

## *Romboutsia timonensis*, a new species isolated from human gut

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### Abstract

The exploration of the human microbiome was recently revolutionized by microbial culturomics and taxonogenomics. Thanks to this approach, we report here the main characteristics of *Romboutsia timonensis* strain Marseille-P326, a new bacterium isolated from the right human colon by colonoscopy in a 63-year-old French man with severe anaemia with melaena.

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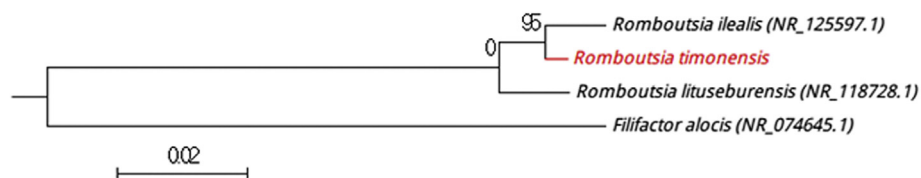
As part of a culturomics study aiming to isolate all bacteria species colonizing the human gut [1], we isolated in pure culture a bacterial strain for which matrix-assisted laser desorption/ionization time-of-flight mass spectrometry identification had failed (<http://www.mediterranee-infection.com/article.php?laref=256&titre=urms-database>) on a MicroFlex spectrometer (Bruker Daltonics, Leipzig, Germany) [2]. This strain was isolated from a sample taken *via* right colon lavage in a 63-year-old French man with severe anaemia with melaena who had undergone exploration by colonoscopy. The patient's clinical history was remarkable for a sleeve gastrectomy performed 4 years before this hospitalization. The patient provided written informed consent; agreement of the National Ethics Committee of the IFR48 (Marseille, France) for this study was obtained under number 09-022.

The strain Marseille-P326 (CSUR-P326) was isolated after 3 days' incubation of the specimen at 37°C on 5% sheep's

blood–Columbia agar medium (bioMérieux, Marcy l'Etoile, France) in an anaerobic atmosphere generated using the GENbag anaer system (bioMérieux), subcultured in pure culture of Marseille-P326 strain for 24 hours at 37°C under anaerobic condition, but no growth was recorded in micro-aerophilic or aerobic conditions. Strain Marseille-P326 colonies observed on 5% sheep's blood–Columbia agar medium were translucent, pale grey and 0.5 to 1 mm in diameter. Bacterial cells were Gram positive, elongated and bacillus shaped, and they ranged in length from 1.5 to 6 µm. Strain Marseille-P326 was catalase and oxidase negative.

The 16S rRNA gene was sequenced using fD1-rP2 primers as previously described [3] using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France). Strain Marseille-P326 exhibited a 98.5% sequence similarity with *Romboutsia ilealis* (GenBank accession no. NR\_125597.1), the phylogenetically closest validated species (Fig. 1). Consequently, we propose to classify the Marseille-P326 strain as a member of a new species within the genus *Romboutsia* in the Firmicutes phylum (<http://www.bacterio.net/index.html>).

The genus *Romboutsia* was created in 2014 by Jacqueline Gerritsen *et al.* after the first isolation of *Romboutsia ilealis* from an ileal digesta sample obtained from a healthy Sprague Dawley



**FIG. 1.** Phylogenetic tree showing position of *Romboutsia timonensis* strain Marseille-P326 relative to other phylogenetically close members among *Romboutsia* genus. GenBank accession numbers are indicated in parentheses. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using maximum-likelihood method within MEGA software. Number at nodes indicates percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. Scale bar = 2% nucleotide sequence divergence.

rat [4], and it currently includes three species, including *R. lituseburensis* (previously *Clostridium litusburensis*) and *R. sedimentorum* (<http://www.bacterio.net/index.html>). In contrast with the other species belonging to this genus, *R. timonensis* strain Marseille-P326 is the only one isolated from human samples; the others were isolated from rat gut (*R. ilealis*), mud (*R. lituseburensis*) and alkaline saline lake sediment (*R. sedimentorum*) [4,5]. All species belonging to this genus are obligate anaerobes.

On the basis of the 16S rRNA sequence divergence of the Marseille-P326 strain (1.5%) with the phylogenetically closest species [6], we propose the creation of the new species *Romboutsia timonensis* sp. nov. (*timonensis* from Hopital de La Timone, Marseille, France, where the strain was first isolated), for which the strain Marseille-P326<sup>T</sup> is the type strain.

The description of new anaerobic gut microbes is critical to deciphering the link between gut microbiota and health because such microbes are involved in gut homeostasis and are suspected to be lost in malnutrition [7].

### Nucleotide sequence accession number

The 16S rRNA gene sequence was deposited in GenBank under accession number LN998074.

### Deposit in a culture collection

Strain Marseille-P326 was deposited in the Collection de Souches de l'Unité des Rickettsies under accession number WDCM 875.

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### Conflict of Interest

None declared.

### References

- Lagier JC, Armougom F, Million M, Hugon P, Pagnier I, Robert C, et al. Microbial culturomics: paradigm shift in the human gut microbiome study. *Clin Microbiol Infect* 2012;18:1185–93.
- Seng P, Abat C, Rolain JM, Colson P, Lagier JC, Gouriet F, et al. Identification of rare pathogenic bacteria in a clinical microbiology laboratory: impact of matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 2013;51:2182–94.
- Mourembou G, Yasir M, Azhar El, Lagier JC, Bibi F, Jiman-Fatani AA, et al. Rise of microbial culturomics: non-contiguous finished genome sequence and description of *Beduini massiliensis* gen. nov., sp. nov. *OMICS* 2015;19:766–76.
- Gerritsen J, Fuentes S, Grievink W, van Niftrik L, Tindall BJ, Timmerman HM, et al. Characterization of *Romboutsia ilealis* gen. nov., sp. nov., isolated from the gastro-intestinal tract of a rat, and proposal for the reclassification of five closely related members of the genus *Clostridium* into the genera *Romboutsia* gen. nov., *Intestinibacter* gen. nov., *Terrisporobacter* gen. nov. and *Asaccharospora* gen. nov. *Int J Syst Evol Microbiol* 2014;64:1600–16.
- Wang Y, Song J, Zhai Y, Zhang C, Gerritsen J, Wang H, et al. *Romboutsia sedimentorum* sp. nov., isolated from an alkaline-saline lake sediment and emended description of the genus *Romboutsia*. *Int J Syst Evol Microbiol* 2015;65:1193–8.
- Huson DH, Auch AF, Qi J, Schuster SC. MEGAN analysis of metagenomic data. *Genome Res* 2007;17:377–86.
- Million M, Diallo A, Raoult D. Gut microbiota and malnutrition. *Microb Pathog* 2016. in press.