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

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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-P4301^T (CSUR P4301^T), Marseille-P4302^T (CSUR P4302^T) and Marseille-P4641^T (CSUR P4641^T), respectively. The strain Marseille-P4482 represents a novel species, *Acidipropionibacterium timonense,* in a previously known genus with type strain being Marseille-P4482^T (CSUR P4482^T).

Keywords: Culturomics, human breast milk, microbiome, microbiota, taxono-genomics
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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 taxonogenomic characterization of strain Marseille-P-4301^T, strain Marseille-P4302^T and strain Marseille-P4641^T as type strains of *Lactimicrobium massiliense* gen. nov., sp. nov. (CSUR 4301), *Anaerolactibacter massiliensis* gen. nov., sp. nov. (CSUR 4302) and *Galactobacillus*

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timonensis gen. nov., sp. nov. (CSUR 4641), close to *Solobacterium moorei* strain JCM 10645 [19] and strain Marseille-P4482^T 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 polypropylene 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 delivery. 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-Méditerranée 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 2016-004 and FMPOS Institutional Ethics Committee (Mali, 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 and 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 (bioMerieux, Marcy l'Etoile, 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 website 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 instructions. Analyses were performed as previously described [22]. Motility assay, Gram-staining, transmission electron microscopy 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 genomes of *Solobacterium moorei* strain RCA59-74^T (NZ_AUKY00000000) [19], *Bulleidia extructa* strain W 1219^T (NZ_ADFR00000000) [26], *Anaerorhabdus furcosa* strain VPI 3253^T (NZ_FUWY00000000) [27], *Holdemania filiformis* strain J1-31B-1^T (NZ_ACCF01000000) [28] and *Holdemania massiliensis* strain AP2^T (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_AOSS00000000), *Pseudopropionibacterium propionicum* F0230a (NC_018142), *Propionibacterium acidifaciens* strain C3M 31 (NZ_AUFR00000000), *Acidipropionibacterium thoenii* strain NCFB 568 (NZ_AUHZ01000000) and *Acidipropionibacterium acidipropionici* strain NCFB 563 (NZ_ATYU01000000) 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

