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Research Article

Description of *Peptoniphilus phoceensis* sp. nov., and *Lentibacillus timonensis* sp. nov., Two New Bacteria Isolated from Fresh Stool of Human

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Received: April 04, 2021; **Published:** June 28, 2021

Abstract

Two bacterial strains were isolated from human stool samples. Strains Marseille-P4043 and Marseille-P2183 are Gram positive bacteria but rod and coccus shaped, respectively. $C_{16:0}$ (56.4%) and $C_{15:0}$ anteiso (77.03%) are the major fatty acids found in the wall cells of the studied strains, respectively. Their genomic sequences measured 3.92 Mbp and 1.73 Mbp with 38.5 mol% and 31.2 mol% of G+C content, respectively. The 16S rRNA gene sequences analysis shows that sequence similarities of strains Marseille-P4043 and Marseille-P2183 were 96.5% with *Lentibacillus populi* strain WD4L (or 96.6% with *Virgibacillus dakarensis* strain Marseille-P3469) and 96.9% with *Peptoniphilus grossensis*, respectively. Further the low 16S rRNA gene sequence similarities (< 98.65%, the cut-off value), the strains had degree of OrthoANI (95 - 96%, the cut-off value) and degree of dDDH (< 70%, the cut-off value) lower than thresholds used to delimit the species barrier in prokaryotic. Basing on the phenotypic, phylogenetic and genomic characteristics, we propose that strain Marseille-P4043 and strain Marseille-P2183 should be classified as new bacterial species for which, *Lentibacillus timonensis* sp. nov., and *Peptoniphilus phoceensis* sp. nov., are the proposed names.

Keywords: *Peptoniphilus phoceensis; Lentibacillus timonensis; Genome; Culturomics; Taxono-Genomics; Gut Microbiota*

Introduction

The microbiota, formerly called “flora” is the subject of more and more studies. It includes a great bacterial diversity which deserves to be more widely exploited to better understand their involvement in the individual state of the healthy or sick subject. To better explore its components, the culturomics method remains an effective strategy because it is to use several culture condition (varying pH, media composition, temperature, atmosphere...) and make in parallel a rapid identification by MALDI-TOF mass spectrometry [1]. The phylum *Firmicutes* regroups the highest number of known bacterial species, therefore its members are sometimes involved in human infections like *Peptoniphilus* spp [2] or retrieved in natural varied environments like *Lentibacillus* spp [3]. However, the genus *Lentibacillus* was created in 2002 by Yoon., *et al* [4]. The members of this genus are Gram variable and halophilic bacteria. They are most often isolated from salty environments. Currently, there are 22 known species of which 17 have a validly published and correct name [5]. In addition, the

genus *Peptoniphilus* was first described by Ezaki., *et al* [6]. *Peptoniphilus* species has been isolated mainly from various human clinical specimens, such as vaginal discharge, abscesses, ovarian, peritoneal, sacral and lacrimal glands [6]. At the time of writing, there are 17 species [7] validly published.

Here, we report the Taxonogenomic description of two new species belonging to the phylum *Firmicutes* for which the names *Lentibacillus timonensis* sp. nov., and *Peptoniphilus phoceencis* sp. nov., are proposed. The later has been previously announced [8] but complete findings from this strain are provided in this current manuscript.

Materials and Methods

Ethics and sample collection

Stool samples were collected from healthy volunteers living in France. They signed an informed consent and the ethics committee of the Institut de Recherche Fédératif 48 (Aix-Marseille University, Marseille, France) approved the study. Furthermore, the methods employed in this study were carried out in accordance with relevant guidelines and recommendations conformed to the Helsinki's Declaration.

Strains isolation and identification

The strain Marseille-P4043 was isolated under aerobic condition from a human stool sample after 24 hours of incubation. The sample was inoculated on Columbia agar with 5% sheep blood (bioMérieux, Marcy l'Etoile, France) and incubated at 37°C. The strain Marseille-P2183 was isolated after 48 hours incubation at 37°C on Columbia agar with 5% sheep blood in an anaerobic atmosphere generated by the GENbagAnaer system (bioMérieux). Both strains were not identified by MALDI-TOF mass spectrometry, despite several attempts. However, generated spectra were imported and analyzed using Biotyper 3.0 software and then incremented in the local database (https://www.mediterranee-infection.com/urms-data-base). Subsequently, the 16S rRNA gene was amplified using the universal primer pairs fD1 and rP2 (Eurogentec, Angers, France) and sequenced with the Big Dye® Terminator v1.1 sequencing kit and the 3500xL Genetic Analyzer capillary sequencer (Thermofisher, Saint-Aubin, France). The nucleotide sequences obtained are assembled and edited with the CodonCode Aligner software (http://www.codoncode.com). The consensus sequence of each strain was used as reference sequence and deposited in Genbank database. To determine the 16S rRNA sequences of the closest species, a comparison (BLASTn) was performed using the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Growth conditions and phenotypic characterization

