol, inulin, L-arabinose, L-arabitol, L-fucose, L-rhamnose, L-Sorbose, L-xylose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, methyl-β-D-xylopyranoside, potassium gluconate, potassium 2-ceto gluconate, salicin, and xylitol. The strain Marseille-P2082 differs from *N. succinicivorans* in its ability to

Table 2 Number of genes associated with the 26 general COG functional categories of strain Marseille-P2082^T

Code	1	2	3	4	5	6	7	Description
J	170	172	172	182	178	172	190	Translation, ribosomal structure and biogenesis
A	0	0	0	0	0	0	0	RNA processing and modification
K	58	60	62	79	98	59	149	Transcription
L	79	76	77	80	104	76	118	Replication, recombination and repair
B	1	1	0	0	0	1	0	Chromatin structure and dynamics
D	20	22	25	21	24	18	40	Cell cycle control, cell division, chromosome partitioning
Y	0	0	0	0	0	0	0	Nuclear structure
V	31	32	37	50	47	46	58	Defence mechanisms
T	45	44	40	57	95	38	131	Signal transduction mechanisms
M	82	85	85	108	122	93	161	Cell wall/membrane/envelope biogenesis
N	8	8	8	10	12	11	80	Cell motility
Z	0	0	0	0	0	0	0	Cytoskeleton
W	4	4	9	6	9	7	12	Extracellular structures
U	22	22	27	25	24	23	40	Intracellular trafficking, secretion, and vesicular transport
O	47	49	47	61	64	43	89	Posttranslational modification, protein turnover, chaperones
X	1	9	7	30	31	2	37	Mobilome: prophages, transposons
C	46	46	45	67	78	42	115	Energy production and conversion
G	40	42	35	45	75	41	143	Carbohydrate transport and metabolism
E	91	96	87	142	195	76	196	Amino acid transport and metabolism
F	62	64	58	64	66	56	75	Nucleotide transport and metabolism
H	84	84	50	105	109	61	129	Coenzyme transport and metabolism
I	38	39	42	41	54	39	52	Lipid transport and metabolism
P	69	72	50	110	84	47	88	Inorganic ion transport and metabolism
Q	16	15	11	15	21	10	31	Secondary metabolites biosynthesis, transport and catabolism
R	90	87	81	125	151	81	181	General function prediction only
S	61	63	49	64	72	49	87	Function unknown
–	241	329	219	439	649	230	556	Not in cog

(1) Strain Marseille-P2082; (2) *N. succinicivorans* strain ADV; (3) *Dialister micraerophilus*; (4) *Dialister invisus*; (5) *Dialister succinatiphilus*; (6) *Dialister pneumosintes*; (7) *Selenomonas bovis*

ferment 14 sugars whereas *N. succinicivorans* is assaccharolytic. The differential characteristics of strain Marseille-P2082^T compared with its close relatives are detailed in Table 1.

The most abundant cellular fatty acids of strain Marseille-P2082^T were identified as $C_{18:1n9}$ (37.2%), $C_{18:0}$ (34.7%), $C_{16:0}$ (21.3%), $C_{18:2n6}$ (6.3%) and a minor amount of $C_{14:0}$ (< 1%).

Genome properties

The draft genome from strain Marseille-P2082 has been deposited in EMBL-EBI under accession number LT700188. The draft genome is 1, 360, 589 bp long with a 51.1% G+C content (Fig. 4). It is composed of 1 scaffold (1 contig). Of the 1353 predicted genes, 1276 are protein-coding genes, 68 are RNAs genes (12 rRNA, 52 tRNA and 4 other RNA) and 9 were pseudogenes. A total of 995 genes (77.9%) were assigned a putative function (by COGs or by NR blast), 23 genes (1.8%) were identified as ORFans, and 5 genes were associated with polyketide synthase (PKS) or non-ribosomal peptide synthetase (NRPS) (Conway and Boddy 2013). The remaining 253 protein coding genes (19.8%) were annotated as hypothetical proteins. Using ARG-ANNOT (Gupta et al. 2014), no antibiotic resistance genes were found.