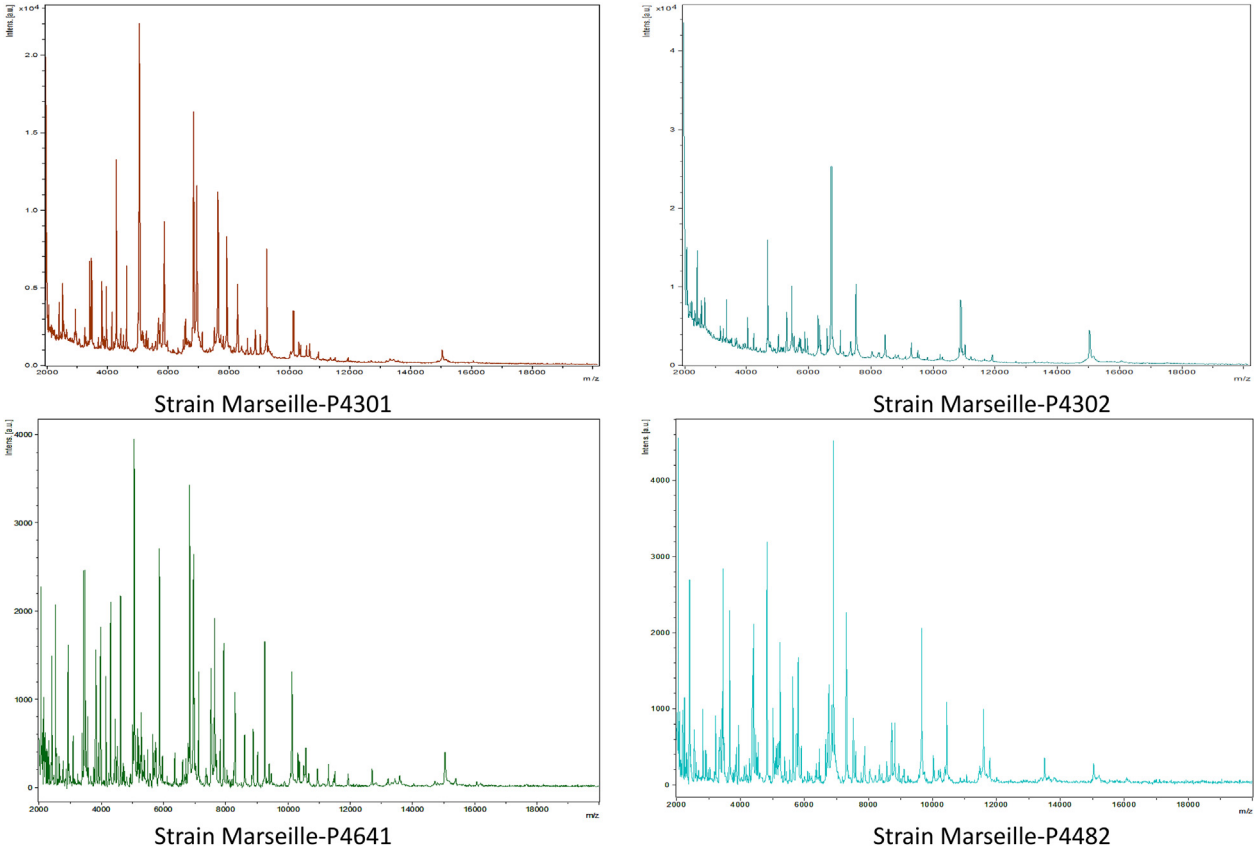

FIG. 1. Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) reference mass spectrum from strains Marseille-P4301, Marseille-P4302, Marseille-P4641 and Marseille-P4482.

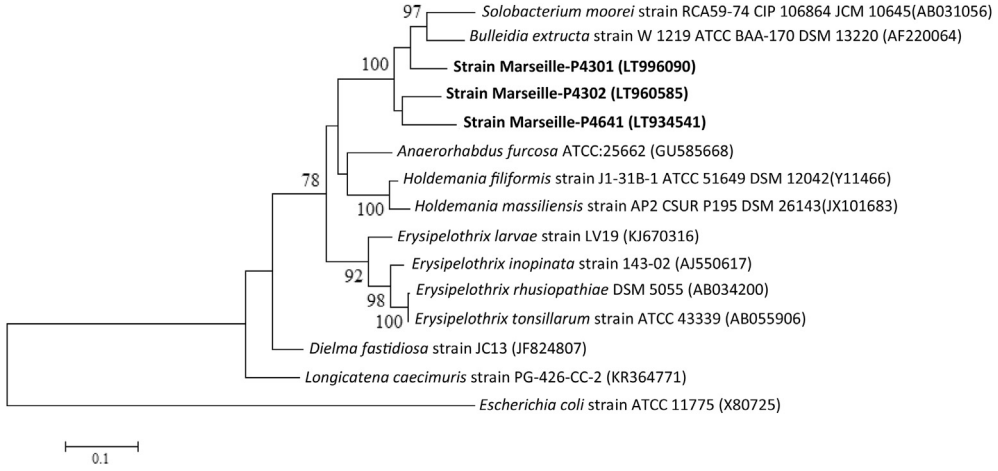

FIG. 2. Maximum likelihood phylogenetic tree highlighting the position of strains Marseille-P4301, Marseille-P4302, and Marseille-P4641 against most closely related species. The evolutionary history was inferred by using the maximum likelihood method based on the Kimura 2-parameter model. A discrete 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.

