Genome sequence and description of *Coprococcus phoceensis* gen. nov., sp. nov., a new bacterial genus isolated from the human left colon

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Abstract

We report here the main characteristics of *Coprococcus phoceensis* strain Marseille-P3062T (CSUR P3062). The 16S rDNA sequencing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry spectrum analysis were used to identify and characterize this new anaerobic bacterial species, which was isolated from the left colon cleansing of a 25-year-old French man with Crohn’s disease. © 2019 The Authors. Published by Elsevier Ltd.

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Isolation and growth conditions

Strain Marseille-P3062T was first isolated after 7 days of incubation on 5% sheep blood-enriched Columbia agar (bioMérieux, Marcy l’Etoile, France) at 37°C in an anaerobic atmosphere (AnaeroGen Compact; Oxoid, Thermo Scientific, Dardilly, France). Growth was not observed under microaerophilic (campyGEN; Oxoid) and aerobic conditions. The bacterial cells tolerated a pH of 5 to 8, with optimum growth at pH 7, and an NaCl concentration <50 mg/L, with optimum growth at 5 g/L. After 20 min of thermal shock at 80°C, this bacterium was not spore-forming and no growth was observed at 37°C on 5% sheep blood-enriched Columbia agar. The electron microscopy then confirmed this negative result.

Phenotypic characteristics

Agar-grown colonies were transparent and crater-shaped with a mean diameter of 3 mm. Bacterial cells were Gram-stain variable, arranged in small chains, rod-shaped, and were 1.3–2.3 μm long and 0.5–0.7 μm wide (Fig. 2). Strain Marseille-P3062T was catalase and oxidase negative. The main
The characteristics of the strain are summarized in Table 1. Using an API ZYM strip, an API 20A strip and an API 50 CH strip, positive enzymatic activities included, alkaline phosphatase, N-acetyl-β-glucosaminidase, α-glucosidase, β-glucosidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. No activity was found for the following enzymes: valine arylamidase, α-fucosidase, β-galactosidase, esterase C4, esterase lipase C8, protease, urease, leucine arylamidase, lipase C14, cystine arylamidase, trypsin, β-glucuronidase, α-chymotrypsin, α-galactosidase and α-mannosidase. No acid production was observed from D-glucose, D-lactose, D-sucrose, D-maltose, salicin, D-xylose, L-arabinose, D-cellobiose, D-mannose, D-raffinose, D-sorbitol, D-trehalose, D-mannitol, D-xylose, L-arabinose, glycerol, D-melezitose and L-rhamnose. Only one carbohydrate was metabolized: potassium 5-ketogluconate, as revealed by an API 50 CH strip. The other tested carbohydrates (D-melibiose, D-ribose, D-tagatose, glycerol, glycogen, D-arabinose, erythritol, L-xylose, D-galactose, D-adenitol, methyl-β-D-xylopyranoside, D-glucose, D-fructose, D-mannose, L-sorbose, dulcitol, L-rhamnose, inositol, D-mannitol, D-sorbitol, methyl-α-D-glucopyranoside, methyl-β-D-mannopyranoside, d-acetylglucosamine, esculin ferric citrate, amygdalin, D-cellobiose, arbutin, salicin, D-maltose, D-sucrose, D-lactose, D-raffinose, D-trehalose, inulin, D-melezitose, starch, xylitol, gentiobiose, D-arabitol, L-arabitol, D-lyxose, D-turanose, D-fucose, L-fucose, potassium gluconate and potassium 2-ketogluconate) were not used.

Strain identification

After three failed identifications by our systematic MALDI-TOF mass spectrometry (MS) screening on a Microflex spectrometer (Bruker Daltonics) [5], the 16S rRNA gene was sequenced, using universal primers FD1 and RP2 (Eurogentec, Angers, France) as previously described [6,7], and a 3130-XL sequencer (Applied Biosciences, Saint-Aubin, France). Strain Marseille-P3062T exhibited a 95.67% sequence identity with Coprococcus comes strain VPI C1-38 (GenBank Accession number NR_044048.1) the phylogenetically closest species with standing in nomenclature (Fig. 3), which putatively classifies it as a new species of the genus Coprococcus in the order of Clostridiales within the Firmicutes phylum.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot with the EZ1 DNA tissue kit (Qiagen, Hilden, Germany) and then sequenced on a MiSeq sequencer (Illumina Inc., San Diego, CA, USA) with the Nextera Mate Pair sample prep kit and Nextera...
**TABLE 1.** Description of *Coprococcus phoceensis* sp. nov., according to the digitized protologue TA00877 at the www.imedea.uib.es/dprotologue website