The optimal growth conditions were determined for studied strains by performing several different tests. The optimum growth temperature is sought among the following temperatures: 28, 37, 42 and 56°C. In parallel, the strains were inoculated on Columbia agar with 5% sheep blood and incubated in different types of atmospheres as aerobic, anaerobic and microaerophilic, as previously tested [9]. API ZYM and API 50 CH strips (bioMérieux, Marcy l'Etoile, France) were used to evaluate the biochemical characteristics of the bacteria according to the manufacturer's recommendations. Gram coloration, catalase and oxidase tests were performed according to standard procedures [10]. The morphology of the strains was visualized using the Hitachi SU5000 scanning electron microscope (Hitachi Group, Krefeld, Germany).

Genome extraction, sequencing and assembly

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit preceded by a lysozyme pre-treatment and incubation at 37°C for 2 hours. The DNA sequencing was performed using MiSeq technology with the Nextra Mate Pair

sample preparation kit and the Nextera XT Paired (Illumina) as described before [11]. Assembly was performed using a pipeline incorporating different softwares, including Velvet [12], Soap Denovo [13] and Spades [14]. Illumina MiSeq data was processed using Trimmomatic software or MiSeq software [15]. GapCloser software was used to reduce assembly deviations. Scaffolds < 800 base pairs and scaffolds with a depth value less than 25% of the average depth, were removed. The best assembly was selected using different criteria (number of scaffolds, N50, number of N).

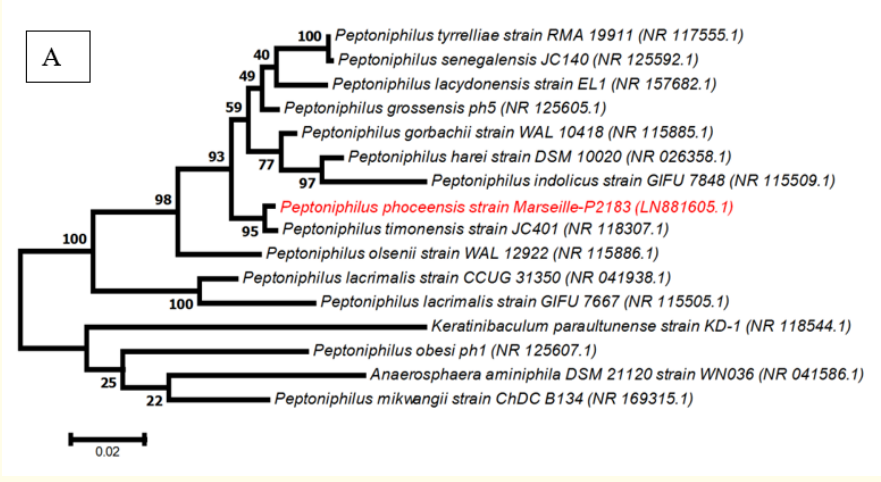
Genome annotation and analysis

Prodigal was used to predict the Open Reading Frame (ORF) with the default settings [16]. Deviations in the sequencing regions predicted by ORFs have been excluded. BlastP was used to predict the bacterial proteome according to the Clusters of Orthologous Groups (COG) database. If there was no match, the search for BlastP in the database [17] was extended with an E-value of 1e03, coverage of 0.7 and percent identity of 30. On the other hand, if the length of the sequence is less than 80 amino acids (aa), an E-value of 1e05 is used. The HMMscan tool is used in the domains that are maintained by the Pfam (Pfam-A and Pfam-B domains). The rRNA and tRNA genes have been retrieved using the RNAmmer [18] and tRNAScanSE tools [19]. The DNA-DNA hybridization (DDH) values of these species were calculated using Genome-to-Genome Distance Calculator (GGDC) (http://ggdc.dsmz.de) [20] with confidence intervals according to the recommended parameters (Formula 2, BLAST). The degree of genomic similarity of *Lentibacillus timonensis* sp. nov., strain Marseille-P4043 and *Peptoniphilus phoceensis* sp. nov., strain Marseille-P2183 with closely related species, was estimated using the OrthoANI software [21].

