**TAXONOGENOMICS: GENOME OF A KNOWN ORGANISM**

*Lactimicrobium massiliense* gen. nov., sp. nov.; *Anaerolactibacter massiliensis* gen. nov., sp. nov.; *Galactobacillus timonensis* gen. nov., sp. nov. and *Acidipropionibacterium timonense* sp. nov. isolated from breast milk from healthy breastfeeding African women

A. H. Togo1,2, A. Diop1,2, A. Camara1,2, E. Kuete1,2, S. Konate1,2, V. Brevaut3, C. Des Robert4, J. Delerce1,2, N. Armstrong1,2, Y. Roussel1,2, P.-E. Fournier1,2, M. A. Thera5, D. Raoult1,2 and M. Million1,2

1) Aix Marseille Univ, IRD, AP-HM, MEPHI, 2) IHU-Méditerranée Infection, 3) APHM, CHU Hôpital Nord, Service de médecine néonatale, 4) APHM, CHU Hôpital de la Conception, Service de médecine néonatale, F-13385, Marseille, France and 5) Malaria Research and Training Center, Department of Epidemiology of Parasitic Diseases, FMOS-FAPH, University of Science, Techniques and Technologies, Bamako, Mali

**Abstract**

Four strains isolated by microbial culturomics from breast milk of healthy mothers from Mali were not identified and characterized by taxono-genomics. This led us to propose the new genera and species *Lactimicrobium massiliense*, *Anaerolactibacter massiliensis* and *Galactobacillus timonensis* containing type strain Marseille-P4301T (CSUR P4301T), Marseille-P4302T (CSUR P4302T) and Marseille-P4641T (CSUR P4641T), respectively. The strain Marseille-P4482 represents a novel species, *Acidipropionibacterium timonense*, in a previously known genus with type strain being Marseille-P4482T (CSUR P4482T).

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**Corresponding author:** M. Million, Aix Marseille Université, Institut Hospitalier Universitaire Méditerranée-Infection, 19-21 Boulevard Jean Moulin, 13005, Marseille, France.
E-mail: matthieumillion@gmail.com

**Introduction**

Human breast milk is a complex biological fluid produced by the mammary glands. Breast milk not only provides the essential nutrients for growth and development in new-borns, it also protects against different infectious diseases [1–5]. Several studies have reported unsuspected diversity, including many bacteria that promote maternal and child health in breast, colostrum and milk [6–9]. The breast-milk microbiota plays a key role in the colonization of the new-born’s digestive tract and in the development of its immunity [10–12]. However, little is known about the composition of the human milk microbiota, and most published studies are limited by the use of metagenomics which does not differentiate between the DNA sequences of live bacteria and dead bacteria [13–16]. To date, to our knowledge, only one bacterial species, *Streptococcus lactarius*, has been officially described with breast milk as the first source of isolation [17]. This suggests that the human milk microbiota is neglected and remains largely unexplored.

We have therefore studied the microbiota of colostrum and breast milk of healthy mothers from France and Mali using the culturomics approach, an approach developed and applied in our laboratory over the past 10 years [18] to decipher the bacterial diversity of colostrum and breast milk. As part of this work, we isolated four new bacterial species from breast milk.

Here, we describe the isolation and taxonomic characterization of strain Marseille-P4301T, strain Marseille-P4302T and strain Marseille-P4641T as type strains of *Lactimicrobium massiliense* gen. nov., sp. nov. (CSUR 4301), *Anaerolactibacter massiliensis* gen. nov., sp. nov. (CSUR 4302) and *Galactobacillus*
timonensis gen. nov., sp. nov. (CSUR 4641), close to Solo-
bacterium moorei strain JCM 10645 [19] and strain Marseille-
P4482T as type strain of Acidipropionibacterium timonense sp.
nov. (CSUR 4482) close to Cutibacterium granulosum. The four
new bacterial species were isolated from a sample of breast
milk from four healthy lactating Malian mothers.

Materials and methods

Sample collection
Milk sample were collected from healthy breastfeeding
mothers in the suburban area of Bamako (Kalabankoro), Mali,
between November 26 and December 1st, 2016. Approximately
20 mL of breast milk were collected aseptically in 50-
mL sterile polypolypropylene conical tubes (Industrial Falcon,
Reynosa, Mexico) containing 1 mL transport medium made
with antioxidants, after breast cleaning by manual expression.
Samples were collected between 5 and 19 months after de-
ivery. All the donors had full-term pregnancies and their
children were apparently healthy. Samples were stored at
−20°C before being sent to our laboratory (IHU-Méditer-
nanée Infection, Marseille, France) for analysis. Written
consent was obtained from each mother before sampling, in
accordance with the Helsinki declaration and CIOMS 2016.
Study and consent procedures were approved by the ethics
committee of IFR 48, under the Consent number 2014/46/CE/FMPOS
under Number 2014/46/CE/FMPOS as at May 22, 2014
(available on request). The material transfer agreement
(MTA) has been signed between IHU-Méditerranée Infection
et Université des Sciences Technique et Technologique de
Bamako (USTTB) and is available on request. The samples
were transferred from Mali to France in accordance with the
Nagoya protocol.