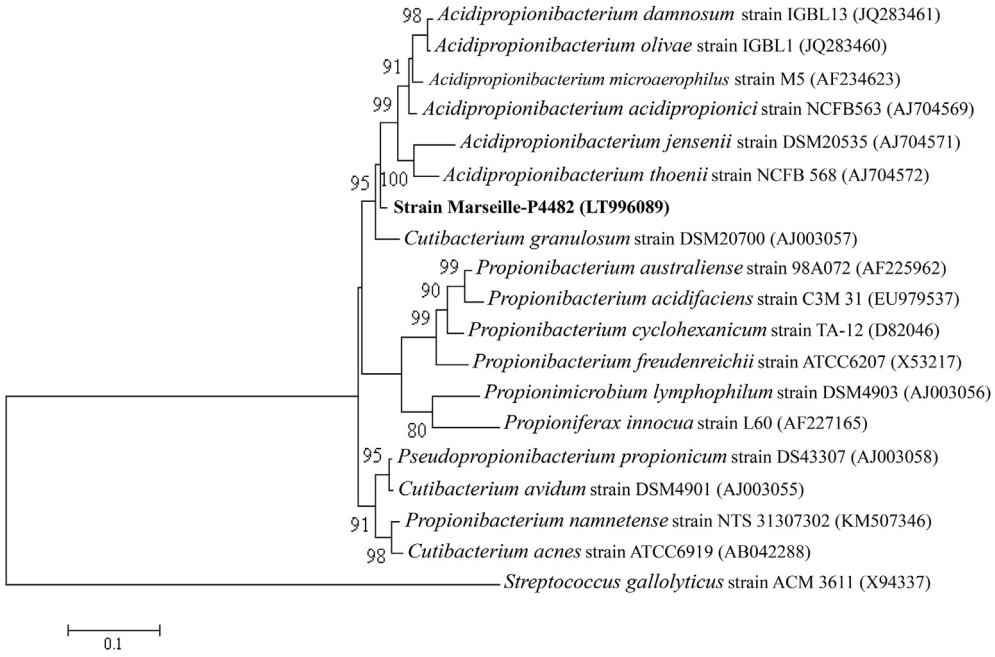

FIG. 3. Maximum likelihood phylogenetic tree highlighting the position of strain Marseille-P4482 against other most closely related species. The evolutionary history was inferred by using the maximum likelihood method based on the Kimura 2-parameter model. A discrete γ distribution was used to model 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.

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 = LMG 16726^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 stain were deposited in EMBL-EBI under accession number: LT996090, LT960585, LT934541 and LT996089 (Strain Marseille-P4301, Marseille-P4302, Marseille-P4641 and Marseille-P4482 respectively).

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	92.5	90.8	91.6	91.9	88.5	87.8	89.2
Strain Marseille-P4302		100	91.9	89.8	90.8	89.3	88.4	89.1
Strain Marseille-P4641			100	88.9	89.5	88.6	87.6	87.7
***S. moorei* strain RCA59-74^T**				100	92.4	87.8	87.1	88.9
***B. extructa* strain W 1219^T**					100	88.4	87.0	89.3
***H. filiformis* strain J1-31B-1^T**						100	97.1	91.7
***H. massiliensis* strain AP2^T**							100	91.0
***A. furcosa* strain VPI 3253^T**								100

1, strain Marseille-P4301; 2, Strain Marseille-P-4302; 3, Strain Marseille-P4641; 4, *Solobacterium moorei* strain RCA59-74^T; 5, *Bulleidia extructa* strain W 1219^T; 6, *Holdemania filiformis* strain J1-31B-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. extructa* strain W 1219^T, Y11466 for *H. filiformis* strain J1-31B-1^T, JX101683 for *H. massiliensis* strain AP2^T and GU585668 for *A. furcosa* strain VPI 3253^T).

TABLE 2. Pairwise comparison of strains Marseille-P4482 for 16S rRNA sequence similarity compared with closely related species. Value (%)

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
***Cutibacterium avidum* ATCC 25577**			100	94.7	99.2	91.8	92.8	94.0
***Cutibacterium granulosum* DSM 20700**				100	94.6	90.6	92.8	94.6
***Pseudopropionibacterium propionicum* F0230a**					100	91.8	92.8	94.0
***Propionibacterium acidifaciens* strain C3M 31**						100	90.3	91.0
***Acidipropionibacterium thoenii* strain NCFB 568**							100	95.5
***Acidipropionibacterium acidipropionici* strain NCFB 563**								100

1, Strain Marseille-P4301; 2, *Cutibacterium acnes* strain ATCC6919^{T}; 3, *Cutibacterium avidum* strain DSM 4901^{T}; 4, *Cutibacterium granulosum* strain DSM 20700^{T}; 5, *Pseudopropionibacterium propionicum* strain F0230a^{T}; 6, *Propionibacterium acidifaciens* strain C3M 31^{T}; 7, *Acidipropionibacterium thoenii* strain NCFB 568^{T}; 8, *Acidipropionibacterium acidipropionici* strain NCFB 563^{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 (LT996089 for strain, AB042288 for *C. acnes*, AJ003055 for *C. avidum*, AJ003057 for C. granulosum, AJ003058 for *P. propionicum*, EU979537 for *P. acidifaciens*, AJ704572 for P. *thoenii* and AJ704569 for *Acidipropionibacterium acidipropionici*).