Results and Discussion

Phylogenetic analysis of strains

16S rDNA-based similarity analysis of strains Marseille-P4043^T and Marseille-P2183^T against Genbank database yielded highest nucleotide sequence similarities of 96.5% with *Lentibacillus populi* strain WD4L (GenBank accession number: NR_153711.1) and 96.9% with *Peptoniphilus grossensis* ph5 (NR_125605.1), respectively. As these values were below the recommended threshold value of 98.65%, established to delineate new bacterial species [22,23], strain Marseille-P4043^T and strain Marseille-P2183^T were considered as potential new species within the families *Bacillaceae* and *Peptoniphilaceae*, respectively. In addition, phylogenetic trees highlighting the position of each of the two strains relative to their closely related species with validly published names are shown in figure 1.



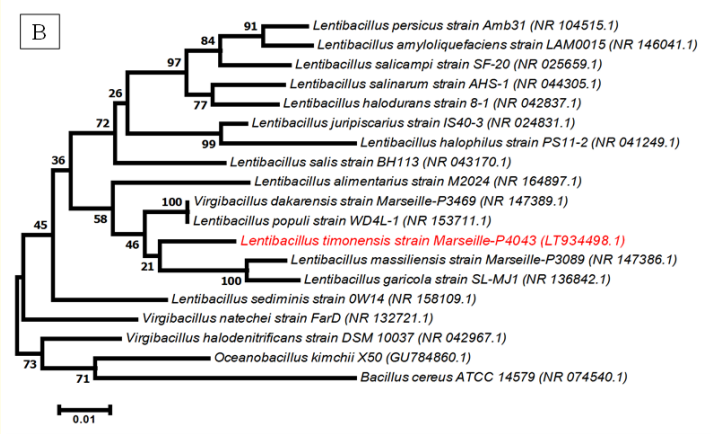


Figure 1: *Phylogenetic tree highlighting the position of Peptoniphilus phoceensis sp. nov., (A) and Lentibacillus timonensis sp. nov., (B) regarding others closely related species. Genbank accession numbers of 16S rRNA sequences are indicated in parenthesis. Sequences were aligned using MUSCLE with default parameters. Phylogenetic inferences were obtained using the Maximum likelihood method and the MEGA X software. Bootstrap values obtained by repeating the analysis 1,000 times to generate a majority consensus tree are indicated at the nodes. The scale bar for each tree is indicated on the figure.*

Phenotypic and biochemical tests

The two strains were tested at temperatures varying from 28 to 56°C and in different atmospheres (aerobic, anaerobic and microaerophilic). Optimal growth was observed at 37°C for both strains. Strain Marseille-P2183 is a Gram-positive coccus-shaped bacterium (Figure 2A). It is catalase positive and oxidase negative and grows well in anaerobiosis after 24 hours of incubation. Its bacterial colonies are opaque and have a diameter of 1 mm on agar plates. Individual cells have a mean diameter of 1.5 µm. They are motile, non spore forming and can grow in media with salt concentration ranging from 0 to 5% and with pH between 6 and 8.5, as previously reported [8]. However, using the API ZYM strip (bioMérieux), strain Marseille-P2183 was positive for leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. But negative reactions were observed for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Using API 50 CH, only esculin ferric citrate and potassium 2-ketogluconate are positive, while remaining carbohydrate substrates are not fermented with strain Marseille-P2183.

Strain Marseille-P4043 is an extremely halotolerant bacillus (Figure 2B). It is Gram-positive, strictly aerobic, rod-shaped bacterium. It was grown in saline media that may contain NaCl concentrations ranging from 0.5 to 25% (w/v) at pH 7.5. The optimal concentration of NaCl for its growth was 5%. Using API ZYM (bioMérieux), positive reactions were observed for alkaline phosphatase, esterase (C4), esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase with the strain Marseille-P4043. Negative reactions were observed for lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase. In addition, the use of API 50 CH strips shows that strain Marseille-P4043 was positive for D-galactose, D-glucose, esculin ferric citrate, D-lactose and negative for glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl αD-mannopyranoside, methyl αD-glucopyranoside,

N-acetyl-glucosamine, amygdalin, arbutin, salicin, D-cellobiose, D-maltose, D-melibiose, sucrose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagalose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. In addition, the phenotypic and biochemical criteria of the two strains are compared with those of other phylogenetically neighbor species (Table 1).