Strains isolation and identification
The first growth of these four strains occurred in May 2017.
Approximately 2 mL of milk samples were preincubated under
anaerobic conditions in blood-culture bottles enriched with 5%
sheep blood and 5% rumen fluid (sterilised by filtration through
a 0.2-μm diameter filter) and later inoculated onto sheep blood
Columbia agar (bioMérieux, Marcy l’Étoile, France) as described
elsewhere [18,20]. The identification procedure was conducted
as previously described [20].

Phylogenetic analysis
The 16S rRNA gene amplification PCR and sequencing were
performed as previously described [21]. Taxonomic assignation
was performed as described elsewhere [20]. Phylogenetic
analysis was performed by ClustalW alignment and the
maximum likelihood method using MEGA7.0.26 software. The
sequences from type strains were downloaded from the web-
site https://www.ncbi.nlm.nih.gov.

Phenotypic, biochemical and chemotaxonomic analysis
Temperature range and atmosphere, pH and salinity for growth
were assessed as previously described. Biochemical analysis
using various strips (API® ZYM, API® 20 A, API® 50 CH and
API Rapid ID 32 A) (bioMérieux) and oxidase and catalase tests
(bioMérieux) were done according to the manufacturer’s in-
structions. Analyses were performed as previously described
[22]. Motility assay, Gram-staining, transmission electron mi-
croscopy and sporulation assay were also performed as
describe elsewhere [23]. Cellular fatty acid methyl ester
(FAME) and metabolic end products analysis were performed
as previously described [20,24].

Genomic analysis
Genome sequencing, assemblage, annotation and comparison
were performed as previously described [22,23,25]. The ge-
nomes of Solobacterium moorei strain RCA59-74T
(NZ_AUKY00000000) [19], Bulleidia extracta strain W 1219T
(NZ_ADFR00000000) [26], Anaerorhabdus fuscosa strain VPI
3253T (NZ_FUYY00000000) [27], Holdemania filiformis strain
J1-31B-1T (NZ_ACCF01000000) [28] and Holdemania massi-
liensis strain AP2T (CALK01000000) [29] were used for genome
comparison of the strains Marseille-P4301, Marseille-P4302 and
Marseille-P4641. The genomes of Cutibacterium acnes ATCC6919
(NZ_CP023676) [30,31], Cutibacterium avidum ATCC 25577
(NZ_AGBA01000000) [32], Cutibacterium granulosum DSM
20700 (NZ_OSS00000000), Pseudopropionibacterium propioni-
fum F0230a (NC_018142), Propionibacterium acidifaciens strain
C3M 31 (NZ_AUFR00000000), Acidipropionibacterium thoennii
strain NCFB 568 (NZ_AUHZ01000000) and Acid-
ipropionibacterium acidipropionici strain NCFB 563 (NZ_A-
TYU01000000) were used for genome comparison of strain
Marseille-P4482.

Results

Strain isolation and identification
The strains were first isolated after 7 days (Marseille-P4301 and
Marseille-P4302 strain) and 10 days (Marseille-P4641 strain
Marseille-P4482 strain) of preincubation of breast milk samples
in an anaerobic blood-culture bottle enriched with 5% rumen
fluid sterilized by filtration at 0.2 μm and 5% sheep blood and
seeded on 5% sheep-blood Columbia agar (bioMérieux) under
anaerobic condition at 37°C. The strains were not identifiable
FIG. 1. Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) reference mass spectrum from strains Marseille-P4301, Marseille-P4302, Marseille-P4641 and Marseille-P4482.

FIG. 2. Maximum likelihood phylogenetic tree highlighting the position of strains Marseille-P4301, Marseille-P4302, and Marseille-P4641 against most closely related species. The evolutionary history was inferred by using the maximum likelihood method based on the Kimura 2-parameter model. A discrete γ distribution was used to model evolutionary rate differences among sites (five categories (+G, parameter = 0.2353)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 15 nucleotide sequences. In total there were 1628 positions in the final dataset. The scale bar represents a 1% nucleotide sequence divergence.

