Peptoniphilus Colimassiliensis sp. nov. and Peptoniphilus Urinimassiliensis sp. nov., Two New Species Isolated From Humans

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Abstract

Strains Marseille-P3761 and Marseille-P3195 are representatives of two bacterial species isolated from human specimens. Strain Marseille-P3761 was isolated from the stool of a healthy volunteer, while strain Marseille-P3915 was cultivated from the urine of a kidney transplant recipient. Both strains are anaerobic Gram-positive cocci bacteria. Both are catalase-negative and oxidase-negative and grow optimally at 37°C in anaerobic conditions. They also metabolize carbohydrates such as galactose, glucose, fructose, and glycerol. The major fatty acids were hexadecanoic acid for both strains, Marseille-P3761 (38%) and Marseille-P3195 (31%). The highest DNA-DNA hybridization values of Marseille-P3761 and Marseille-P3195 strains when compared to their closest phylogenetic relatives were 52.3% and 56.4%, respectively. The morphological, biochemical, phenotypic and genomic characteristics strongly support that these strains are new members of the Peptoniphilus genus. Thus, we suggest that strains Marseille-P3761 (CSUR P3761 = CCUG71569) and Marseille-P3195 (CSUR P3195 = DSM 103468) are the type strains of two new Peptoniphilus species, for which we propose the names Peptoniphilus colimassiliensis sp. nov. and Peptoniphilus urinimassiliensis sp. nov., respectively.

Introduction

Understanding the role that bacterial diversity plays in diseases that affect humans is essential (Turnbaugh et al. 2007). The implementation of the Culturomics strategy, a concept which is based on diversified culture conditions in order to enlarge our knowledge of the human microbiota, has enabled the discovery of many previously uncultured bacteria (Lagier et al. 2016, 2018). In order to describe new bacterial isolates, we used the taxono-genomics approach that includes matrix-assisted laser desorption-ionisation time-of-flight (MALDI-TOF) mass spectrometry (MS) analysis, phylogenetic inference, description of the main phenotypic characteristics and genome sequencing and comparison to describe them (Fournier and Drancourt 2015; Fournier et al. 2015).

The genus Peptoniphilus was described by Ezaki et al. as early as 2001 (Ezaki et al. 2001). Members of this genus are Gram-positive anaerobic cocci, non-motile, non-saccharolytic, and their major energy source results from the use of peptones and oligopeptides (Song, Liu and Finegold 2007). Peptoniphilus spp. have mostly been isolated from human clinical samples, notably vaginal discharges and peritoneal and gland abscesses (Ezaki et al. 2001). Currently, there are 20 species validly published with standing in the nomenclature (https://lpsn.dsmz.de/genus/peptoniphilus).

Herein, we report a broad description of two new Peptoniphilus species named Peptoniphilus urinimassiliensis sp. nov., strain Marseille-P3195 (previously reported) and Peptoniphilus colimassiliensis sp. nov., strain Marseille-P3761, both of which were isolated from human samples.

Materials And Methods

Strain isolation and identification

Strain Marseille-P3195 was isolated in a urine sample from a young man who had just received a kidney transplant for genetic focal segmental glomerulosclerosis (Brahimi et al. 2017), while strain Marseille-P3761 was isolated in a fresh stool sample from a volunteer living in France. These two bacterial strains were retrieved seven days after pre-incubation in an anaerobic blood culture bottle (Becton-Dickinson Diagnostics, Le Pont-de-Clai, France) supplemented with 5% sheep blood at 37°C. Individuals gave their free and informed consent for the project before sampling. The study was approved by the ethics committee of Institut Fédératif de Recherche IFR48 under number 2016-010 as part of a long term culturomics study of the human microbiota.

Attempts were made to identify bacterial colonies using MALDI-TOF MS (Bruker Daltonics, Bremen, Germany), as previously reported (Lo et al. 2015). The obtained spectra were imported and analysed using Biotyper 3.0 software. They were then incremented in our local mass spectrometry database (https://www.mediterranee-infection.com/urms-data-base).

No identification was successful using MALDI-TOF MS. Therefore, sequencing of the 16S rRNA gene was performed for each of the studied strains using the primer pair fD1 and rP2 (Weisburg et al. 1991). They were sequenced using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xL Genetic Analyzer capillary3500xL sequencer (Thermo Fisher, Saint-Aubin, France), as previously described (Lo et al. 2016). The 16S rRNA nucleotide sequences were assembled and corrected using CodonCode Aligner software (http://www.codoncode.com).

