# *'Marasmitruncus massiliensis'* gen. nov., sp. nov., *'Clostridium culturomicum'* sp. nov., *'Blautia provencensis'* sp. nov., *'Bacillus caccae'* sp. nov. and *'Ornithinibacillus massiliensis'* sp. nov., isolated from stool samples of undernourished African children

**T.-P.-T. Pham[1], F. Cadoret[1], M. Tidjani Alou[1], S. Brah[2], B. Ali Diallo[3], A. Diallo[4], C. Sokhna[4], J. Delerce[1], P.-E. Fournier[1], M. Million[1] and D. Raoult[1]**

1) *Aix-Marseille Université, URMITE, UM63, CNRS 7278, IRD 198, INSERM 1095, IHU Méditerranée Infection, Marseille, France,* 2) *Service de médecine interne, Hôpital National de Niamey,* 3) *Laboratoire de Microbiologie, Département de Biologie, Université Abdou Moumouni de Niamey, Niamey, Niger and* 4) *Institut de Recherche pour le Développement, UMR 198 (URMITE), Campus International de Hann, Dakar, Senegal*

## Abstract

We report here the main characteristics of five new species, '*Marasmitruncus massiliensis*' strain Marseille-P3646$^T$ (CSUR P3646), '*Clostridium culturomicum*' strain Marseille-P3545$^T$ (CSUR P3545), '*Blautia provencensis*' strain Marseille-P3502$^T$ (CSUR P3502), '*Bacillus caccae*' strain Marseille-P3604$^T$ (CSUR P3604) and '*Ornithinibacillus massiliensis*' strain Marseille-P3601$^T$ (CSUR P3601), which were isolated recently from undernourished children's stool samples from Niger using microbial culturomics.


**Keywords:** *Bacillus caccae*, *Blautia provencensis*, *Clostridium culturomicum*, *Marasmitruncus massiliensis*, *Ornithinibacillus massiliensis*
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**Corresponding author**. D. Raoult, Aix-Marseille Université, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes (URMITE), CNRS 7278, IRD 198, INSERM 1095, UM63, Institut Hospitalo-Universitaire Méditerranée-Infection, Faculté de médecine, 27 Boulevard Jean Moulin, 13385, Marseille cedex 5, France
**E-mail:** didier.raoult@gmail.com

In 2016, as a part of culturomics study of the human microbiome, we isolated five bacterial strains from stool samples of patients in Niger with malnutrition diseases (marasmus and kwashiorkor) that could not be identified by our systematic matrix-assisted laser desorption–ionization time-of-flight mass spectrometry screening on a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [1–4]. This work, which contributes to the characterization of the malnourished human microbiota, is the first step in understanding the involved mechanisms. Indeed, gut microbiota alteration, probably associated with a diet low in antioxidants, is highly suspected to explain the vicious cycle of diarrhoea, infection and malnutrition and the resilience of severe acute malnutrition despite therapeutic diet [5,6]. The patients provided signed informed consent, and the study was validated by the ethics committee of the Institut Federatif de Recherche IFR48 under number 09-022.

Here we describe five bacterial strains which were deposited in the open Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under the reference numbers P3646 ('*Marasmitruncus massiliensis*' strain Marseille-P3646$^T$), P3545 ('*Clostridium culturomicum*' strain Marseille-P3545$^T$), P3502 ('*Blautia provencensis*' strain Marseille-P3502$^T$), P3604 ('*Bacillus caccae*' strain Marseille-P3604$^T$) and P3601 ('*Ornithinibacillus massiliensis*' strain Marseille-P3601$^T$) respectively.

All five strains were isolated from two different Nigerian children with clinical aspects of marasmus but missing anthropometric criteria.

For each strain, the following conditions for initial growth were as follows. Strain Marseille-P3646$^T$ was isolated after 15 days of culture in marine broth, anaerobe condition, 37°C.



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Strain Marseille-P3545$^T$ was isolated after 10 days in blood culture bottle, anaerobe condition, 37°C. Strain Marseille-P3502$^T$ was isolated after 1 day in blood culture bottle + sheep's blood + rumen, anaerobic condition, 37°C. Strain Marseille-P3604$^T$ was isolated after 3 days of culture in marine broth, aerobe condition, 37°C. Strain Marseille-P3601$^T$ was isolated after 1 day of culture in marine broth, aerobe condition, 37°C.