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using matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS). The spectra from these strains did not match any of the spectra in our database, either at the species level (strain Marseille-P4482) or at the genus level (strains Marseille-P4301, Marseille-P4302 and Marseille-P4641) (Fig. 1). In an attempt to identify these four strains, their 16S rRNA gene was sequenced, and the sequences obtained showed a similarity of 91.6%, 89.8% and 88.9% with *Solobacterium moorei* strain RCA59-74 (= CIP 106864 T = JCM 10645 T) for strains Marseille-P4301, Marseille-P4302, and Marseille-P4641, respectively, 96.28% with *Cutibacterium granulosum* strain ATCC 25564 T (= CCUG 32987 T = CIP 103262 T = DSM 20700 T = JCM 6498 T = DSM 18657 T = NCTC 11865 T) for the strain Marseille-P4482 (Fig. 2, Fig. 3, Table 1, Table 2), the closest phylogenetically validated species with standing in nomenclature. The 16S rRNA gene sequences of these stains were deposited in EMBL-EBI under accession number: LT996090, LT960585, LT934541 and LT996089 (Strain Marseille-P4301, Marseille-P4302, Marseille-P4641 and Marseille-P4482 respectively).

![FIG. 3. Maximum likelihood phylogenetic tree highlighting the position of strain Marseille-P4482 against other most closely related species. The evolutionary history was inferred by using the maximum likelihood method based on the Kimura 2-parameter model. A discrete γ distribution was used to model evolutionary rate differences among sites (five categories (+G, parameter = 0.2353)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 15 nucleotide sequences. In total there were 1628 positions in the final dataset. The scale bar represents a 1% nucleotide sequence divergence.](image_url)

**TABLE 1.** Pairwise comparison of strains Marseille-P4301, Marseille-P4302 and Marseille-P4641 for 16S rRNA sequence similarity with closely related species. Value (%)

| Species                        | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   |
|-------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Strain Marseille-P4301        | 100 |     |     |     |     |     |     |     |
| Strain Marseille-P4302        | 100 | 92.5| 90.8|     |     |     |     |     |
| Strain Marseille-P4641        | 100 | 91.9| 89.8| 91.6| 91.9| 88.5| 87.8| 89.2|
| S. moorei strain RCA59-74 T   |     |     |     | 90.8| 90.8| 90.4|     |     |
| B. extroct strain W 1219 T    |     |     |     | 89.5| 89.5| 88.6| 88.6| 87.7|
| H. filiformis strain J-31B-1 T|     | 100 | 88.9| 88.9| 87.2| 88.6| 87.4| 90.1|
| H. massiliensis strain AP2 T  |     | 100 | 97.1|     |     |     |     |     |
| A. furcosa strain VPI 3253 T  |     | 100 |     |     |     |     |     | 100 |

1, strain Marseille-P4301; 2, Strain Marseille-P4302; 3, Strain Marseille-P4641; 4, Solobacterium moorei strain RCA59-74 T; 5, Bulleidia extroctua strain W 1219 T; 6, Holdemania filiformis strain J-31B-1 T; 7, Holdemania massiliensis strain AP2 T; 8, Anaerorhabdus furcosa strain VPI 3253 T. Identity was obtained using blastn suite-2 sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&BLAST_SPEC=blast2seq&LINK_LOC=align2seq) with the following sequences (LT996090 for strain P4301, LT960585 for P4302, LT934541 for P4641, AB031056 for S. moorei strain RCA59-74 T, AF220064 for B. extroctua strain W 1219 T, Y11466 for H. filiformis strain J-31B-1 T, JX101683 for H. massiliensis strain AP2 T and GU585668 for A. furcosa strain VPI 3253 T).
Phenotypic and biochemical characterization

Cells from the strains Marseille-P4301, Marseille-P4302 and Marseille-P4482 are Gram-negative staining, non-motile, non-sporing, strictly anaerobic rods. Those from strain Marseille-P4482 are Gram-positive staining, non-motile, non-sporing and facultatively anaerobic coccobacilli. Strains Marseille-P4301, Marseille-P4302, Marseille-P4641 and Marseille-P4482 measure 0.5/1.5, 0.5/2, 0.4/0.8 and 0.8/1.2 μm width/length respectively by electron microscopy (Fig. 4). The four strains have no catalase or oxidase activity. Strain growth occurred between 28 and 45°C, but optimal growth was observed at 37°C after 24 or 48 h incubation in an anaerobic