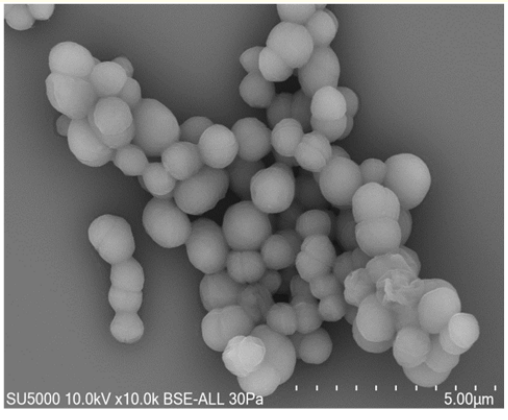


Figure 2A: *Peptoniphilus phoceensis* sp. nov.

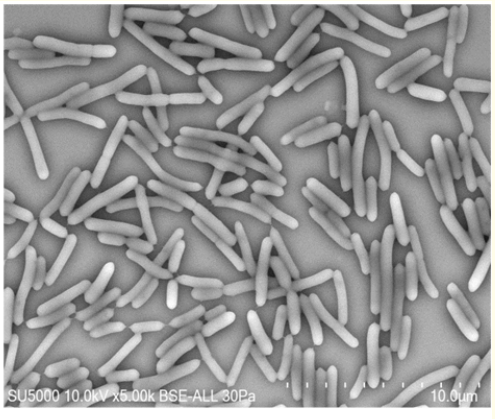


Figure 2B: *Lentibacillus timonensis* sp. nov.

Figure 2: *Transmission electron microscopy of Peptoniphilus phoceensis (2A) and Lentibacillus timonensis (2B). Cells are observed under Hitachi SU5000 transmission electron microscope. Scales are displayed on figure.*

Fatty acids	Names	1	2
$C_{16:0}$	Hexadecanoic acid	56.1 ± 3.2	34.2 ± 3.9
$C_{18:1n9}$	9-Octadecenoic acid	17.2 ± 3.5	31.8 ± 1.6
$C_{18:2n6}$	9,12-Octadecadienoic acid	15.5 ± 0.3	20.8 ± 2.9
$C_{14:0}$	Tetradecanoic acid	4.3 ± 0.1	2.2 ± 0.5
$C_{18:0}$	Octadecanoic acid	4.1 ± 0.2	6.0 ± 1.6
$C_{15:0}$	Pentadecanoic acid	1.2 ± 0.2	TR
$C_{17:0}$	Heptadecanoic acid	TR	-
$C_{16:1n7}$	9-Hexadecenoic acid	TR	-
$C_{18:1n7}$	11-Octadecenoic acid	-	1.8 ± 0.7
$C_{12:0}$	Dodecanoic acid	-	TR
$C_{15:0\ anteiso}$	12-methyl-tetradecanoic acid	-	TR
$C_{15:0\ iso}$	13-methyl-tetradecanoic acid	-	TR
$C_{4:1n1}$	Butenoic acid	-	TR
$C_{5:0\ anteiso}$	2-methyl butenoic acid	-	TR

Table 2: *Cellular fatty acid profiles (%) of Peptoniphilus phoceensis strain Marseille-P2183 compared with Peptoniphilus lacydonensis [29].*

1, Peptoniphilus phoceensis sp. nov., strain Marseille-P2183; 2, Peptoniphilus lacydonensis strain EL1; TR: Trace Amounts < 1%; -: Not Detected.

The most abundant fatty acid for strain Marseille P2183 was saturated structure Hexadecanoic acid (56%). Then, 9-octadecenoic acid (17%) and 9,12-octadecadienoic acid (16%) were unsaturated branched chains found but no branched structures were detected. Minor amounts of other fatty acids were also described (Table 2). For strain Marseille-P4043, major fatty acids were saturated iso/anteiso branched structures: 12-methyl-tetradecanoic acid (77%), 14-methyl-hexadecanoic acid (16%) and 14-methyl-pentadecanoic acid (4%). No unsaturated fatty acids were detected for strain Marseille-P4043 (Table 3).