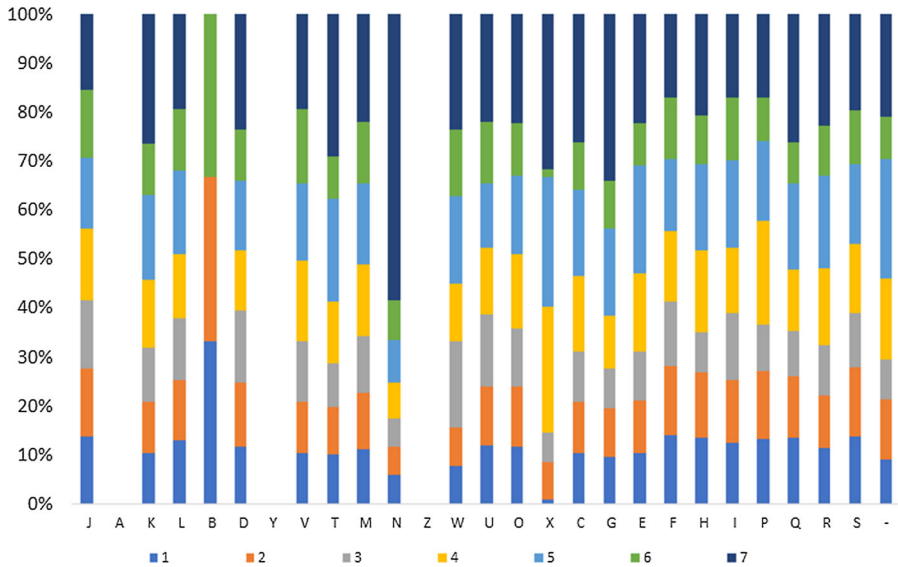

Fig. 5 Distribution of functional classes of predicted genes according to the COGs of strain Marseille-P2082^T compared with closely related species. (1) Strain Marseille-P2082; (2) *N. succinicivorans* strain ADV; (3) *Dialister micraerophilus*; (4) *Dialister invisus*; (5) *Dialister succinatiphilus*; (6) *Dialister pneumosintes*; (7) *Selenomonas bovis*

Table 3 Pairwise comparison of strain Marseille-P2082 with other species using GGDC, formula 2 (dDDH estimates based on identities/HSP length)*

	1	2	3	4	5	6	7
Strain Marseille-P2082	100%	53.7% ± 5.3	33.2% ± 4.9	30.5% ± 4.9	30.1% ± 4.9	27.7% ± 4.8	23.2% ± 4.8
N. succinicivorans		100%	34.1% ± 4.9	23.9% ± 4.8	27.8% ± 4.9	27% ± 4.9	22.4% ± 4.8
D. micraerophilus			100%	20.9% ± 4.6	23% ± 4.7	19% ± 4.6	28.8% ± 4.9
D. invisus				100%	22.7% ± 4.8	22.9% ± 4.7	28.7% ± 4.8
D. succinatiphilus					100%	24.5% ± 4.8	25.6% ± 4.8
D. pneumosintes						100%	26.3% ± 4.9
S. bovis							100%

(1) Strain Marseille-P2082; (2) *N. succinicivorans* strain ADV; (3) *Dialister micraerophilus*; (4) *Dialister invisus*; (5) *Dialister succinatiphilus*; (6)*Dialister pneumosintes*; (7) *Selenomonas bovis*

The distribution of genes into COGs functional categories is presented in Table 2.

Genome comparison

The draft genome (1.36 Mb) of the strain Marseille-P2082 is larger than those of *D. succinatiphilus*, *D. pneumosintes* and *D. micraerophilus* (1.25, 1.27 and 1.28 Mb respectively) but smaller than those of *N. succinicivorans, D. invisus* and *S. bovis* (1.48, 1.89 and 2.69 Mb). The G+C content of strain Marseille-P2082^T (51.1%) is higher than those of *D. pneumosintes*, *D. micraerophilus*, *D. invisus* and *D. succinatiphilus* (35.2%, 35.4%, 45.5% and 50.4% respectively), similar to that of *N. succinicivorans*, but smaller than that of *S. bovis* (59.2%). The gene content of strain Marseille-P2082 (1353) is smaller than those of *N. succinicivorans*, *D. invisus*, *D. succinatiphilus* and *S. bovis* (1454, 1829, 2280, and 2569 respectively) but higher than those of *D. micraerophilus* and *D. pneumosintes* (1237 and 1263 respectively). However, the gene distribution into COGs categories was similar among all compared genomes (Table 2, Fig. 5).