| Value (%) | Strain Marseille-P4482 | Cutibacterium acnes ATCC6919 | Cutibacterium odium ATCC 25577 | Cutibacterium granulosum DSM 20700 | Propionibacterium propionicum F0330a | Propionibacterium acidipropionici strain NCFB 568 | Acidipropionibacterium acidipropionici strain NCFB 563 |
|-----------|------------------------|-----------------------------|-------------------------------|-----------------------------------|--------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Species   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Strain Marseille-P4482 | 100 | 95.0 | 95.8 | 96.3 | 95.6 | 92.2 | 94.9 | 95.6 |
| Cutibacterium acnes ATCC6919 | 100 | 96.1 | 93.9 | 96.1 | 92.0 | 92.0 | 92.0 | 92.0 |
| Cutibacterium odium ATCC 25577 | 100 | 94.7 | 99.2 | 91.8 | 92.8 | 92.8 | 94.0 | 92.0 |
| Cutibacterium granulosum DSM 20700 | 100 | 94.6 | 90.6 | 92.8 | 92.8 | 92.8 | 94.0 | 94.0 |
| Propionibacterium propionicum F0330a | 100 | 91.8 | 91.8 | 92.8 | 92.8 | 90.3 | 91.0 | 91.0 |
| Propionibacterium acidipropionici strain NCFB 568 | 100 | 90.3 | 91.0 | 90.3 | 91.0 | 91.0 | 91.0 | 91.0 |
| Acidipropionibacterium acidipropionici strain NCFB 563 | 100 | 91.0 | 91.0 | 91.0 | 91.0 | 91.0 | 91.0 | 91.0 |

1. Strain Marseille-P4301; 2. Cutibacterium acnes strain ATCC6919; 3. Cutibacterium odium strain DSM 4900; 4. Cutibacterium granulosum strain DSM 20700; 5. Propionibacterium propionicum strain F0330a; 6. Propionibacterium acidipropionici strain C3M 31; 7. Acidipropionibacterium thoenii strain NCFB 568; 8. Acidipropionibacterium acidipropionici strain NCFB 563. Identity was obtained using blastn suite-2 sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&BLAST_SPEC=blast2seq&LINK_LOC=align2seq) with the following sequences (LT996089 for strain, AB042288 for C. acnes, AJ003055 for C. odium, AJ003057 for C. granulosum, AJ003058 for P. propionicum, EU979537 for P. acidipropionici, AJ004572 for P. acidipropionici and AJ004569 for Acidipropionibacterium acidipropionici).
atmosphere on 5% sheep-blood Columbia agar (bioMérieux). The strain Marseille-P4482 is able to grow under aerobic conditions but the strains Marseille-P4301, Marseille-P4302 and Marseille-P4641 are not. Colonies from strain Marseille-P4301 and Marseille-P4641 were translucent, non-haemolytic, regular and umbilicate with a mean diameter from 1 to 1.5 mm. Colonies from strain Marseille-P4302 were grey, regular, with a mean of 1-2 mm, the agar plate looks like blood burnt after 48 of incubation. Colonies from strain Marseille-P4482 were cream-coloured, regular and non-haemolytic with a mean diameter of 3–5 mm. No growth was observed beyond 10 g/L of NaCl concentration on Schaedler agar (bioMérieux) for the strains Marseille-P4301, Marseille-P4641 and Marseille-P4482, but growth was observed up to 50 g/L for strain Marseille-P4302. These strains were able to grow at pH levels ranging from 6.5 to 8, but the optimum was observed at pH 7.5. Using API strip; aesculin and gelatine are hydrolysed, indole is produced, but none of the four species produces urease or reduces nitrate. Cellulobiose, maltose, succrose and trehalose are fermented while arabinose, rhamnose, sorbitol and xylose are not for any of the four species. All strains exhibited acid phosphatase, naphthol-AS-BI-phosphohydrolase, esterase (C-4), esterase lipase (C-8), α-galactosidase and leucine arylamidase activity. Table 3 displays the phenotypic and chemical characteristics of the four strains. The major cellular fatty acid are: C16:0 (45.6, 39.3 and 45.9%), C18:1ω7c (21.8, 27.6 and 26.6%) and C18:1ω9c (20.2, 19.4 and 12.3%) for the strains Marseille-P4301, Marseille-P4641 and Marseille-P4482, respectively.

### Genomic analysis

Draft genomes of these strains were deposited in EMBL-EBI under accession numbers OEPX00000000, OLMH00000000, OUNG00000000 and UWPF00000000 (Fig. 5). Genomes are 2 457 574 bp, 3 334 468 bp, 2 581 777 bp and 2 816 504 bp with 47%, 48.5%, 50% and 69% G+C content for the strains Marseille-P4301, Marseille-P4302, Marseille-P4641 and Marseille-P4482 respectively. After final assembly, the genomes are composed of 7, 2, 10 and 15 scaffolds for strains Marseille-P4301, Marseille-P4302, Marseille-P4641 and Marseille-P4482 respectively. The coding gene contents were 2346, 3121, 2370, and 2592, including 48, 53, 57 and 54 RNA genes for strains Marseille-P4301, Marseille-P4302, Marseille-P4641 and Marseille-P4482 respectively; the distribution of genes into clusters of orthologous groups (COG) functional categories is presented in Table 5.