Phenotypic and biochemical characterization

Cells from the strains Marseille-P4301, Marseille-P4302 and Marseille-P4641 are Gram-negative staining, non-motile, non-spore-forming, strictly anaerobic rods. Those from strain Marseille-P4482 are Gram-positive staining, non-motile, non-spore forming 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

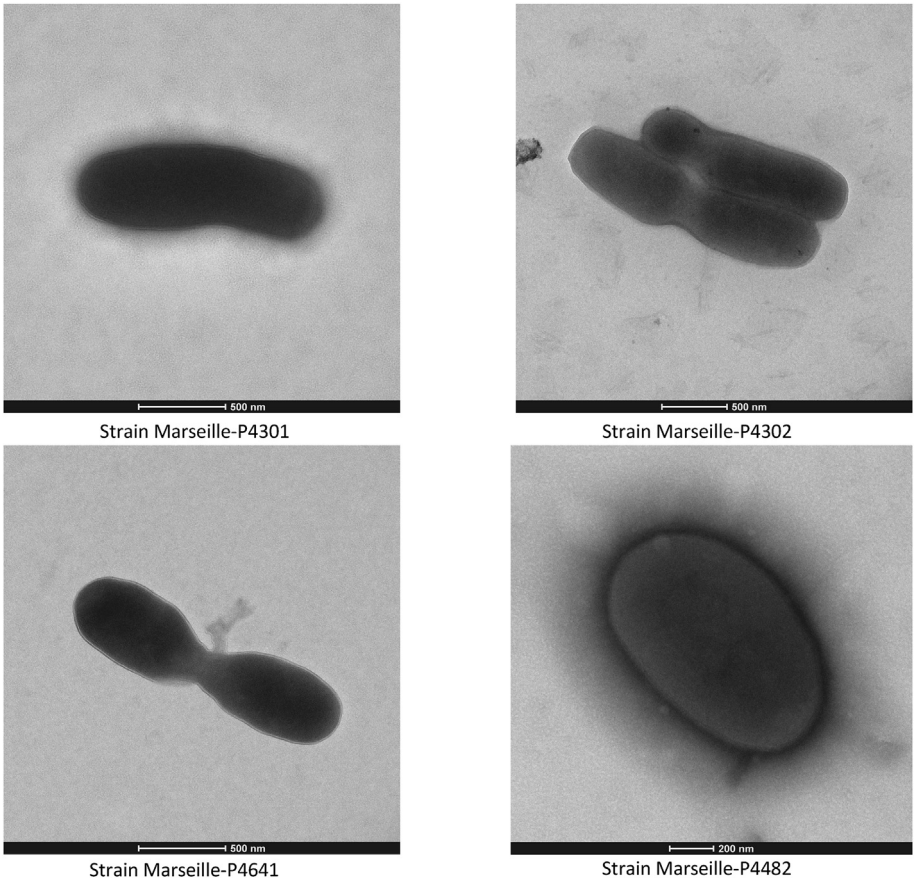

FIG. 4. Transmission electron microscopy of strains Marseille-P4301, Marseille-P4302, Marseille-P4641 and Marseille-P4482 using Tecnai G20 (FEI Company) at operating voltage of 60 kV. Scale bar = 500 nm (Strain marseille-P4301, Maeseille-P4302 and marseille-P4641) and 200nm (Strain Marseille-P4482).

atmosphere on 5% sheep-blood Columbia agar (bioMérieux). The strain Marseille-P4482 is able to growth 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. Cellobiose, maltose, sucrose 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), β-galactosidase and leucine arylamidase activity but not cystine arylamidase, lipase (C-14), glutamic acid decarboxylase, trypsin, α-chymotrypsin and β-glucuronidase activity. Table 3 displays the phenotypic and chemical characteristics of the four strains. The major cellular fatty acid are: $C_{16:0}$ (45.6, 39.3 and 45.9%), $C_{18:1n9}$ (21.8, 27.6 and 26.6%) and $C_{18:1n7}$ (20.2, 19.4 and 12.3%) for the strains Marseille-P4301, Marseille-P4302 and Marseille-P4641 respectively and iso-$C_{15:0}$ (62.5%) and anteiso-$C_{15:0}$ (18.4%) for the strain Marseille-P4482. Cellular fatty composition of the four species is shown in Table 4.