Phenotypic and biochemical characteristics

Bacterial colonies of strains Marseille-P3761 and Marseille-P3195 appear distinctly on 5% sheep blood-enriched Columbia (bioMérieux, Marcy l’Étoile, France). Phenotypic tests such Gram staining, sporulation, catalase and oxidase reactions were performed for each strain, as previously reported (Brahimi et al. 2017). The optimal temperature and pH for the growth of each strain was sought. Strains were inoculated on 5% sheep blood-enriched Columbia agar (bioMérieux) and incubated under different temperatures (20, 28, 32, 37, 45, and 56°C) and different pH levels (5, 6, 7, 7.5, 8, and 8.5). In addition, biochemical properties such as carbohydrate metabolism and enzymatic activities of these two strains were revealed using the API strips test (ZYM and 50 CH, bioMérieux). The morphology of the bacterial cells of each strain was highlighted using a
TM4000 microscope (Hitachi Group, Krefeld, Germany). For each strain, 50 mg of bacterial biomass was collected from several culture plates in order to prepare the fatty acid methyl ester analysis, as previously described (Dione et al. 2018).

**Genomes sequencing and analysis**

The total DNA of the genomes was recovered using the EZ1 biorobot (Qiagen, Courtaboeuf, France) and the EZ1 DNA tissue kit. Sequencing was performed using MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit and Nextera XT paired end (Illumina), as previously described (Morel et al. 2015). Several bioinformatic tools, including Velvet (Zerbino and Birney 2008), Spades (Bankevich et al. 2012), and SOAPdenovo (Luo et al. 2012) were used to assemble the genomes. GapCloser software (Xu et al. 2019) was used to reduce assembly gaps. Scaffolds which had fewer than 800 base pairs (bp) or had a depth value lower than 25% of the mean depth were removed. The best assembly was selected using different criteria (number of scaffolds, N50, number of N). The degree of genomic similarity of each strain was evaluated by processing sequences using the Orthologous ANI Tool (OAT) software (Lee et al. 2016). Furthermore, the Genome-to-Genome Distance Calculator (GGDC) web server, which is available online (http://ggdc.dsmz.de), was used to calculate DNA-DNA hybridisation (DDH) values between the genomes of closest species, as previously described (Meier-Kolthoff et al. 2013).

**Results And Discussion**

**Growth conditions of strains**

Different growth temperatures and pH levels were tested with these strains. They all grew optimally at 37°C in anaerobic conditions. The optimal pH was 7 for strain Marseille-P3761 and Marseille-P3195. Bacterial strains grew well, with distinct colonies on 5% sheep blood-enriched Columbia agar.

**Morphology and biochemical characteristics**

The colonies of the two bacterial strains are similar; they appear grey and circular on 5% sheep blood-enriched Columbia agar. The two strains are Gram-positive cocci, catalase and oxidase negative. They do not produce spores. Using API ZYM, esterase (C4), α-chymotrypsin, and naphthol-AS-BI-phosphohydrolase were positive for strain Marseille-P3761, while strain Marseille-P3195 had a positive reaction to esterase (C4), esterase lipase (C8), acid phosphatase, and naphthol-AS-BI-phosphohydrolase. In addition, weak reactions were observed for alkaline phosphatase and trypsin in both strains. Furthermore, the use of 50 CH strips revealed that strain Marseille-P3761 fermented glycerol, D-galactose, D-glucose, D-fructose, D-mannitol, esculin ferric citrate, D-trehalose, D-melezitose, and D-turanose, while strain Marseille-P3195 had a positive reaction to glycerol, L-fucose, D-raffinose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, esculin ferric citrate, N-acetyl-glucosamine, D-mannitol, D-sorbitol, L-rhamnose, dulcitol, D-galactose, D-glucose, D-fructose, D-mannose, D-arabinose, L-arabinose, D-ribose, and D-xylose. All reactions observed with API strips tests are reported in the Supplementary Table S1.