The genomic characteristics of the strains were compared to those of the other closest species for which the genomes are available. The distribution of genes into COG categories is similar for all species compared, with the exception of the...
TABLE 4. Cellular fatty acid composition (%) of (1) strain Marseille-P4301, (2) strain Marseille-P4302, (3) strain Marseille-P4641, and (4) strain Marseille-P4482

| Fatty acids     | 1     | 2     | 3     | 4     |
|-----------------|-------|-------|-------|-------|
| C16:0           | 45.6  | 39.3  | 45.9  | 7.4   |
| C18:0           | 21.8  | 27.6  | 26.6  | 2.6   |
| Iso-C15:0       | ND    | ND    | ND    | 62.5  |
| Antiso-C15:0    | ND    | ND    | ND    | 67.9  |
| C15:0           | 20.2  | 19.4  | 12.3  | ND    |
| C18:1n9         | 9.2   | 7.8   | 8.7   | 1.8   |
| C16:0           | 1.7   | 2.9   | 2.8   | <1    |
| C15:0           | <1    | 1.2   | 1.1   | 1.1   |
| Iso-C15:0       | ND    | ND    | ND    | 1.4   |
| C16:1n7         | <1    | <1    | <1    | ND    |
| C16:0           | <1    | <1    | <1    | <1    |
| C16:1n7         | <1    | <1    | <1    | <1    |
| C18:0           | <1    | <1    | <1    | ND    |
| C18:2/3:6       | <1    | <1    | <1    | <1    |
| C17:0 iso       | ND    | ND    | ND    | 3.0   |
| Antiso-C17:0    | ND    | ND    | ND    | <1    |
| Iso-C13:0       | ND    | ND    | ND    | <1    |

ND, not detected.

FIG. 5. Graphical circular map of the genome of (a) strain Marseille-P4301, (b) strain Marseille-P4302, (c) strain Marseille-P4641, and (d) strain Marseille-P4482. From outside to the centre: Contigs (red/grey), clusters of orthologous groups (COGs) category of genes on the forward strand (three circles), genes on forward strand (blue circle), genes on the reverse strand (red circle), COGs category on the reverse strand (three circles), G+C content.

The digital DNA–DNA hybridization (dDDH) values ranged from 17.4% between S. moorei and A. furcosa to 50% between strain Marseille-P4301 and strain Marseille-P4302, and 67.9% between strain Marseille-P4302 and S. moorei (Table 6). These values are certainly high but remain below the 75% threshold for defining whether two strains are of the same species. This value ranges from 19.7% between P. propionicum and strain Marseille-P4482 to 22.7% between C. granulosum and strain Marseille-P4482 when strain Marseille-P4482 is compared with its closest neighbours (Table 7).
### TABLE 5. Number of genes associated with the 25 general COG functional categories

| Description                                      | 1   | 2   | 3   | 4   |
|--------------------------------------------------|-----|-----|-----|-----|
| Translation, ribosomal structure and biogenesis  | 187 | 218 | 198 | 190 |
| RNA processing and modification                  | 0   | 0   | 0   | 0   |
| Transcription                                    | 161 | 220 | 231 | 168 |
| Replication, recombination and repair            | 134 | 171 | 152 | 108 |
| Chromatin structure and dynamics                 | 0   | 0   | 0   | 0   |
| Cell cycle control, cell division, chromosome partitioning | 35  | 48  | 57  | 39  |
| Nuclear structure                                | 0   | 0   | 0   | 0   |
| Defence mechanisms                               | 113 | 118 | 81  | 87  |
| Signal transduction mechanisms                   | 92  | 148 | 89  | 106 |
| Cell wall/membrane/envelope biogenesis           | 115 | 154 | 128 | 122 |
| Cell motility                                    | 17  | 20  | 13  | 11  |
| Cytoskeleton                                     | 1   | 2   | 2   | 2   |
| Extracellular structures                         | 8   | 14  | 8   | 8   |
| Intracellular trafficking, secretion, and vesicular transport | 19  | 33  | 32  | 25  |
| Posttranslational modification, protein turnover, chaperones | 74  | 86  | 77  | 94  |
| Mobilome: prophages, transposons                 | 164 | 230 | 45  | 97  |
| Energy production and conversion                 | 100 | 129 | 115 | 127 |
| Carbohydrate transport and metabolism            | 187 | 254 | 116 | 240 |
| Amino acid transport and metabolism              | 166 | 185 | 144 | 203 |
| Nucleotide transport and metabolism              | 73  | 94  | 58  | 82  |
| Coenzyme transport and metabolism                | 71  | 76  | 93  | 135 |
| Lipid transport and metabolism                   | 57  | 67  | 59  | 76  |
| Inorganic ion transport and metabolism            | 83  | 102 | 92  | 107 |
| Secondary metabolites biosynthesis, transport and catabolism | 22  | 19  | 21  | 30  |
| General function prediction only                 | 195 | 249 | 154 | 201 |
| Function unknown                                 | 112 | 124 | 124 | 122 |
| Hypothetical protein                             | 433 | 714 | 541 | 513 |