TABLE 3. Differential characteristics of the new strains

Properties	1	2	3	4
Cells size (μm)	0.5/1.5	0.5/2	0.4/0.8	0.8/1.2
Gram stain	—	—	—	+
Motility	—	—	—	—
Urease	—	—	—	—
Aesculin hydrolase	+	+	+	+
Gelatine hydrolase	—	+	+	+
Indole production	+	+	+	—
Nitrates reduction	—	—	—	—
Acid from:				
Arabinose	—	—	—	—
Cellobiose	+	+	+	+
Glucose	—	+	+	+
Glycerol	—	—	+	+
Lactose	+	—	+	+
Maltose	+	+	+	+
Mannitol	—	—	+	+
Mannose	—	—	v	+
Melezitose	—	—	+	+
Raffinose	—	—	—	+
Rhamnose	—	—	—	—
Saccharose	+	+	+	+
Salicin	—	+	+	+
Sorbitol	—	—	—	—
Trehalose	+	+	+	+
Xylose	—	—	—	—
Acid phosphatase	+	+	+	+
Alkaline phosphatase	v	—	—	+
N-Acetyl-β-glucosaminidase	+	—	—	+
Naphthol-AS-BI-phosphohydrolase	+	+	+	+
Esterase (C-4)	+	+	+	+
Esterase lipase (C-8)	+	+	+	+
Lipase (C-14)	—	—	—	—
Arginine dihydrolase	+	+	—	—
Alanine arylamidase	—	—	—	+
Arginine arylamidase	+	—	+	+
Cystine 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	—	—	—	+
Glutamic acid decarboxylase	—	—	—	—
α-Arabinosidase	—	—	+	+
Trypsin	—	—	—	—
α-Chymotrypsin	—	—	—	—
α-Fucosidase	—	+	—	—
β-Galactosidase	+	+	+	+
β-Galactosidase 6 phosphate	+	—	—	—
α-Glucosidase	+	—	—	+
β-Glucosidase	+	—	+	+
β-Glucuronidase	—	—	—	—
α-Mannosidase	—	—	—	+
α-Galactosidase	+	—	—	+
Genome G+C %	47	48.5	50	69
Isolated from	Human milk	Human milk	Human milk	Human milk

+, Positive reaction; – negative reaction; v, variable reaction; 1, strain Marseille-P4301; 2, strain Marseille-P-4302; 3, strain Marseille-P4641; 4, strain Marseille-P4482.

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. At final assembly, the genomes are composed of 7, 2, 10 and 15 scaffolds for strains Marseille-P4301, Marseille-P4302, Marseille-P4641 and Mareille-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-P-4302, (3) strain Marseille-P4641, and (4) strain Marseille-P4482

Fatty acids	1	2	3	4
$C_{16:0}$	**45.6**	**39.3**	**45.9**	7.4
$C_{18:1n9}$	**21.8**	**27.6**	**26.6**	2.6
Iso-$C_{15:0}$	ND	ND	ND	**62.5**
Anteiso-$C_{15:0}$	ND	ND	ND	**18.4**
$C_{18:1n7}$	**20.2**	**19.4**	**12.3**	ND
$C_{18:0}$	9.2	7.8	8.7	1.8
$C_{14:0}$	1.7	2.9	3.8	<1
$C_{15:0}$	<1	1.3	1.1	1.1
Iso-$C_{5:0}$	ND	ND	ND	1.4
$C_{16:1n7}$	<1	<1	<1	ND
$C_{17:0}$	<1	<1	<1	<1
$C_{12:0}$	<1	<1	<1	ND
$C_{18:2n6}$	<1	<1	<1	<1
$C_{17:0\ iso}$	ND	ND	ND	3.0
Anteiso-$C_{17:0}$	ND	ND	ND	<1
Iso-$C_{13:0}$	ND	ND	ND	<1

ND, not detected.

presence of the RNA processing and modification gene for the strain Marseille-P4482, the absence of the chromatin structure and dynamics gene for the strains Marseille-P4301 and Marseille-P4302 and the extracellular structures gene for the strain Marseille-P4641 (Table 5).

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).