The colony morphologies of these five bacterial species are as follows. Colonies of strain Marseille-P3646$^T$ were circular, smooth, very small and white with a mean diameter of 0.3 to 0.5 mm. Colonies of strain Marseille-P3545$^T$ were white, circular, smooth and convex, with intact edges and a larger diameter of 2 to 5 mm. Colonies of strain Marseille-P3502$^T$ were circular, smooth, crateriform and pale grey with intact edges and a mean diameter of 1 to 3.5 mm. Colonies of strain Marseille-P3604$^T$ were circular, convex, smooth and grey, with irregular edges and a mean diameter of 3 to 6 mm. Colonies of strain Marseille-P3601$^T$ were circular, smooth and pale rose, and had a raised form with intact edges and a mean diameter of 3 to 5 mm.

All five bacterial strains were Gram positive; strains were rod shaped for strains Marseille-P3646$^T$, Marseille-P3545$^T$, Marseille-P3604$^T$, and Marseille-P3601$^T$; and coccus shaped for strain Marseille-P3502$^T$. The 16S rRNA gene was sequenced in these five strains using fD1-rP2 primers as previously described, using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France).

Strain Marseille-P3646$^T$ exhibited a 94.40% 16S rRNA gene sequence identity with *Anaerotruncus colihominis* strain WAL 14565 (GenBank accession no. NR_027558), the phylogenetically closest species with standing in nomenclature (Fig. 1), which putatively classifies it as a member of the family *Ruminococcaceae* in the phylum *Firmicutes*. Strain Marseille-P3646$^T$ exhibits a 16S rRNA gene sequence divergence >5% with its phylogenetically closest species with standing in nomenclature [7]. Thus, we propose the creation of the new genus '*Marasmitruncus*' (Ma.ras.mi.trun'cus, from *marasmi,* L. masc. adj. for 'marasmus,' and *truncus,* L. masc. n., 'stick'; '*Marasmitruncus,*' a stick isolated from a patient with marasmus). '*Marasmitruncus massiliensis*' is proposed as the type species of the '*Marasmitruncus*' genus. Strain Marseille-P3646$^T$ is the type strain of the new species '*Marasmitruncus massiliensis*' (mas.si.li.en'sis, L. gen. masc. n., *massiliensis,* pertaining to Massilia, the ancient name of the city of Marseille, where this bacterium was discovered).

Strain Marseille-P3545$^T$ exhibited a 97.63% 16S rRNA gene sequence identity with *Clostridium histolyticum* strain JCM 1403 (GenBank accession no. NR_113187), the phylogenetically closest species with standing in nomenclature (Fig. 2), which putatively classifies it as a member of the genus *Clostridium*