1. Marseille-P4301; 2. Marseille-P4302; 3. Marseille-P46413; 4. Marseille-P4482.

### TABLE 6. Pairwise comparison of strains Marseille-P4301, Marseille-P4302 and Marseille-P46413 with other species using the genome-to-genome distance calculator (GGDC), formula 2 (digital DNA–DNA hybridization (dDDH) estimates based on identities/high-scoring segment pairs (HSP) length)^

|          | 1          | 2          | 3          | 4          | 5          | 6          | 7          | 8          |
|----------|------------|------------|------------|------------|------------|------------|------------|------------|
| Marseille-P4301 | 100%       | 50.0±2.7   | 24.8±2.4   | 28.0±2.4   | 37.3±2.5   | 26.1±2.4   | 19.0±2.3   | 27.1±2.5   |
| Marseille-P4302 | 100%       | 38.9±2.3   | 67.9±2.3   | 21.8±2.4   | 26.2±2.4   | 16.3±2.2   | 23.4±2.4   |            |
| Marseille-P4641 | 100%       | 20.3±2.3   | 34.0±2.3   | 18.9±2.3   | 18.9±2.3   | 26.5±2.4   |            |            |
| S. moorei     |            | 100%       | 24.5±2.4   | 26.4±2.5   | 24.6±2.4   | 17.4±2.2   |            |            |
| H. filiformis  |            |            | 20.3±2.3   | 26.5±2.4   | 24.6±2.4   |            |            |            |
| H. massiliensis|            |            |            | 100%       | 28.8±2.4   |            |            |            |

1. Marseille-P4301; 2. Marseille-P4302; 3. Marseille-P46413; 4. Solobacterium moorei strain RCA59-74T; 5. Bulleidia extructa strain W1219T; 6. Anaerorhabdus furcosa strain VPI 3253T; 7. Holdemania filiformis strain J1-31B-1T; 8. Holdemania massiliensis AP2T.

a Confidence intervals indicate inherent uncertainty in estimating DDH values from intergenomic distances based on models derived from empirical test data sets. These results are consistent with the 16S rRNA and phylogenomic analyses as well as the GGDC results.

### TABLE 7. Pairwise comparison of strains Marseille-P4482 with closest species using the genome-to-genome distance calculator (GGDC), formula 2 (digital DNA–DNA hybridization (dDDH) estimates based on identities/high-scoring segment pairs (HSP) length)^

|          | 1          | 2          | 3          | 4          | 5          | 6          | 7          | 8          |
|----------|------------|------------|------------|------------|------------|------------|------------|------------|
| Strain Marseille-P4482 | 100%       | 21.5±2.4   | 21.2±2.3   | 21.6±2.4   | 21.4±2.3   | 21.8±2.4   | 22.7±2.4   | 19.8±2.4   |
| A. acidipropionici | 100%       | 20.8±2.4   | 23.0±2.4   | 20.0±2.3   | 20.9±2.4   | 19.3±2.3   | 18.8±2.3   | 18.4±2.3   |
| C. avidum         | 100%       | 20.7±2.4   | 23.6±2.4   | 23.3±2.4   | 23.8±2.4   | 18.9±2.3   | 20.4±2.4   |            |
| A. thoenii        | 100%       | 20.7±2.4   | 21.1±2.4   | 19.5±2.3   | 20.3±2.4   |            |            |            |
| C. acnes          | 100%       | 22.2±2.4   | 20.1±2.3   | 22.1±2.4   |            |            |            |            |
| C. granulosum     | 100%       | 19.1±2.3   | 20.5±2.4   |            |            |            |            |            |
| P. acidifaciens   | 100%       | 19.1±2.3   |            |            |            |            |            |            |
| P. propionicum    | 100%       |            |            |            |            |            |            |            |

