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-P3062[T] (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.
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M. Bonnet and Davide Ricaboni participated equally in the study.

Introduction

In March 2016, as part of the culturomic study to assess the microbial diversity of the human microbiota [1–3], we isolated a new bacterial species from the left colon cleansing of a 25-year-old French man with Crohn's disease. The strain isolated here could not be identified by our systematic matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) screening on a MicroFlex spectrometer (Bruker Daltonics, Bremen, Germany) [4]. The spectra obtained (Fig. 1) were imported and analysed using the Biotyper 3.0 software against the Bruker database that was continually incremented with MEPHI database. The study was approved by the Institut Fédératif de Recherche 48 (agreement number 09–022, Marseille, France) and the patient's consent was obtained.

Isolation and growth conditions

Strain Marseille-P3062[T] was first isolated after 7 days of incubation on 5% sheep blood-enriched Columbia agar (bioMérieux, Marcy l'Etoile, France) at 37°C in anaerobic atmosphere (AnaeroGen Compact; Oxoid, Thermo Scientific, Dardilly, France). Growth was not observed under micro-aerophilic (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-P3062[T] was catalase and oxidase negative. The main
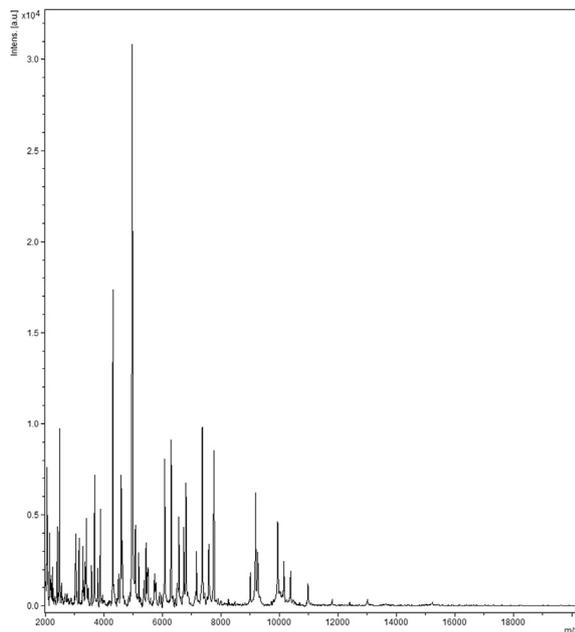
FIG. 1. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry reference spectrum of *Coprococcus phoceensis* sp. nov. The reference spectrum was generated by comparison of spectra from 12 individual colonies.
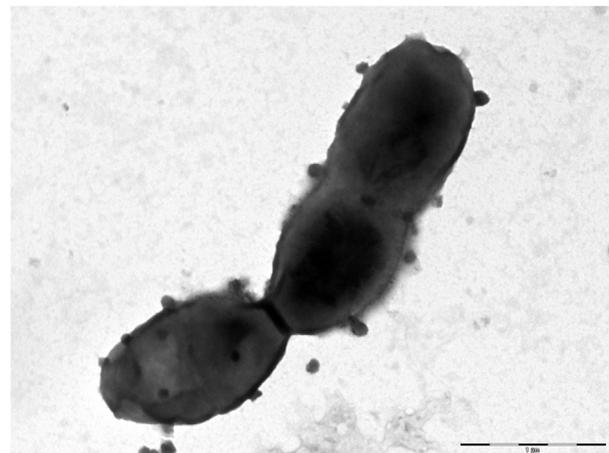

FIG. 2. Scanning electron microscopy (SEM) of stained *Coprococcus phoceensis* sp. nov. A colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. Then a drop of the suspension was directly deposited on a poly-L-lysine-coated microscope slide for 5 min and treated with 1% phosphotungstic acid aqueous solution (pH 2.0) for 2 min to increase SEM image contrast. The slide was gently washed in water; air-dried and examined in a tabletop SEM (Hitachi TM4000) of approximately 60 cm in height and 33 cm in width to evaluate the bacterial structure. Scales and acquisition settings are shown in the figures.

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-adonitol, 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-α-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-P3062[T] 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 I. 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
Draft number/Date	001
Version	Submitted
Species name	*Coprococcus phoceensis*
Genus name	*Coprococcus*
Specific epithet	*phoceensis*
Species status	sp. nov.
Species etymology	pho.ce.en.sis, L., neut., adj., *phoceensis*, based on the acronym of the Phocean 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	
Country of origin	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	28°C
Highest temperature for growth	37°C
Temperature optimum	37°C
Lowest pH for growth	5
Highest pH for growth	8
Relationship to O_2	Anaerobe
O_2 conditions for strain testing	Aerobiosis, anaerobiosis, microaerophilic
Oxidase	Negative
Catalase	Negative