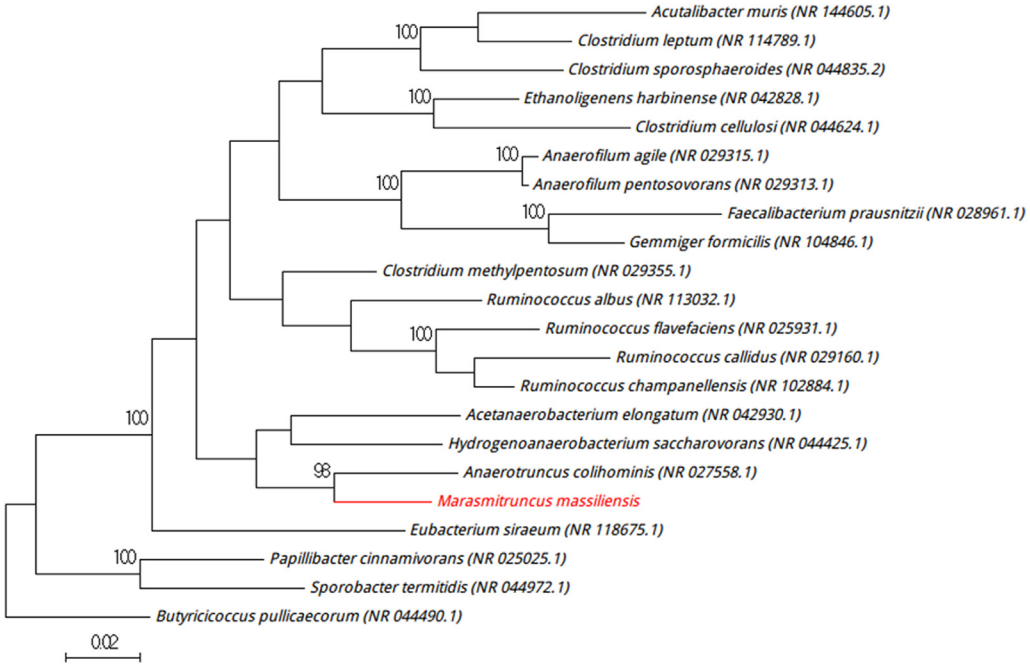


**FIG. 1.** Phylogenetic tree showing position of '*Marasmitruncus massiliensis*' strain Marseille-P3646$^T$ relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW and phylogenetic inferences obtained using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. Only bootstrap scores of at least 90% were retained. Scale bar indicates 2% nucleotide sequence divergence.

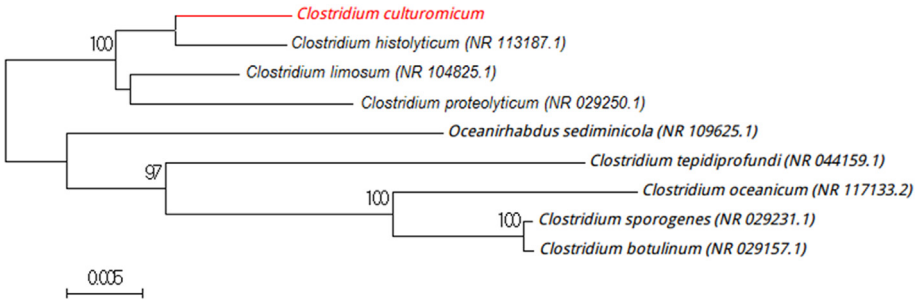


**FIG. 2.** Phylogenetic tree showing position of '*Clostridium culturomicum*' Marseille-P3545$^T$ relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW and phylogenetic inferences obtained using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. Only bootstrap scores of at least 90% were retained. Scale bar indicates 0.5% nucleotide sequence divergence.

within the family *Clostridiaceae* in the phylum *Firmicutes*. Strain Marseille-P3545$^T$ exhibiting a 16S rRNA gene sequence divergence >1.3% with its phylogenetically closest species with standing in nomenclature [7]. Thus we propose the creation of the new species '*Clostridium culturomicum*' (cul.tu.ro.mi'cum, L. masc. adj., to refer to the microbial culturomics approach performed to cultivate this strain). Strain Marseille-P3545$^T$ is the type strain of the new species '*Clostridium culturomicum*.'

Strain Marseille-P3502$^T$ exhibited a 97.41% 16S rRNA gene sequence identity with *Blautia luti* strain DSM 14534 (GenBank accession no. NR_114135), the phylogenetically closest species with standing in nomenclature (Fig. 3), which putatively classifies it as a member of the genus *Blautia* within the family *Lachnospiraceae* in the phylum *Firmicutes*. Strain Marseille-P3502$^T$ exhibited a 16S rRNA sequence divergence >1.3% with its phylogenetically closest species with standing in nomenclature [7]. Thus, we propose the creation of the new species '*Blautia provencensis*' (pro.ven.cen'sis, N.L. masc. adj., *provencensis*, pertaining to Provence, the region of France where the type strain was isolated). Strain Marseille-P3502$^T$ is the type strain of the new species '*Blautia provencensis*.'