The main biochemical characteristics of these strains were compared with those of other closely related *Peptoniphilus* species (Table 1). The cell morphology of each strain was revealed by scanning electron microscope. They are sphere-shaped bacteria and can aggregate in duplicate (Supplementary Figure S1). Hexadecanoic acid was detected as a major fatty acid for strains Marseille-P3761 (38%) and Marseille-P3195 (31%). Minor amounts of unsaturated and other saturated structures were also detected (Table 2).
Comparison of differential characteristics of *Peptoniphilus colimassiliensis* sp. nov. Marseille-P3761, *Peptoniphilus urinimassiliensis* Marseille-P3195, and other bacterial species including *Peptoniphilus coxii* CCUG 59622^T^, *Peptoniphilus obesi* ph1^T^, and *Peptoniphilus asaccharolyticus* DSM 20463.

| Properties                  | *P. colimassiliensis* | *P. urinimassiliensis* | *P. coxii* | *P. obesi* | *P. asaccharolyticus* |
|-----------------------------|-----------------------|------------------------|------------|------------|-----------------------|
| Cell diameter (µm)          | 0.5                   | 0.8                    | 0.7        | 0.7-0.9    | na                    |
| Oxygen requirement          | -                     | -                      | -          | -          | -                     |
| Gram stain                  | +                     | +                      | +          | +          | +                     |
| Salt requirement            | -                     | -                      | -          | -          | -                     |
| Motility                    | -                     | -                      | -          | -          | -                     |
| Endospore formation         | -                     | -                      | -          | -          | -                     |
| Alkaline phosphatase        | -                     | -                      | -          | -          | -                     |
| Catalase                    | -                     | -                      | -          | -          | -                     |
| Oxidase                     | -                     | -                      | -          | -          | -                     |
| β-galactosidase             | -                     | -                      | -          | -          | -                     |
| N-acetyl-glucosamine        | -                     | -                      | -          | -          | na                    |
| Arabinose                   | -                     | -                      | -          | -          | na                    |
| Lipase (C8)                 | -                     | w                      | na         | na         | na                    |
| Mannose                     | -                     | -                      | +          | +          | -                     |
| Mannitol                    | +                     | -                      | na         | na         | na                    |
| Sucrose                     | +                     | -                      | na         | -          | -                     |
| D-glucose                   | +                     | -                      | na         | na         | -                     |
| D-fructose                  | +                     | -                      | na         | na         | -                     |
| D-maltose                   | +                     | -                      | na         | na         | -                     |
| Source                      | Human                 | Human                  | Human      | Human      | Human                 |

+, positive; -, negative; w, weak; na, no available data.

**Table 2**

Fatty acid profiles (%) of *Peptoniphilus colimassiliensis* sp. nov., strain Marseille-P3761 and *Peptoniphilus urinimassiliensis* sp. nov., strain Marseille-P3195.

| Fatty acids | Name                          | *P. colimassiliensis* | *P. urinimassiliensis* |
|-------------|-------------------------------|-----------------------|------------------------|
| C_16:0      | Hexadecanoic acid             | 38                    | 31                     |
| C_18:1n9    | 9-Octadecenoic acid           | 30                    | 25                     |
| C_18:2n6    | 9,12-Octadecadienoic acid    | 9.7                   | 24.2                   |
| C_14:0      | Tetradecanoic acid            | 8.2                   | 5.7                    |
| C_18:0      | Octadecanoic acid             | 4.1                   | 2.6                    |
| C_10:0      | Decanoic acid                 | 1.6                   | 1.2                    |
| C_12:0      | Dodecanoic acid               | TR                    | TR                     |
| C_15:0      | Pentadecanoic acid            | TR                    | TR                     |
| C_5:0 iso   | 3-methyl-butanoic acid        | ND                    | 7.1                    |

ND = not detected; TR = trace amounts < 1

Phylogenetic identification
The Blastn against the 16S GenBank database revealed that strain Marseille-P3761 and strain Marseille-P3195 both exhibited 95.72% and 96.02% sequence identity with *Peptoniphilus coxii* RMA 16757 (GenBank accession number NR_117556.1). The values obtained were below the threshold value of 98.65% recommended to delimit new prokaryotic species. Given these phylogenetic criteria, we consequently classified these strains as new members belonging to the genus *Peptoniphilus* within the phylum *Firmicutes*. In addition, the phylogenetic tree (Figure 1) shows the positions of these two new species among related *Peptoniphilus* species with a validly published name.