Fatty acids	Names	1	2
$C_{16:0}$	Hexadecanoic acid	56.1 ± 3.2	34.2 ± 3.9
$C_{18:1n9}$	9-Octadecenoic acid	17.2 ± 3.5	31.8 ± 1.6
$C_{18:2n6}$	9,12-Octadecadienoic acid	15.5 ± 0.3	20.8 ± 2.9
$C_{14:0}$	Tetradecanoic acid	4.3 ± 0.1	2.2 ± 0.5
$C_{18:0}$	Octadecanoic acid	4.1 ± 0.2	6.0 ± 1.6
$C_{15:0}$	Pentadecanoic acid	1.2 ± 0.2	TR
$C_{17:0}$	Heptadecanoic acid	TR	-
$C_{16:1n7}$	9-Hexadecenoic acid	TR	-
$C_{18:1n7}$	11-Octadecenoic acid	-	1.8 ± 0.7
$C_{12:0}$	Dodecanoic acid	-	TR
$C_{15:0\ anteiso}$	12-methyl-tetradecanoic acid	-	TR
$C_{15:0\ iso}$	13-methyl-tetradecanoic acid	-	TR
$C_{4:1n1}$	Butenoic acid	-	TR
$C_{5:0\ anteiso}$	2-methyl butenoic acid	-	TR

Table 2: *Cellular fatty acid profiles (%) of Peptoniphilus phoceensis strain Marseille-P2183 compared with Peptoniphilus lacydonensis [29].*

1, Peptoniphilus phoceensis sp. nov., strain Marseille-P2183; 2, Peptoniphilus lacydonensis strain EL1; TR: Trace Amounts < 1%; -: Not Detected.

Fattyacids	Names	1	2
15:0 anteiso	12-methyl-tetradecanoic acid	77.3 ± 2.6	42.41
17:0 anteiso	Methyl hexadecanoic acid	14 - 15.5 ± 1.3	17.17
16:0 iso	14-methyl-pentadecanoic acid	3.6 ± 0.8	7.73
14:0 iso	12-methyl-Tridecanoic acid	1.3 ± 0.2	3.1
16:00	Hexadecanoic acid	1.3 ± 0.2	2.16
13:00	anteiso 10-methyl-Dodecanoic acid	TR	-
15:00	iso 13-methyl-tetradecanoic acid	TR	-
15:00	Pentadecanoic acid	TR	
15:0 iso	13-methyl-tetradecanoic acid	-	20.73
17:0 iso	15-Methylhexadecanoic acid	-	5.3
14:00	Tetradecanoic acid	TR	-

Table 3: *Cellular fatty acid profiles (%) of Lentibacillus timonensis strain Marseille-P4043 compared with Lentibacillus halodurans [30].*

1, Lentibacillus timonensis strain Marseille-P4043; 2, Lentibacillus halodurans strain 8-1^T ; TR: Trace Amounts < 1%; -: Not Detected.

Genomic analysis

Strain Marseille-P4043 was 3,924,819 bp with 38.5 mol% G+C content. The genome assembly of this strain was achieved on 3 contigs. Of the 3,976 predicted genes, 3,805 were protein-coding genes and 114 were RNAs (8 5S rRNA, 8 16S rRNA, 7 23S rRNA, 86 tRNA and 5 ncRNA genes) and 57 pseudo genes. Strain Marseille-P2183 was 1,739,566 bp with 31.2 mol% G+C content. The genome assembly of this strain was achieved on 11 contigs. Of the 1,685 predicted genes, 1,597 were protein-coding genes and 54 were RNAs (3 5S rRNA, 4 16S rRNA, 2 23S rRNA, 41 tRNA and 4 ncRNA genes) and 34 pseudo genes. A graphical circular map shows the different characteristics of the two genomes studied (Figure 3). Analysis of DDH values of species close to strain Marseille-P4043 shows that these values ranged from 18.1% between *L. sediminis* and *L. persicus* to 25.5% between *L. salicampi* and *L. jeotgali* (Table 4). For strain Marseille-P2183, the DDH values of neighbor species ranged from 20.9% between *P. indolicus* and *P. lacydonensis* to 32.6% between *P. senegalensis* and *P. gorbachii* (Table 5). These DDH values obtained are below the threshold of 70% recommended for delineating the species barrier in prokaryotes, thus confirming that strains Marseille-P4043 and Marseille-P2183 represent new bacterial species [24]. Analysis of the Clusters of Orthologous Groups (COGs) categories (Figure 4) shows that the genes involved in amino acid transport, metabolism and translation elements are most present in both genomes of the bacteria studied. In addition, calculation of OrthoANI values among closely related species reveals that the highest average nucleotide identity (ANI) for the *Lentibacillus* sp. was 80.6%, shared between *L. timonensis* and *L. populi*, while for the *Peptoniphilus* sp. was 86.8%, shared between *P. gorbachii* and *P. senegalensis* (Figure 5). Furthermore, *L. timonensis* sp. nov., had the highest OrthoANI value (77.3%) shared with *L. populi* and the lowest OrthoANI value (69.7%) is shared with *L. sediminis*. Then *P. phoceensis* sp. nov., had as highest OrthoANI value of 83.5% with *P. harei* and lower OrthoANI value of 68.3% with *P. indolicus*. All values obtained are below the thresholds (95 - 96%) recommended for delineating the species barrier in bacteria [24,25].

