Description of '*Gorbachella massiliensis*' gen. nov., sp. nov., '*Fenollaria timonensis*' sp. nov., '*Intestinimonas timonensis*' sp. nov. and '*Collinsella ihuae*' sp. nov. isolated from healthy fresh stools with culturomics

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Abstract

We report here the main characteristics of '*Gorbachella massiliensis*' GD7^T gen. nov., sp. nov., '*Fenollaria timonensis*' GD5^T sp. nov., '*Intestinimonas timonensis*' GD4^T sp. nov., and '*Collinsella ihuae*' sp. nov. GD8^T isolated from one fresh stool of a French volunteer. We used a bacterial culturomics approach combined with taxono-genomics.

Keywords: Collinsella ihuae, fenollaria timonensis, gorbachella massiliensis, gut microbiota, intestinimonas timonensis'
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In our study concerning the intolerant oxygen species from the human gut microbiota, we isolated four bacteria in 2015 using a bacterial culturomics approach. These bacteria could not be identified by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS; http://www.mediterranee-infection.com/article.php?laref=256&titre=urms-database (last access 01/30/17)) on a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [1,2]. These species were isolated from the same fresh stool from a healthy volunteer. The individual has signed informed consent and the study has been validated by the Ethics Committee of the IFR48 Federative Research Institute under the number 09-022.

Strain GD7^T was isolated from a dilution of the fresh sample. The species was grown after 48 h on Columbia agar supplemented with 5% sheep blood at 37°C under strict anaerobic conditions. The colonies appeared translucent and rough, non-haemolytic, non-motile, nonspore-forming, and 1 mm size. The cells were Gram-negative, rod-shaped. The strain did not show catalase or oxidase activity. The 16S rRNA gene was sequenced using fD1-rP2 primers as described previously using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France) [3]. The strain GD7^T had a 16S rRNA gene sequence identity of 93.4% with *Subdoligranulum variabile* strain BI 114^T (NR_028997), the phylogenetically closest species with standing in nomenclature (Fig. 1). This similarity <98.65% leads us to putatively classify GD7^T as a new member in the *Ruminococcaceae* family of *Firmicutes* [4]. Therefore we propose the creation of the new genus '*Gorbachella*' (Gor.ba.chel'la. NL gen fem, in honour of the microbiologist Sherwood Gorbach of the Tufts University School of Medicine, Boston, MA, USA). GD7^T is the type strain of the species *Gorbachella massiliensis* (ma.ssi.li.en'sis L. adj. fem to Massilia, the Latin name of Marseille, France, where this strain was isolated).

Strain GD5^T was isolated from the fresh sample after 48 h anaerobic growth on Columbia agar supplemented with 5% sheep blood at 37°C. The colonies appeared to be translucent, rough, non-haemolytic, motile, non-spore-forming, and 1 mm in size. The cells were rod-shaped with Gram-negative staining. Oxidase and catalase activities were negative. Strain GD5^T showed 97.4% sequence homology with the 16S RNA of *Fenollaria massiliensis* strain 9401234^T (NR_133038) (Fig. 2) [6]. So we propose to classify GD5^T as a new species within the genus *Fenollaria* in the phylum *Firmicutes* [4]. GD5^T is the type