**Comparison and genomic specificities**

The genome size of *Peptoniphilus colimassiliensis* sp. nov., strain Marseille-P3761 was 1,986,843 bp long with a 48.6 mol% G+C content, whereas the genome of *Peptoniphilus urinimassiliensis* sp. nov., strain Marseille-P3195, was 1,822,830 bp with a 49.7 mol% G+C content (Figure 1).

The dDDH values ranged from 17.5% between *Peptoniphilus ivorii* (DSM 10022T) and *Peptoniphilus coxii* (CCUG 59622T), to 60.2% between *P. colimassiliensis* and *P. obesi* (ph1T). Strains Marseille-P3761 and Marseille-P3195 had the highest DDH values of 60.2% and 52.4%, respectively, in this analysis (Table 3).

|                | *P. obesi* | *P. urinimassiliensis* | *P. lacrimalis* | *P. timonensis* | *P. colimassiliensis* | *P. coxii* | *P. asaccharolyticus* | *P. ivorii* |
|----------------|------------|------------------------|-----------------|-----------------|----------------------|-----------|-----------------------|------------|
| *P. obesi*     | 100%       | 39.8±2.5%              | 32.3±2.4%       | 25.2±2.4%       | 60.2±2.8%            | 40.4±2.5% | 27.7±2.4%             | 37.5±2.5%  |
| *P. urinimassiliensis* | 100%      | 56.4±2.7%              | 34.3±2.4%       | 27.2±2.4%       | 20.1±2.3%            | 32.3±2.4% | 18.0±2.2%             |            |
| *P. lacrimalis* | 100%       | 21.9±2.3%              | 52.3±2.6%       | 40.6±2.5%       | 31.9±2.4%            | 30.9±2.4% |                        |            |
| *P. timonensis* | 100%       | 51.1±2.6%              | 27.3±2.4%       | 27.2±2.4%       | 33.7±2.4%            |           |                       |            |
| *P. colimassiliensis* | 100%     | 20.4±2.3%              | 45.1±2.6%       | 18.1±2.2%       |                      |           |                       |            |
| *P. asaccharolyticus* | 100%      | 35.4±2.4%              | 17.5±2.2%       |                 |                      |           |                       |            |
| *P. ivorii*    | 100%       |                        |                 |                 |                      |           |                       |            |

The degree of genomic similarity of strain Marseille-P3761 and Marseille-P3195 with closely related species was estimated. OrthoANI values among closely related species (Figure 3) ranged from 83.5% between *Peptoniphilus colimassiliensis* and *Peptoniphilus urinimassiliensis* to 63% between *Peptoniphilus lacrimalis* (GIFU 7667T) and *Peptoniphilus ivorii*. However, *P. colimassiliensis* had higher genomic similarity with *P. urinimassiliensis* (83.5%). *P. colimassiliensis* and *P. urinimassiliensis* had lower values with *P. timonensis*, ranging from 67.4–64.6%, respectively.

COG analysis showed that genes encoding extracellular structures, prophages, transposons, and general function prediction only were most present in the genomes of strains Marseille-P3761 and Marseille-P3195. The distribution of genes in the 25 general COG categories is illustrated in Supplementary Figure S2.

**Conclusion**

Based on the results from unique phenotypic criteria, MALDI-TOF spectra, phylogenetic and genomic characteristics such as 16S rRNA sequence similarity lower than 98.65% and OrthoANI value lower than 95% with the phylogenetically closest species with standing in the nomenclature, we formally proposed strain Marseille-P3761T and Marseille-P3195T as respectively the type strains of *Peptoniphilus colimassiliensis* sp. nov., and *Peptoniphilus urinimassiliensis* sp. nov.

**Description of Peptoniphilus colimassiliensis** sp. nov.

*Peptoniphilus colimassiliensis* (co.li.mas.si.li.en’sis), is a name composed of co.li. N. L. mas. adj. the Latin name of colon, and mas.si.li.en’sis. L. fem. adj. massiliensis, from Massilia, the Latin name for Marseille, where the strain was isolated. Bacterial colonies appear grey and circular. Bacterial cells are Gram-positive sphere-shaped, non-spore forming. Catalase and oxidase activities are not detected. Optimal growth is obtained at 37°C in anaerobic atmosphere on 5% sheep blood-enriched Columbia agar.
Strain Marseille-P3761 is a Gram-positive sphere-shaped bacterium with a mean length of 1.2 µm and a mean diameter of 0.5 µm.