1. Marseille-P4482; 2. Acidipropionibacterium acidipropionici strain DSM4900T; 3. Cutibacterium avidum strain ATCC25577T; 4. Solobacterium heitneri strain DSM20276T; 5. Cutibacterium acnes strain ATCC6919T; 6. Cutibacterium granulosum strain DSM20700T; 7. Propionibacterium acidipropionici strain F0230aT.

a Confidence intervals indicate inherent uncertainty in estimating DDH values from intergenomic distances based on models derived from empirical test data sets. These results are consistent with the 16S rRNA and phylogenomic analyses as well as the GGDC results.
Conclusion

Considering the specific phenotypic, biochemical, genomic and phylogenetic characteristics of new bacteria, we propose the creation of three new genera named:

Lactimicrobium, with the type species Lactimicrobium massiliense, type strain Marseille-P4301ᵀ (CSUR P4301ᵀ); Anaerolactibacter with the type species Anaerolactibacter massiliensis, type strain Marseille-P4302ᵀ (CSUR P4302ᵀ); Galactobacillus, with the type species Galactobacillus timonensis, type strain Marseille-P4641ᵀ (CSUR P4641ᵀ). The main characteristics of this new species have been previously published with the former name Lactomassilus timonensis [33]. The name was changed following the advice of a world expert in taxonomy (we thank Professor A. Oren). We also proposed the creation of a new species: Acidpropionibacterium timonense, with type strain Marseille-P4482ᵀ (CSUR P4482ᵀ).

Taxonomic and nomenclatural proposals

Description of Lactimicrobium gen. nov. Lactimicrobium (Lac.ti.-micro.bium. L. masc. n. lac, lactis milk; N.L. neut. n. microbium a microbe; N.L. neut. n. Lactimicrobium a microbe from milk). Cells are Gram-positive, non-motile, non-spore-forming and anaerobic rod-shaped bacteria. They are mesophilic and do not require NaCl for growth. pH tolerance ranges from pH 6.5 to pH 8. Cells do not produce catalase or oxidase activity and measure approximately 0.5/1.5 μm width/length. The type species is Lactimicrobium massiliense. The taxonomic classification is Bacteria; Terrabacteria group; Firmicutes; Erysipelotrichi; Erysipelotrichales; Erysipelotrichaceae; Lactimicrobium.

Description of Lactimicrobium massiliense sp. nov. Lactimicrobium massiliense (mas.si.li.en.se. L. neut. adj. massiliense of Massilia, the Latin name for Marseille). Colonies grown on 5% sheep blood Columbia agar plates (bioMérieux) after 48 h incubation under anaerobic conditions are circular, around 1–2 mm in diameter; the agar plate takes the colour of burnt blood after 48 h of incubation. Using an API strip (ZYM, 20 A and Rapid ID 32 A), indole is produced while urease is not. Aesculin and gelatine are hydrolysed. Cellobiose, glucose, maltose, saccharose, salicin and trehalose are fermented. Acid phosphatase, naphthol-AS-Bl-phosphohydrolase, esterase (C-4), esterase lipase (C-8), arginine dihydrolase, leucine arylamidase, α-fucosidase and β-galactosidase activities were positive. Their major fatty acids are C₁₆:0, C₁₈:1n9 and C₁₈:1n7. The DNA G+C content of the type strain is 48.5 % (genome sequence). The type strain is Marseille-P4302ᵀ (CSUR P4302ᵀ) isolated from a milk sample from a healthy lactating Malian mother.

Description of Anaerolactibacter gen. nov. Anaerolactibacter (An.æ.ro.lacti.bacter. Gr. pref. an; Gr. masc. or fem. n. aer air; L. masc. n. lac, lactis milk; N.L. masc. n. bacter a rod; N.L. masc. n. Anaerolactibacter an anaerobic rod from milk). Cells are Gram-positive, non-motile, non-spore-forming and anaerobic rod-shaped bacteria. They are mesophilic and do not need NaCl for their growth but tolerate up to 50 g/L of salt. pH tolerance ranges from pH 6.5 to pH 8. Cells do not produce catalase or oxidase activity and measure approximately 0.5/2μm width/length. The type species is Anaerolactibacter massiliensis. The taxonomic classification is Bacteria; Terrabacteria group; Firmicutes; Erysipelotrichia; Erysipelotrichales; Erysipelotrichaceae; Anaerolactibacter.