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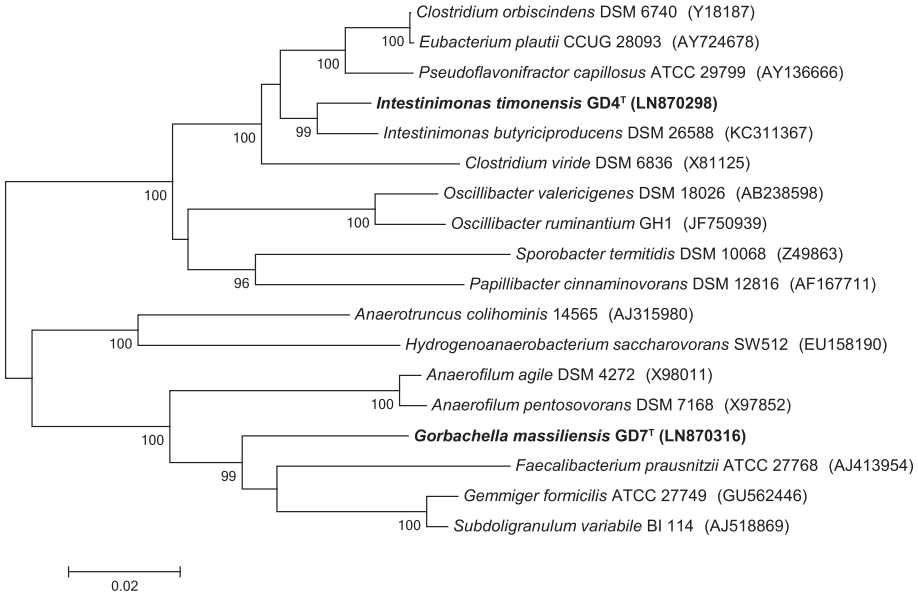

FIG. 1. Phylogenetic tree showing the position of '*Intestinimonas timonensis*' GD4^T and '*Gorbachella massiliensis*' GD7^T relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained with Kimura two-parameter models using the maximum-likelihood method within the MEGA software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. Only bootstrap values >95% are displayed. The scale bar indicates a 2% nucleotide sequence divergence.

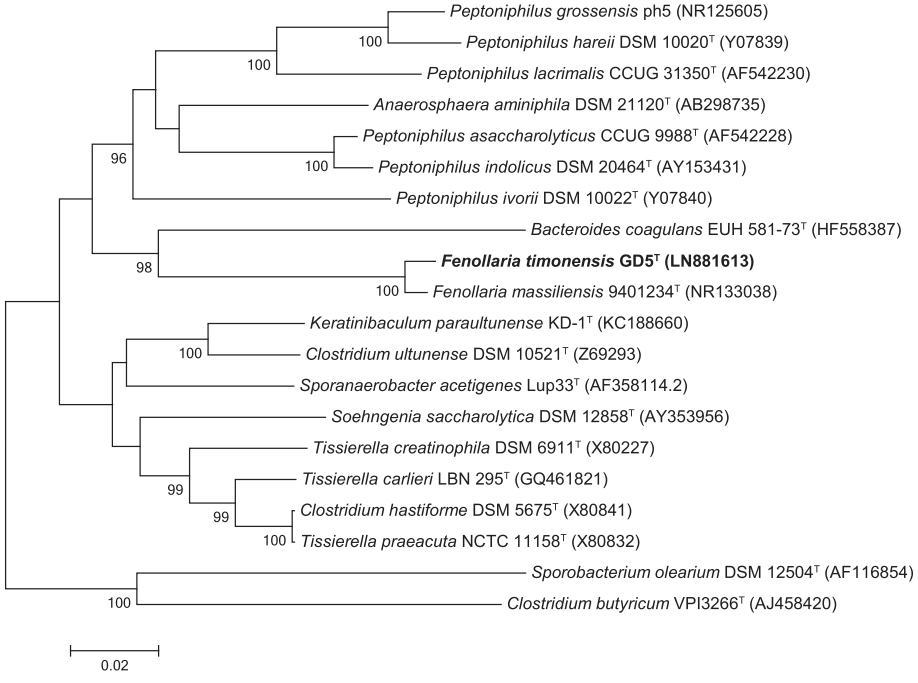

FIG. 2. Phylogenetic tree showing the position of '*Fenollaria timonensis*' GD5^T relative to other phylogenetically close neighbours. Alignment and phylogenetic inferences were made as described for Fig. 1.