FIG. 3. Phylogenetic tree showing the position of *Coprococcus phoceensis* strain Marseille-P3062[T] 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.
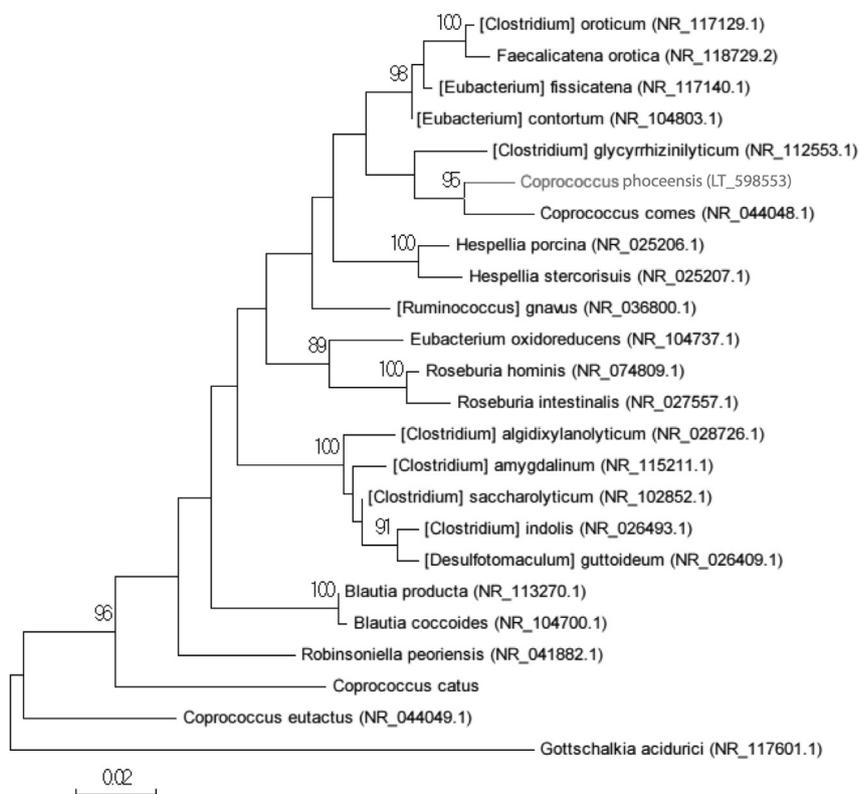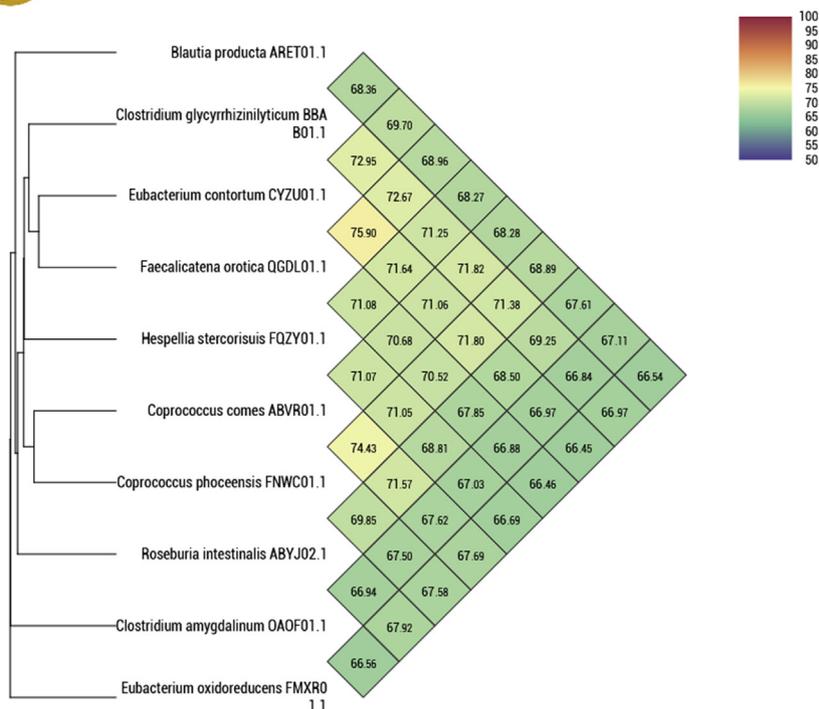
FIG. 4. Heatmap generated with ORTHOANI values calculated using the OAT software between *Coprococcus phoceensis* sp. nov., and other closely related species with standing in nomenclature.

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 un-trimmed 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-P3062^T was 3 601 259 bp long with 40.21% G+C content. The degree of genomic similarity of strain Marseille-P3062^T 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 *Eubacterium oxidoreducens* to 75.9% between *Eubacterium contortum* and *Faecalicatena orotica*. When *Coprococcus phoceensis* was compared with these closely related species, values ranged from 67.58% with *E. oxidoreducens* to 70.52% with *F. orotica*.

Conclusion

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

[5,14], we propose the strain Marseille-P3062^T as a representative of a new species within the genus *Coprococcus*. Consequently, 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-P3062^T 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-P3062^T 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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