Description of Anaerolactibacter massiliensis sp. nov. Anaerolactibacter massiliensis (mas.si.li.en sis. L. masc. adj. massiliensis of Massilia, the Latin name for Marseille). Colonies grown on 5% sheep blood Columbia agar plates (bioMérieux) after 48 h incubation under anaerobic conditions are grey, circular, around 1–2 mm in diameter; the agar plate takes the colour of burnt blood after 48 h of incubation. Using an API strip (ZYM, 20 A and Rapid ID 32 A), indole is produced while urease is not. Aesculin and gelatine are hydrolysed. Cellobiose, glucose, maltose, saccharose, salicin and trehalose are fermented. Acid phosphatase, naphthol-AS-Bl-phosphohydrolase, esterase (C-4), esterase lipase (C-8), arginine dihydrolase, leucine arylamidase, α-fucosidase and β-galactosidase activities were positive. Their major fatty acids are C₁₆:0, C₁₈:1n9 and C₁₈:1n7. The DNA G+C content of the type strain is 48.5 % (genome sequence). The type strain is Marseille-P4302ᵀ (CSUR P4302ᵀ) isolated from a milk sample from a healthy lactating Malian mother.

Description of Galactobacillus gen. nov. Galactobacillus (Ga.lac.to.bacillus. Gr. neut. n. gala, galaktos milk; L. masc. n. bacillus, a small rod. N.L. masc. n. Galactobacillus a rod from milk). Cells are Gram-positive, non-motile, non-spore-forming, anaerobic rod-shaped bacteria. They are mesophilic and do not require NaCl for growth. pH tolerance ranges from pH 6.5 to pH 8. Cells do not produce catalase or oxidase activity and measure approximately 0.4/0.8μm width/length. The type species is Galactobacillus timonensis. The taxonomic classification is Bacteria; Terrabacteria group; Firmicutes; Erysipelotrichia; Erysipelotrichales; Erysipelotrichaceae; Galactobacillus.

Description of Galactobacillus timonensis sp. nov. Galactobacillus timonensis (ti.mon.en sis. L. masc. adj. timonensis of quarter La Timone where the strain was isolated). Colonies grown on 5% sheep blood Columbia agar plate (bioMérieux) after 48 h incubation under anaerobic conditions are regular and umbilicate, translucent, non-haemolytic, around 1–1.5 mm.
in diameter. Using an API strip (ZYM, 20 A and Rapid ID 32 A), indole is produced but urease is not. Gelatine and aesculin are hydrolysed. Cellobiose, glucose, glycerol, lactose, maltose, mannitol, melezitose, saccharose, salicin and trehalose are fermented. Acid phosphatase, naphthol-AS-Bl-phosphohydrolase, esterase (C-4), esterase lipase (C-8), arginine arylamidase, glutamyl glutamic acid arylamidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, α-arabinosidase, β-galactosidase, β-glucosidase activity are positive. Their major fatty acid are C_{16:0}, C_{18:1\alpha9} and C_{18:1\alpha7}. The DNA G+C content of the type strain is 50 % (genome sequence). The type strain is Marseille-P4482T (CSUR P4482T) isolated from a milk sample from a healthy lactating Malian mother.

### Description of Acidipropionibacterium timonense sp. nov.

**Acidipropionibacterium timonense** (ti.mon‘ense. N.L. neut. adj. timon‘ense of quarter La Timone where the strain was isolated).

Colonies grown on 5% sheep blood Colombia agar plat (bio-Mérieux) after 24 h incubation under anaerobic or aerobic conditions are creamy, non-haemolytic, circular, around 3–5 mm in diameter. Cells are Gram-positive, non-motile, non-spore-forming, facultatively anaerobic coccobacilli 0.8/1.2 μm in width/length. pH tolerance ranges from pH 6.5 to pH 8. Using API strip (ZYM, 20 A and Rapid ID 32 A), gelatine and aesculin are hydrolysed but indole and urease are not produced. Cellobiose, glucose, glycercol, lactose, maltose, mannitol, mannose, melezitose, raffinose, saccharose, salicin and trehalose are fermented. Acid phosphatase, alkaline phosphatase, N-acetyl-β-glucosaminidase, naphthol-AS-Bl-phosphohydrolase, esterase (C-4), esterase lipase (C-8), alanine arylamidase, arginine arylamidase, glutamyl glutamic acid arylamidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, proline arylamidase, pyroglutamic acid arylamidase, serine arylamidase, tyrosine arylamidase, valine arylamidase, α-arabinosidase, β-galactosidase, α-glucosidase, β-glucosidase, α-mannosidase and α-galactosidase activities were positive. The major cellular fatty acids of strain Marseille-P4482 are C_{15:0} iso and anteiso-C_{15:0}.

The type strain is Marseille-P4482T (CSUR P4482T) isolated from a milk sample from a healthy Malian mother.

### Transparency declaration

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