Table 4 OrthoANI values calculated from the OAT software of strain Marseille-P2082 compared with those of its phylogenetically close neighbours

	1	2	3	4	5	6	7
Strain Marseille-P2082	100%	93.78%	64.35%	64.67%	64.83%	63.86%	64.22%
N. succinicivorans		100%	64.52%	64.46%	64.87%	63.35%	63.95%
D. micraerophilus			100%	67.90%	67.05%	68.17%	62.13%
D. invisus				100%	70.81%	67.46%	62.88%
D. succinatiphilus					100%	66.62%	63.98%
D. pneumosintes						100%	61.51%
S. bovis							100%

(1) Strain Marseille-P2082; (2) *N. succinicivorans* strain ADV; (3) *Dialister micraerophilus*; (4) *Dialister invisus*; (5) *Dialister succinatiphilus*; (6) *Dialister pneumosintes*; (7) *Selenomonas bovis*

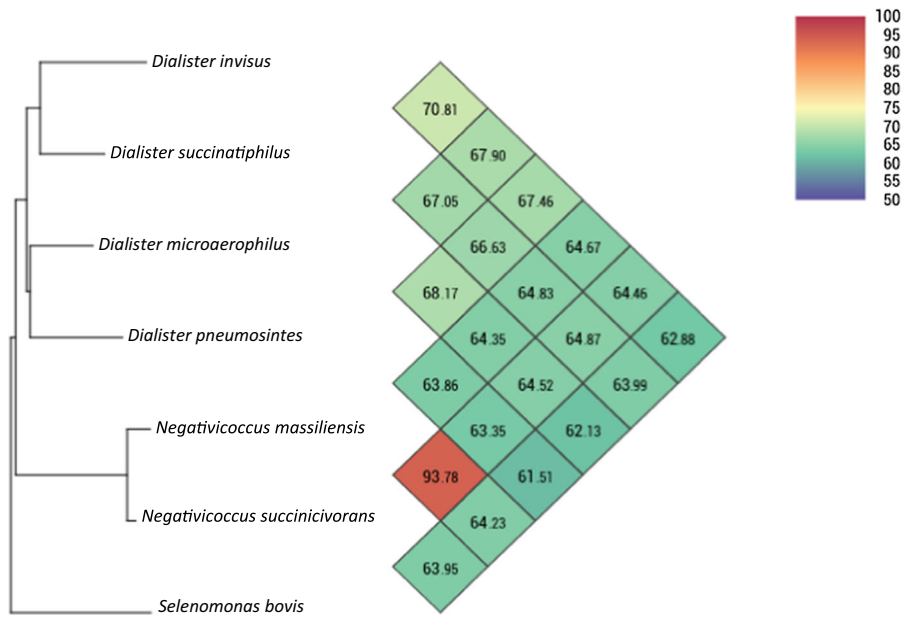

Fig. 6 Heatmap generated with OrthoANI values calculated using the OAT software between strain Marseille-P2082 and other closely related species with standing in nomenclature

The dDDH values of strain Marseille-P2082 ranged from 23.2% ± 4.8% with *S. bovis* to 53.7 ± 5.3% with *N. succinicivorans* strain ADV (Table 3). The OrthoANI values ranged from 63.35% between strain Marseille-P2082 and *D. pneumosintes* to 93.78% with *N. succinicivorans*. These values are lower than the threshold value (95–96%) recommended to delineate prokaryotic species (Konstantinidis and Tiedje 2005; Rodriguez-R and Konstantinidis 2014). The OrthoANI values between strain Marseille-P2082 and closely related species are shown in Table 4 and Fig. 6.

Based on phenotypic, chemotaxonomic, genomic and phylogenetic characteristics, strain Marseille-P2082^T (= CSUR P2082^T, DSM 100853^T) is designated as the type strain of a novel species, here named *Negativicoccus massiliensis*.

Description of "*Negativicoccus massiliensis*" sp. nov

Negativicoccus massiliensis (*mas.si.li.en'sis*. L. masc. adj. *massiliensis*, of Massilia, the Latin name of Marseille, where the type strain was isolated).