Strain Marseille-P3761 exhibits positive reactions for esterase (C4), α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, glycerol, galactose, glucose, fructose, mannitol, esculin ferric citrate, trehalose and D-turanose. The major fatty acids of strain Marseille-P3761 are C\textsubscript{16:0} (38%) and C\textsubscript{18:1\textsubscript{n9}} (30%). The genome size and G + C content of strain Marseille-P3761 are 1,986,843 bp and 48.6 mol%, respectively.

The type strain Marseille-P3761\textsuperscript{T} (= CSUR P3761 = CCUG 71569) was isolated in a stool sample from a healthy French volunteer.

The genome and 16S rRNA sequence were deposited in GenBank under accession numbers OPYI00000000 and LT972121, respectively.

\textbf{Description} of \textit{Peptoniphilus urinimassiliensis} sp. nov.

\textit{Peptoniphilus urinimassiliensis} (u.r.i.ni.mas.si.li.en'sis) composed of u.r.i.ni L. masc. adj. for urine, from which strain Marseille-P3195 was isolated and mas.si.li.en'sis, L. masc. adj. massiliensis from Massilia, the Latin name for Marseille, France, where the type strain was first isolated).

Colonies are grey and circular. Bacterial cells are Gram-positive sphere-shaped, non-spore forming. Catalase or oxidase activities are not detected. Optimal growth is at 37°C in anaerobic atmosphere on sheep blood-enriched Columbia agar. Strain Marseille-P3195 exhibits positive reactions for glycerol, fucose, raffinose, maltose, lactose, melibiose, D-trehalose, esculin ferric citrate, N-acetyl-glucosamine, sorbitol, dulcitol, galactose, glucose, fructose, arabinose, ribose, xylose, esterase lipase (C8), acid phosphatase, and naphthol-AS-BI-phosphohydrolase. The major fatty acids are C\textsubscript{16:0} (31%), C\textsubscript{18:1\textsubscript{n9}} (25%), and C\textsubscript{18:2\textsubscript{n6}} (24.2%). The genome size and G + C content of strain Marseille-P3195 are 1,822,830 bp and 49.7 mol%, respectively. The type strain Marseille-P3195\textsuperscript{T} (= CSUR P3195 = DSM 103468) was isolated in a urine sample from a young man who had just received a kidney transplant for genetic focal segmental glomerulosclerosis.

The genome and 16S rRNA sequence were deposited in GenBank under accession numbers FTPC00000000 and LT598577, respectively.

\textbf{Declarations}

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\textbf{Author Contributions:} Conceptualisation, V. M., F.F. and D.R.; methodology, V. M., F.F., C.I.L. and P.E.F.; validation, F.F., and P.E.F.; formal analysis, B.M., C.I.L., N.D., N.A. and S.B.A.; investigation, C.I.L., S.A. and B.M.; strain isolation and culture, B.M., N.D., and S.B.; writing and original draft preparation, B.M. and C.I.L.; writing, review and editing, C.I.L. and F.F.; supervision, M.M., P.E.F. and F.F.; funding acquisition, D.R. All authors have read and agreed to the published version of the manuscript.

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**Figures**
Figure 1

Phylogenetic trees highlighting the position of Peptoniphilus colimassiliensis sp. nov. and Peptoniphilus urinimassiliensis sp. nov. based on the 16S rRNA gene sequences relative to the most closely related type strains within the genus Peptoniphilus. Genbank accession numbers are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters, phylogenetic inference were obtained using the maximum likelihood method and the MEGA 7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1,000 times to generate a majority consensus tree. The scale bar indicates a 1% nucleotide sequence.
Figure 2

Graphical circular map of Peptoniphilus strains. The fourth circle shows the G + C% content plot. The innermost circle shows GC skew, purple indicates negative values and olive indicates positive values. A. Peptoniphilus urinimassiliensis sp. nov. B. Peptoniphilus colimassiliensis sp. nov.
Figure 3

Heatmap generated with OrthoANI values calculated using the OAT software between *Peptoniphilus colimassiliensis* sp. nov. and *Peptoniphilus urinimassiliensis* sp. nov. and other closely related species with standing in the nomenclature.

Supplementary Files

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