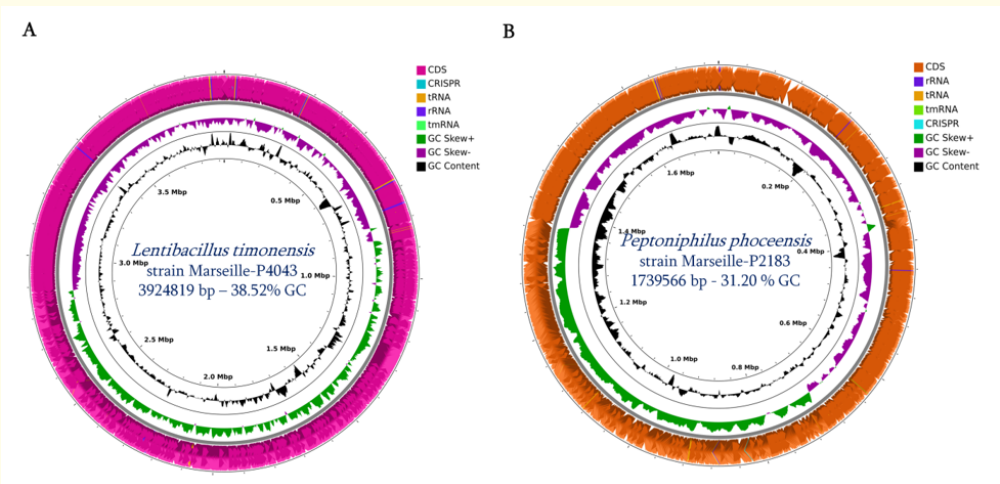


Figure 3: *A circular map generated using the CGView tool [26]. Server showing a complete view of the genome of Lentibacillus timonensis sp. nov. (A), Peptoniphilus phoceensis sp. nov. (B).*

	Lti	***Lju***	***Les***	***Lpo***	***Lha***	***Lsa***	***Lpe***	***Lje***
Lti	100%	23.6 [21.3 - 26.1%]	21 [18.8 - 23.4%]	22.2 [19.9 - 24.6%]	20 [17.8 - 22.5%]	22.5 [20.2 - 25%]	20.6 [18.3 - 23%]	23.8 [21.5 - 26.2%]
Lju		100%	20.8 [18.6 - 23.3%]	21.8 [19.5 - 24.2%]	19.7 [17.5 - 22.1%]	21.4 [19.2 - 23.9%]	19 [16.9 - 21.4%]	21 [18.7 - 23.4%]
Les			100%	21.5 [19.3 - 24%]	19.1 [17 - 21.5%]	22.1 [19.8 - 24.5%]	18.1 [16 - 20.5%]	22.2 [20 - 24.7%]
Lpo				100%	19.8 [17.6 - 22.2%]	22.4 [20.2 - 24.9%]	19.7 [17.5 - 22.1%]	23.7 [21.4 - 26.1%]
Lha					100%	22.2 [20 - 24.7%]	20.2 [17.9 - 22.6%]	22.1 [19.8 - 24.5%]
Lsa						100%	20.7 [18.4 - 23.1%]	25.5 [23.2 - 28%]
Lpe							100%	20.9 [18.7 - 23.3%]
Lje								100%

Table 4*: Numerical DNA-DNA hybridization values (%) obtained by comparison between L. timonensis sp. nov. strain Marseille-P4043 and other closely related species using GGDC formula 2 software (DDH estimates based on HSP identities/length).*

Lti: Lentibacillus timonensis sp. nov.: Marseille-P4043; Lju: Lentibacillus juripiscarius JCM 12147; Lse: Lentibacillus sediminis 0W14; Lpo: Lentibacillus populi DSM 101738; Lha: Lentibacillus halodurans DSM 18342; Lsa; Lentibacillus salicampi ATCC BAA-719; Lpe; Lentibacillus persicus DSM 22530; Lje: Lentibacillus jeotgali Grbi.