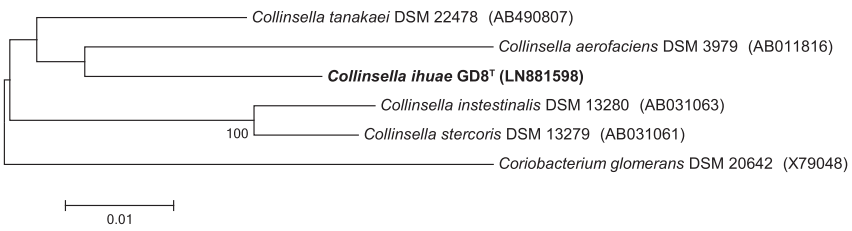

FIG. 3. Phylogenetic tree showing the position of '*Collinsella ihuae*' GD8^T relative to other phylogenetically close neighbours. Alignment and phylogenetic inferences were made as described for Fig. 1. The scale bar indicates a 1% nucleotide sequence divergence.

strain of the species '*Fenollaria timonensis*' (ti.mo.nen'sis L. adj. fem to Timone, the name of the main hospital of Marseille, France, where this strain was isolated).

Strain GD4^T was isolated after 48 h anaerobic growth on Columbia agar supplemented with 5% sheep blood and 5% rumen fluid at 37°C. The colonies appeared translucent, rough, non-haemolytic, motile, non-spore-forming, and 1 mm in size. The cells were Gram-negative. Catalase and oxidase activities were negative. Strain GD4^T presented a sequence identity of 97.08% with 16S rRNA sequence of *Intestinimonas butyriciproducens* DSM 26588^T (NR_118554), the closest species with a valid name (Fig. 1). We propose to putatively classify GD4^T as a new member of the genus *Intestinimonas* in the phylum *Firmicutes* [4]. GD4^T is the type strain of the species '*Intestinimonas timonensis*' (ti.mo.nen'sis L. adj. fem to Timone, the name of the main hospital of Marseille, France, where this strain was isolated).

'*Collinsella ihuae*' strain GD8^T was isolated after 48 h of anaerobic growth on Columbia agar supplemented with 5% sheep blood and 5% rumen fluid at 37°C. Colonies appeared as microcolonies rough, non-haemolytic, motile, non-spore-forming, and 0.5 mm in size. The cells were rod-shaped with Gram-positive staining. Catalase and oxidase activities were negative. The 16S rRNA sequence of the strain GD8^T presented an identity of 96.2% with the 16S rRNA sequence of *Collinsella tanakaei strain* JCM 16071 (NR_113273), the closest phylogenetic species with nomenclature (Fig. 3) [5]. Then we propose the creation of the new species '*Collinsella ihuae*' within the phylum *Actinobacteria* [4]. GD8^T is the type strain of the species '*Collinsella ihuae*' (i.hu.ae L. adj. fem to Institut Hospitalo-Universitaire (IHU), the name of the name of the laboratory (Marseille, France) where this strain was isolated).

MALDI-TOF-MS spectra accession numbers. The MALDI-TOF-MS spectra of these species are available at http://mediterranee-infection.com/article.php? laref=256&titre=urms-database. Last access 7 December 2016.

Nucleotide sequence accession number. The 16S r RNA gene sequences were deposited in GenBank under accession numbers: '*Gorbachella massiliensis*' GD7^T (LN870316), '*Fenollaria timonensis*' GD5^T (LN881613), '*Intestinimonas timonensis*' GD4^T (LN870298) and '*Collinsella ihuae*' GD8^T (LN881598).

Deposit in a culture collection. The strains were deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under the following numbers: '*Gorbachella massiliensis*' GD7^T (P2021), '*Fenollaria timonensis*' GD5^T (P2133), '*Intestinimonas timonensis*' GD4^T (P2010) and '*Collinsella ihuae*' GD8^T (P2019).

Transparency declaration

The authors have no conflicts of interest to declare.

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