| Taxonumber | TA00877 |
|------------|---------|
| Date of the entry | 2019-01-23 |
| First submission date | 2019-01-23 |
| Version | 001 |
| Species name | *Coprococcus phoceensis* |
| Genus name | *Coprococcus* |
| Specific epithet | *phoceensis* |
| Species status | sp. nov. |
| Species etymology | *phoceensis*, L., neut., adj., phoceensis, based on the acronym of the Phocian city where the type strain was first isolated |
|Submitter | Bonnet Marion |
| E-mail of the submitter | marioncg.bonnet@yahoo.fr |
| Designation of the type strain | Strain Marseille-P3062 |
| Strain collection numbers | CSUR P3062 = DSM 103635 |
| 16S rRNA gene Accession number | LT598553 |
| Genome Accession number (EMBL) | FNWC01000000 |
| Genome status | Draft |
| Genome size | 3 601 259 bp |
| GC mol % | 40.21 |
| Data on the origin of the sample from which the strain was isolated | France |
| Region of origin | Marseille |
| Date of isolation | 2016-03 |
| Source of isolation | Human left colon cleansing sample |
| Sampling date | 2016-03 |
| Growth medium, incubation conditions (temperature, pH and further information) used for standard cultivation | Columbia agar supplemented with 5% sheep blood, 37°C for 48 h of incubation |
| Gram stain | Variable |
| Cell shape | Small chain rod |
| Cell size (length or diameter) | 1.3–2.3 × 0.5–0.7 (μm) |
| Motility | Non-motile |
| Colony morphology | Transparent and crater-shaped |
| Temperature range | 37°C |
| Lowest temperature for growth | 38°C |
| Highest temperature for growth | 37°C |
| Temperature optimum | 37°C |
| Lowest pH for growth | 5 |
| Highest pH for growth | 8 |
| Relationship to O₂ | Anaerobe |
| O₂ conditions for strain testing | Aerobiosis, anaerobiosis, microaerophilic |
| Oxidase | Negative |
| Catalase | Negative |

**FIG. 3.** Phylogenetic tree showing the position of *Coprococcus phoceensis* strain Marseille-P3062T relative to other phylogenetically close neighbours. Sequences were aligned using MUSCLE, and phylogenetic inferences were obtained using the maximum-likelihood method within the MEGA software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 100 times to generate a majority consensus tree. Only the bootstrap scores of at least 70% were retained. The scale bar indicates a 2% nucleotide sequence divergence.

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XT Paired End (Illumina Inc.), as previously described [8]. The assembly was performed using a pipeline containing several software (Velvet [9], Spades [10] and Soap Denovo [11]), on trimmed data (MiSeq and TRIMMOMATIC [12] software) or untrimmed data (only MiSeq software). GapCloser was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value <25% of the mean depth were removed. The best assembly was selected by using different criteria (number of scaffolds, N50, number of N). The genome of strain Marseille-P3062T was 3,601,259 bp long with 40.21% G+C content. The degree of genomic similarity of strain Marseille-P3062T with closely related species was estimated using the ORTHOANI software [13]. ORTHOANI values among closely related species (Fig. 4) ranged from 66.54% between *Blautia producta* and *Eu-
bacterium oxidoreducens* to 75.9% between *Eubacterium con-
tortum* and *Faecalibacterium oroticum*. When *Coprococcus phoceensis* was compared with these closely related species, values ranged from 67.58% with *E. oxidoreducens* to 70.52% with *F. oroticum*.

**Conclusion**

As the sequence identity with the phylogenetically closest validated species was <98.7%, which is the threshold recom-
mended to define a species according to the nomenclature

[5,14], we propose the strain Marseille-P3062T as a representa-
tive of a new species within the genus *Coprococcus*. Conse-
quently, we suggest the creation of the new species named

"*Coprococcus phoceensis" sp. nov., strain Marseille-P3062 T

(pho.ce.en.sis, L., neut., adj., phoceensis, based on the acronym of the Phocean city where the type strain was first isolated).

**Nucleotide sequence Accession number**

The 16S rRNA gene sequence was deposited in GenBank under Accession number LT598553.

**Deposit in a culture collection**

Strain Marseille-P3062T was deposited in the Collection de Souches de l’Unité des Rickettsies (CSUR, WDCM 875) under number P3062.

**MALDI-TOF-MS spectrum**

The MALDI-TOF-MS spectrum of ‘*Coprococcus phoceensis*’

Marseille-P3062T is available online at http://backup.mediterranee-
infection.com/article.php?larub=280&titre=urms-database.
Conflict of interest

None to declare.

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