	Pp	***Ph***	***Pi***	***Ps***	***P l***	***Pg***	***Pla***
Pp	100,00%	26.6 [24.2 - 29.1%]	21.7 [19.4 - 24.1%]	24.7 [22.3 - 27.1%]	23.6 [21.3 - 26.1%]	25.3 [23 - 27.8%]	24.7 [22.4 - 27.2%]
Ph		100,00%	24.1 [21.8 - 26.6%]	23.6 [21.3 - 26%]	21.9 [19.6 - 24.3%]	24.4 [22.1 - 26.9%]	26.5 [24.1 - 29%]
Pi			100,00%	24.5 [22.2 - 27%]	20.9 [18.7 - 23.3%]	23.8 [21.5 - 26.3%]	26 [23.7 - 28.5%]
Ps				100,00%	24.1 [21.8 - 26.6%]	32.6 [30.2 - 35.1%]	28.9 [26.6 - 31.4%]
P l					100,00%	24.6 [22.3 - 27.1%]	26.5 [24.2 - 29%]
Pg						100,00%	24.4 [22.1 - 26.9%]
Pla							100,00%

Table 5*: Numerical DNA-DNA hybridization values (%) obtained by comparison between, P. phoceensis sp. nov., strain Marseille-P2183 and other closely related species using GGDC formula 2 software (DDH estimates based on HSP identities / length)*

Pp: Peptoniphilus phoceensis Marseille-P2183; Ph: Peptoniphilus harei DSM 10020; Pi: Peptoniphilus indolicus DSM20464; Ps: Peptoniphilus senegalensis DSM 25694; Pl: Peptoniphilus lacydonensis DSM 25694; Pg: Peptoniphilus gorbachii DSM 21461; Pla: Peptoniphilus lacrimalis NCTC 131.

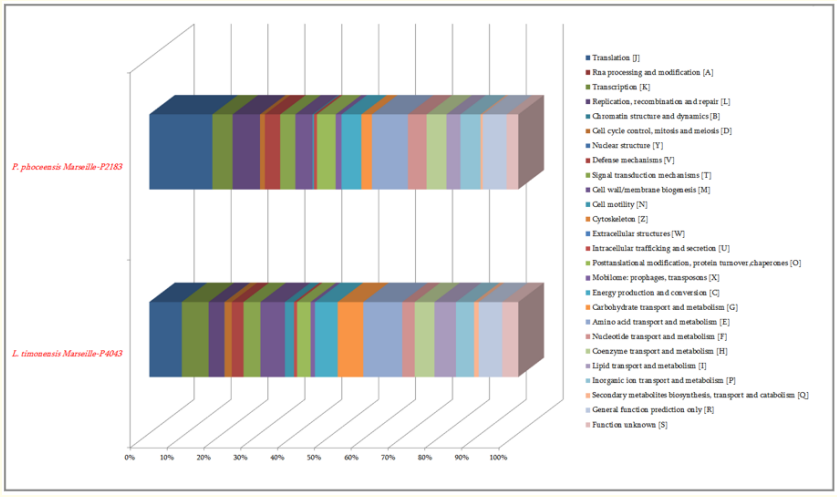


Figure 4: *Distribution of functional classes of the predicted genes in Lentibacillus timonensis sp. nov., and Peptoniphilus phoceensis sp. nov., according to the clusters of orthologous groups of proteins.*