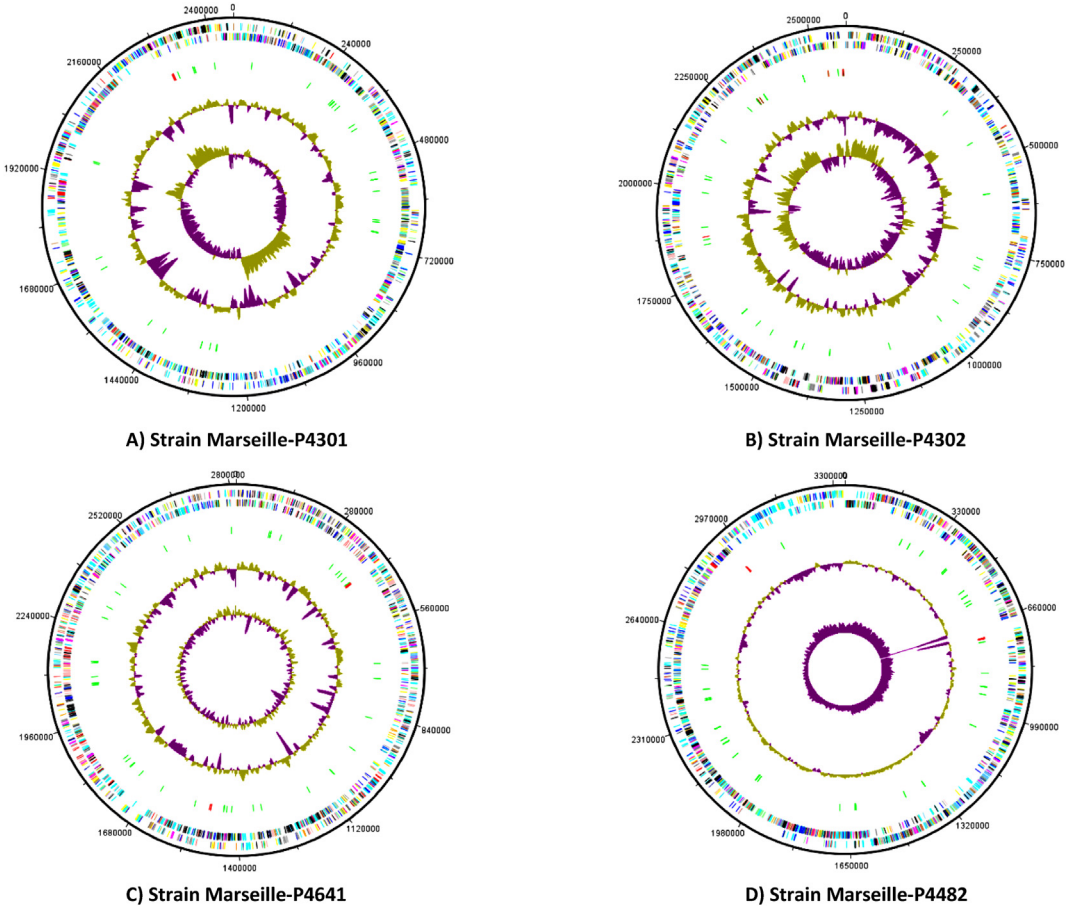

FIG. 5. Graphical circular map of the genome of (a) strain Marseille-P4301, (b) strain Marseille-P4302, (c) strain Marseille-P4641, and (d) strain Marseille-P4482. From outside to the centre: Contigs (red/grey), clusters of orthologous groups (COGs) category of genes on the forward strand (three circles),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

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	1
Transcription	161	220	231	168
Replication, recombination and repair	134	171	152	108
Chromatin structure and dynamics	0	0	1	1
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	0	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)[a]

	1	2	3	4	5	6	7	8
Marseille-P4301	**100%**	50.0%±2.7	24.8%±2.4	28.0%±2.4	27.3%±2.5	26.1%±2.4	19.0%±2.3	27.1%±2.5
Marseille-P4302		**100%**	38.9%±2.3	67.9%±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
B. extructa					**100%**	29.5%±2.5	27.7%±2.4	26.4%±2.5
H. filiformis						**100%**	24.5%±2.4	28.6%±2.5
H. massiliensis							**100%**	28.8%±2.4
A. furcosa								**100%**

1, Marseille-P4301; 2, Marseille-P4302; 3, Marseille-P4641; 4, *Solobacterium moorei* strain RCA59-74^T; 5, *Bulleidia extructa* strain W 1219^T; 6, *Anaerorhabdus furcosa* strain VPI 3253^T; 7, *Holdemania filiformis* strain J1-31B-1^T; 8, *Holdemania massiliensis* AP2^T