Strain Marseille-P3604$^T$ exhibited a 98.38% 16S rRNA gene sequence identity with *Bacillus persicus* strain B48 (GenBank accession no. NR_109140), the phylogenetically closest species with standing in nomenclature (Fig. 4), which putatively classifies it as a member of the genus *Bacillus* within the family

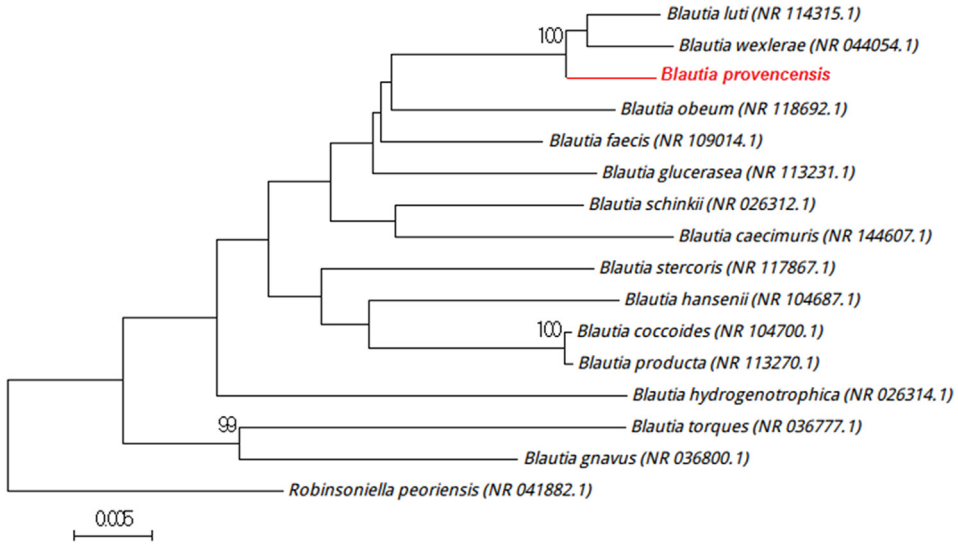


**FIG. 3.** Phylogenetic tree showing position of '*Blautia provencensis*' Marseille-P3502$^T$ relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW and phylogenetic inferences obtained using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. Only bootstrap scores of at least 90% were retained. Scale bar indicates 0.5% nucleotide sequence divergence.

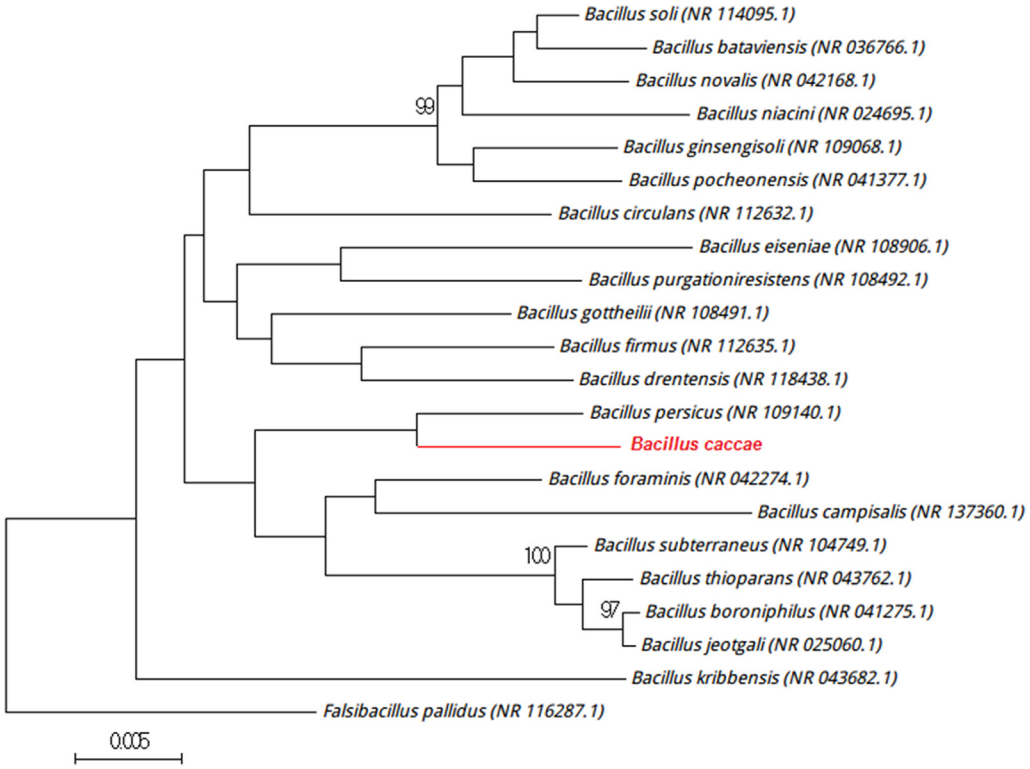


**FIG. 4.** Phylogenetic tree showing position of '*Bacillus caccae*' Marseille-P3604$^T$ relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW and phylogenetic inferences obtained using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. Only bootstrap scores of at least 90% were retained. Scale bar indicates 0.5% nucleotide sequence divergence.