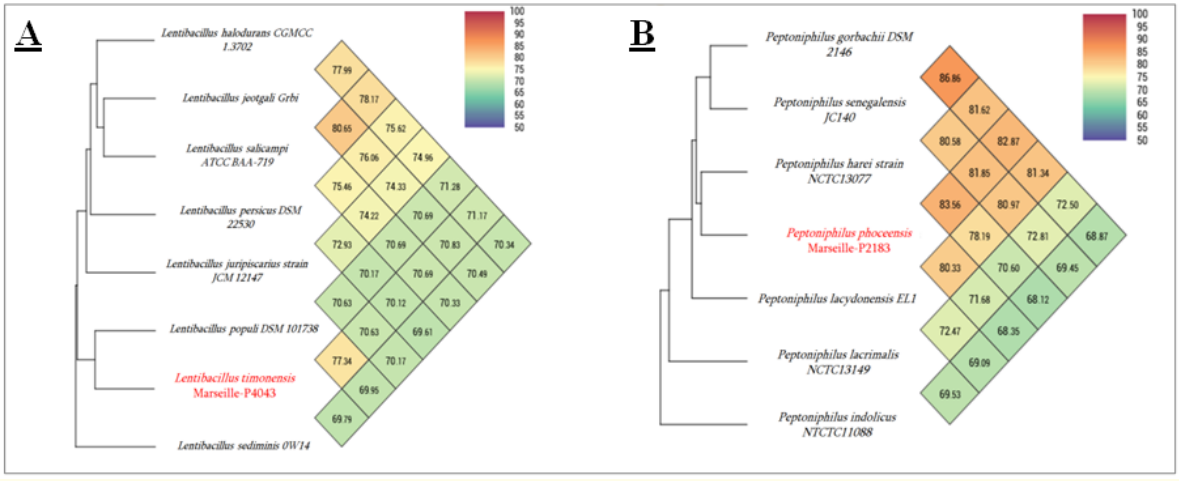


Figure 5: *Heatmap generated with OrthoANI values calculated using the OAT software between Lentibacillus timonensis sp. nov., (A), Peptoniphilus phoceensis sp. nov., (B) with other closely related species validly described.*

Conclusion

Finally, the phenotypic, morphologic and genomic characteristics of strains Marseille-P4043 and Marseille-P2183 have concurred to confirm that these strains are new members of *Firmicutes* phylum. Moreover, comparative study with the phylogenetically closest species

with standing in nomenclature, lead us formally to the creation of *Lentibacillus timonensis* sp. nov., and *Peptoniphilus phoceensis* of which Marseille-P4043 and Marseille-P2183 are assigned as their respective type strains.

Description of *Lentibacillus timonensis* sp. nov.

Lentibacillus timonensis (ti.mo.nen'sis, L. masc. adj., *timonensis* pertaining to La Timone, the name of the University Hospital of Marseille, France). It is a Gram-positive, rod-shaped, strictly aerobic and halophilic bacterium with cell diameter varying between 0.4 to 0.7 µm. Cell is motile and able to form endospore. The strain grows optimally under aerobic condition after 24 hours on Columbia agar at 37°C. Positive reactions are observed for alkaline phosphatase, esterase (C4), esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase, D-galactose, D-glucose, esculin ferric citrate and D-lactose. The 12-methyl-tetradecanoic acid (77%) and 14-methyl-hexadecanoic acid (16%) were the main saturated branched fatty acids found in cell wall. The genome size of strain Marseille-P4043 was 3,924,819 bp long with 38.5 mol% G+C content.

The type strain is Marseille-P4043^T. It was isolated from a fresh stool sample.

The 16S rRNA and genome sequences are deposited in the GenBank database under accession numbers LT934498 and OIXC00000000, respectively.

Description of *Peptoniphilus phoceensis* sp. nov.

Peptoniphilus phoceensis (pho.ce.en'sis L. neutr. adj. *phoceensis*, referring to Phocea, the Greek name of Marseille the city where this strain was isolated). It is a Gram-positive coccus-shaped bacterium. It develops under anaerobic conditions after 24 hours. Individual colonies are opaque and have a diameter of 1 mm on agar plates. Cells have a mean diameter of 1.5 µm. They are motile and spore-free. It can grow in medium with salinity ranging from 0 to 5% and at a pH between 6 and 8.5. Positive reactions are observed for leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, esculin ferric citrate and potassium 2-ketogluconate. The most abundant fatty acid found through the wall cell of strain Marseille-P2183 was hexadecanoic acid (56%). However, unsaturated structures as 9-octadecenoic acid (17%) and 9,12-octadecadienoic acid (16%) are also present.

Strain Marseille-P2183 is designated as the type strain of *Peptoniphilus phoceensis* sp. nov., which was isolated from a fresh stool sample.

Its genome size is 1,739,566 bp long with 31.2 mol% G+C content. The 16S rRNA and genome sequences are deposited in the GenBank database under accession numbers LN881605 and FCEX00000000, respectively.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgments

The authors thank Amael FADLANE for culturing the strains and Aurelia CAPUTO for submitting the genomic sequence to GenBank.

Funding Sources

This study was supported by the Institut Hospitalo-Universitaire (IHU) Méditerranée Infection, the National Research Agency under the program Investissements d'avenir reference ANR-10-IAHU-03, the Région Provence-Alpes-Côte d'Azur and European funding FEDER PRIMI.

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