Cells are Gram-negative, non-motile, asporogenous and coccoid, with a mean diameter of 0.5 μm. Columbia agar-grown colonies are circular, flat, translucent with a mean diameter smaller than 0.5 mm after 72 h of incubation in anaerobic conditions. Catalase and oxidase activities are absent. The optimum growth temperature is 37 °C. Arginine hydrolase activity is positive. Not able to reduce nitrate to nitrite. Indole and urease are not produced, gelatin is not liquefied and aesculin is not hydrolysed. The G+C content of the draft genome of the type strain is 51.1%.

The type strain Marseille-P2082^T (= CSUR P2082^T, = DSM 100853^T) was isolated from a stool sample of a morbidly obese French woman. The habitat of this microorganism is the human gut. The 16S rRNA gene sequence and whole-genome shotgun sequence of strain Marseille-P2082^T have been deposited in EMBL-EBI under accession numbers LN876651 and LT700188, respectively.

Acknowledgements The authors thank the Xegen Company (www.xegen.fr) for automating the genomic annotation process. The authors also thank Noémie Labas for the genome sequencing. We are grateful to Professor Hélène Marchandin for providing the *Negativicoccus succinicivorans* strain ADV 07/08/06-B-1388^T.

Author's contributions AHT isolated for the first time the strain Marseille-P2082, performed its phenotypic characterization and wrote the manuscript; AD and MLD actively participated to genome analysis; SK actively participated in the laboratory project in which the strain was isolated; MM and MM actively participated to the specimen collection and the study design; DR designed and directed the project; PEF corrected the manuscript, verified the accuracy of the Latin name of the strain; GD corrected the manuscript and acted as the corresponding author.

Funding This work was supported by the French Government under the « Investissements d'avenir » (Investments for the Future) program managed by the Agence Nationale de la Recherche (ANR, fr: National Agency for Research) under the number IHU Méditerranée Infection 10-IAHU-03.

Data deposit The whole-genome shotgun sequence of *N. succinicivorans* strain ADV 07/08/06-B-1388^T has been deposited in NCBI under accession number PRJEB34910.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Auch AF, von Jan M, Klenk H-P, Göker M (2010) Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. Stand Genomic Sci 2:117–134. https://doi.org/10.4056/sigs.531120

Cecil JE, Tavendale R, Watt P et al (2008) An obesity-associated FTO gene variant and increased energy intake in children. N Engl J Med 359:2558–2566. https://doi.org/10.1056/NEJMoa0803839

Conway KR, Boddy CN (2013) ClusterMine360: a database of microbial PKS/NRPS biosynthesis. Nucleic Acids Res 41:D402–D407

Drancourt M, Bollet C, Carlioz A et al (2000) 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. J Clin Microbiol 38:3623–3630

Frayling TM, Timpson NJ, Weedon MN et al (2007) A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 316:889–894. https://doi.org/10.1126/science.1141634

Gupta SK, Padmanabhan BR, Diene SM et al (2014) ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob Agents Chemother 58:212–220

Hyatt D, Chen G-L, Locascio PF et al (2010) Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:119. https://doi.org/10.1186/1471-2105-11-119

Kent WJ (2002) BLAT—the BLAST-like alignment tool. Genome Res 12:656–664

Kong L-C, Tap J, Aron-Wisnewsky J et al (2013) Gut microbiota after gastric bypass in human obesity: increased richness and associations of bacterial genera with adipose tissue genes. Am J Clin Nutr 98:16–24. https://doi.org/10.3945/ajcn.113.058743

Konstantinidis KT, Tiedje JM (2005) Towards a genome-based taxonomy for prokaryotes. J Bacteriol 187:6258–6264. https://doi.org/10.1128/JB.187.18.6258-6264.2005

Lagier J-C, Hugon P, Khelaifia S et al (2015) The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. Clin Microbiol Rev 28:237–264. https://doi.org/10.1128/CMR.00014-14

Lechner M, Findeiss S, Steiner L et al (2011) Proteinortho: detection of (co-)orthologs in large-scale analysis. BMC Bioinformatics 12:124. https://doi.org/10.1186/1471-2105-12-124