*Bacillaceae* in the phylum *Firmicutes*. Strain Marseille-P3604$^T$ exhibits a 16S rRNA gene sequence divergence >1.3% with its phylogenetically closest species with standing in nomenclature [7]. Thus, we propose the creation of the new species '*Bacillus caccae*' (cac'cae, pronounced kak'ka, Gr. n., from *kakke*, 'faeces': N.L. gen. n. *caccae*, 'of faeces,' to refer to the clinical sample from which this bacterium was isolated). Strain Marseille-P3604$^T$ is the type strain of the new species '*Bacillus caccae*.'

Strain Marseille-P3601$^T$ exhibited a 98.68% 16S rRNA gene sequence identity with *Ornithinibacillus scapharcae* strain TW25 (GenBank accession no. NR_117927), the phylogenetically closest species with standing in nomenclature (Fig. 5), which putatively classifies it as a member of the genus *Ornithinibacillus* within the family *Bacillaceae* in the phylum *Firmicutes*. Strain Marseille-P3601$^T$ exhibiting a 16S rRNA gene sequence divergence >1.3% with its phylogenetically closest species with

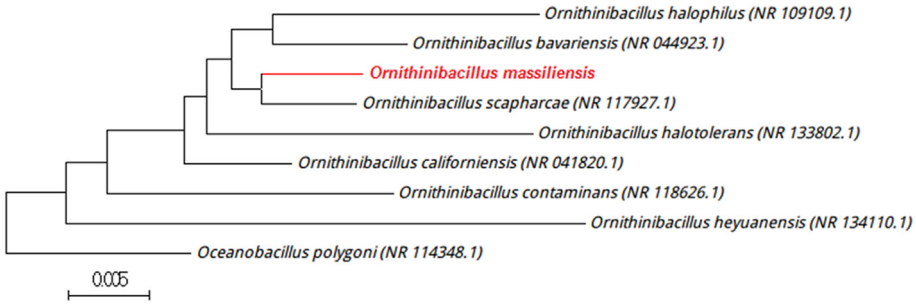


**FIG. 5.** Phylogenetic tree showing position of '*Ornithinibacillus massiliensis*' Marseille-P3601$^T$ relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW and phylogenetic inferences obtained using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. Only bootstrap scores of at least 90% were retained. Scale bar indicates 0.5% nucleotide sequence divergence.

standing in nomenclature [7]. Thus, we propose the creation of the new species '*Ornithinibacillus massiliensis*' (mas.si.li.en'sis, L. gen. masc. n., *massiliensis,* pertaining to Massilia, the ancient name of the city of Marseille, where this bacterium was discovered). Strain Marseille-P3601$^T$ is the type strain of the new species '*Ornithinibacillus massiliensis.*'

## Nucleotide sequence accession number

All the 16S rRNA gene sequences were deposited in GenBank under the following accession numbers: LT725660 ('Marasmitruncus massiliensis' strain Marseille-P3646T), LT797537 ('Clostridium culturomicum' strain Marseille-P3545T), LT714168 ('Blautia provencensis' strain Marseille-P3502T), LT714169 ('Bacillus caccae' strain Marseille-P3604T) and LT725658 ('Ornithinib*acillus massiliensis*' strain Marseille-P3601$^T$).

## Deposit in a culture collection

All strains were deposited in the open Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under reference numbers P3646 ('*Marasmitruncus massiliensis*' strain Marseille-P3646$^T$), P3545 ('*Clostridium culturomicum*' strain Marseille-P3545$^T$), P3502 ('*Blautia provencensis*' strain Marseille-P3502$^T$), P3604 ('*Bacilluscaccae*' strain Marseille-P3604$^T$) and P3601 ('*Ornithinibacillus massiliensis*' strain Marseille-P3601$^T$) respectively.

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## Conflict of Interest

None declared.

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