[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 trains 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)[a]

	1	2	3	4	5	6	7	8
Strain Marseille-P4482	**100%**	21.5%±2.4	21.2%±2.3	21.6%±2.4	21.40%±2.3	22.7%±2.4	19.8%±2.4	19.7%±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
C. avidum			**100%**	20.7%±2.4	23.6%±2.4	23.3%±2.4	18.8%±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%**	18.4%±2.3
P. propionicum								**100%**

1, Marseille-P4482^T; 2, *Acidipropionibacterium acidipropionici* strain DSM4900^T; 3, *Cutibacterium avidum* strain ATCC25577^T; 4, *Acidipropionibacterium thoenii* strain DSM20276^T; 5, *Cutibacterium acnes* strain ATCC6919^T; 6, *Cutibacterium granulosum* strain DSM20700^T; 7, *Propionibacterium acidifaciens* strain C3M_31^T; 8, *Pseudopropionibacterium propionicum* strain F0230a^T.

[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-P4301^T (=CSUR P4301^T); *Anaerolactibacter* with the type species *Anaerolactibacter massiliensis*, type strain Marseille-P4302^T (=CSUR P4302^T); *Galactobacillus*, with the type species *Galactobacillus timonensis*, type strain Marseille-P4641^T (=CSUR P4641^T). 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: *Acidipropionibacterium timonense*, with type strain Marseille-P4482^T (= CSUR P4482^T).

Taxonomic and nomenclatural proposals

Description of Lactimicrobium *gen. nov.*. *Lactimicrobium* (Lac.ti.mi.cro'bi.um. 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 0.5/1.5µm width/length. The type species is *Lactimicrobium massiliense*. The taxonomic classification is *Bacteria*; *Terrabacteria* group; *Firmicutes*; *Erysipelotrichia*; *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 of incubation under anaerobic conditions are regular, umbilicate, translucent, non-haemolytic, around 1–1.5 mm in diameter. Using API strip (ZYM, 20 A and Rapid ID 32 A), indole is produced but urea is not. Aesculin is hydrolysed but gelatine is not. Cellobiose, lactose, maltose, saccharose and trehalose are fermented. Acid phosphatase, N-acetyl-β-glucosaminidase, naphthol-AS-BI-phosphohydrolase, esterase (C-4), esterase lipase (C-8), arginine dihydrolase, arginine arylamidase, leucine arylamidase, β-galactosidase, β-galactosidase 6-phosphate, α-glucosidase, β-glucosidase and α-galactosidase activity were positive. Their major fatty acid are $C_{16:0}$, $C_{18:1n9}$ and $C_{18:1n7}$. The DNA G+C content of the type strain is 47 % (genome sequence). The type strain is Marseille-P4301^T (CSUR P4301^T) isolated from a milk sample from a healthy lactating Malian mother.

Description of Anaerolactibacter *gen. nov.*. *Anaerolactibacter* (An.ae.ro.lac.ti.bac'ter. Gr. pref. *an* not; 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-BI-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 $C_{16:0}$, $C_{18:1n9}$ and $C_{18:1n7}$. The DNA G+C content of the type strain is 48.5 % (genome sequence). The type strain is Marseille-P4302^T (CSUR P4302^T) isolated from a milk sample from a healthy lactating Malian mother.

Description of Galactobacillus *gen. nov.*. *Galactobacillus* (Ga.lac.to.ba.cil'lus. 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. N.L. masc. adj. *timonensis* the name of quarter La Timone where the strain was isolated). Colonies grown on 5% sheep blood Colombia agar plat (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-BI-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 $C_{16:0}$, $C_{18:1n9}$ and $C_{18:1n7}$. The DNA G+C content of the type strain is 50 % (genome sequence). The type strain is Marseille-P4641^T (CSUR P4641^T) 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. *timonense* of quarter La Timone where the strain was isolated). Colonies grown on 5% sheep blood Colombia agar plat (bioMérieux) 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 0.8/1.2µm 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, glycerol, lactose, maltose, mannitol, mannose, melezitose, raffinose, saccharose, salicin and trehalose are fermented. Acid phosphatase, alkaline phosphatase, N-acetyl-β-glucosaminidase, naphthol-AS-BI-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-P4482^T (CSUR P4482^T) isolated from a milk sample from a healthy Malian mother.

Transparency declaration

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