Lee I, Ouk Kim Y, Park S-C, Chun J (2016) OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 66:1100–1103. https://doi.org/10.1099/ijsem.0.000760

Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006) Microbial ecology: human gut microbes associated with obesity. Nature 444:1022–1023. https://doi.org/10.1038/4441022a

Marchandin H, Teyssier C, Campos J et al (2010) *Negativicoccus succinicivorans* gen. nov., sp. nov., isolated from human clinical samples, emended description of the family *Veillonellaceae* and description of *Negativicutes* classis nov., *Selenomonadales* ord. nov. and *Acidaminococcaceae* fam. nov. in the bacterial phylum Firmicutes. Int J Syst Evol Microbiol 60:1271–1279

Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60. https://doi.org/10.1186/1471-2105-14-60

Million M, Maraninchi M, Henry M et al (2012) Obesity-associated gut microbiota is enriched in *Lactobacillus reuteri* and depleted in *Bifidobacterium animalis* and *Methanobrevibacter smithii*. Int J Obes 36:817–825. https://doi.org/10.1038/ijo.2011.153

Ng M, Fleming T, Robinson M et al (2014) Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. The Lancet 384:766–781. https://doi.org/10.1016/S0140-6736(14)60460-8

Pattengale ND, Alipour M, Bininda-Emonds ORP et al (2010) How many bootstrap replicates are necessary? J Comput Biol J Comput Mol Cell Biol 17:337–354. https://doi.org/10.1089/cmb.2009.0179

Ramasamy D, Mishra AK, Lagier J-C et al (2014) A polyphasic strategy incorporating genomic data for the taxonomic description of novel bacterial species. Int J Syst Evol Microbiol 64:384–391

Rodriguez-R LM, Konstantinidis KT (2014) Bypassing cultivation to identify bacterial species: culture-independent genomic approaches identify credibly distinct clusters, avoid cultivation bias, and provide true insights into microbial species. Microbe Mag 9:111–118. https://doi.org/10.1128/microbe.9.111.1

Rogosa M (1971) Transfer of *Veillonella Prevot* and *Acidaminococcus Rogosa* from *Neisseriaceae* to *Veillonellaceae* fam. nov., and the inclusion of *Megasphaera Rogosa* in *Veillonellaceae*. Int J Syst Bacteriol 21:231–233. https://doi.org/10.1099/00207713-21-3-231

Seng P, Drancourt M, Gouriet F et al (2009) Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 49:543–551. https://doi.org/10.1086/600885

Swofford D (2002) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sinauer Associates, Sunderland

Thompson JD, Gibson TJ, Higgins DG (2002) Multiple sequence alignment using ClustalW and ClustalX. Curr Protoc Bioinforma Chapter 2: Unit 2.3. https://doi.org/10.1002/0471250953.bi0203s00

Togo AH, Diop A, Dubourg G et al (2016a) *Butyricimonas phoceensis* sp. nov., a new anaerobic species isolated from the human gut microbiota of a French morbidly obese patient. New Microbes New Infect 14:38–48. https://doi.org/10.1016/j.nmni.2016.07.010

Togo AH, Khelaifia S, Lagier J-C et al (2016b) Noncontiguous finished genome sequence and description of *Paenibacillus ihumii* sp. nov. strain AT5. New Microbes New Infect 10:142–150. https://doi.org/10.1016/j.nmni.2016.01.013

Togo AH, Khelaifia S, Valero R et al (2016c) '*Negativicoccus massiliensis*', a new species identified from human stool from an obese patient after bariatric surgery. New Microbes New Infect 13:43–44. https://doi.org/10.1016/j.nmni.2016.05.018

Togo AH, Durand G, Khelaifia S et al (2017) *Fournierella massiliensis*, gen. nov., sp. nov., a new human-associated member of the family *Ruminococcaceae*. Int J Syst Evol Microbiol 67:1393–1399. https://doi.org/10.1099/ijsem.0.001826

Turnbaugh PJ, Ley RE, Mahowald MA et al (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444:1027–1031. https://doi.org/10.1038/nature05414

Zhang H, DiBaise JK, Zuccolo A et al (2009) Human gut microbiota in obesity and after gastric bypass. Proc Natl Acad Sci USA 106:2365–2370. https://doi.org/10.1073/pnas.0812600106