

# ‘Enterococcus timonensis’ sp. nov., ‘Actinomyces marseillensis’ sp. nov., ‘Leptotrichia massiliensis’ sp. nov., ‘Actinomyces pacaensis’ sp. nov., ‘Actinomyces oralis’ sp. nov., ‘Actinomyces culturomici’ sp. nov. and ‘Gemella massiliensis’ sp. nov., new bacterial species isolated from the human respiratory microbiome

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# *'Enterococcus timonensis'* sp. nov., *'Actinomyces marseillensis'* sp. nov., *'Leptotrichia massiliensis'* sp. nov., *'Actinomyces pacaensis'* sp. nov., *'Actinomyces oralis'* sp. nov., *'Actinomyces culturomici'* sp. nov. and *'Gemella massiliensis'* sp. nov., new bacterial species isolated from the human respiratory microbiome

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## Abstract

We report the main characteristics of *'Enterococcus timonensis'* strain Marseille-P2817$^T$ (CSUR P2817), *'Leptotrichia massiliensis'* sp. nov., strain Marseille-P3007$^T$ (CSUR P3007), *'Actinomyces marseillensis'* sp. nov., strain Marseille-P2818$^T$ (CSUR P2818), *'Actinomyces pacaensis'* sp. nov., strain Marseille-P2985$^T$ (CSUR P2985), *'Actinomyces oralis'* sp. nov., strain Marseille-P3109$^T$ (CSUR P3109), *'Actinomyces culturomici'* sp. nov., strain Marseille-P3561$^T$ (CSUR P3561) and *'Gemella massiliensis'* sp. nov., strain Marseille-P3249$^T$ (CSUR P3249) which were isolated from human sputum samples.

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Culturomics has proven its efficiency in the description of the human microbiome at different levels and has enlarged the known human prokaryotic repertoire [1,2]. Nevertheless, our laboratory succeeded in identifying a significant number of bacterial species that could not be identified by our systematic matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) screening on a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [3,4]. The corresponding reference spectrum is available online (http://mediterraner-infection.com/article.php?laref=256&titre=urms-database). As part of the project aiming to describe the human respiratory microbiome by culturomics, we were able to isolate several new species not previously reported.

Before we began our study, it was validated by the ethics committee of the Institut Fédératif de Recherche IFR48 under number 09-022. Sputum samples were collected from different participants and incubated in a modified blood culture bottle (Becton Dickinson, Le Pont de Claix, France). A follow-up of 30 days was performed, and colony identification was done by MALDI-TOF MS and 16S rRNA gene sequencing in case of failure as previously described. All strains that we report here failed to be identified by MALDI-TOF MS.

Strain Marseille-P2817 was isolated on 5% sheep's blood–enriched Columbia agar (bioMérieux, Marcy l'Etoile, France) at 37°C from a sputum sample of a healthy Frenchman after incubation of 10 days in an aerobic blood culture bottle (Becton Dickinson) supplemented with filtered rumen. The colonies were smooth with a 1 mm diameter. Moreover, bacterial cells were Gram-positive cocci, motile and non–spore forming, with a mean diameter of 0.65 µm and a mean length of 1.1 µm. Strain Marseille-P2817 did not


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exhibit either catalase or oxidase activities and showed a 96.00% sequence identity with *Enterococcus casseliflavus* strain Kd7 TUC-EEAOC (GenBank accession no. KM096606), which is phylogenetically the closest species with standing in nomenclature (Fig. 1). Because strain Marseille-P2817 has a 16S rRNA gene sequence divergence of >1.3% with its phylogenetically closest species with standing in nomenclature [5], we suggest the creation of a new species called *'Enterococcus timonensis'* (ti.mo.nen'sis, N.L. masc. adj., *timonensis* from the Latin name of Hôpital de la Timone, where strain Marseille-P2817 was isolated). Strain Marseille-P2817$^T$ is the type strain of the new species *'Enterococcus timonensis.'*

Likewise, strain Marseille-P3007 was cultured directly on 5% sheep's blood–enriched Columbia agar (bioMérieux) at 30°C under anaerobic atmosphere. Although growth was observed at 37°C, it was optimal at 30°C. Colonies had a diameter ranging from 0.5 to 3 mm with a rough-edged appearance. Bacterial cells were nonmotile, spore-forming, Gram-positive rods and were catalase positive and oxidase negative with a diameter ranging from 0.67 to 0.8 µm and a length ranging from 3.5 to 11.5 µm. Strain Marseille-P3007 showed a 98.3% sequence identity with *Leptotrichia buccalis* strain C-1013-b (GenBank accession no. NR_114394), which is the phylogenetically closest species with standing in nomenclature (Fig. 2). Because it had a sequence similarity of <98.65% [6], we propose *'Leptotrichia massiliensis'* (mas.il.i.en'sis, L. gen. neutr. n., *massiliensis,* 'of Massilia,' the Latin name of Marseille, the place where the strain was isolated) strain Marseille-P3007 as new species. Strain Marseille-P3007$^T$ is the type strain of the new species *'Leptotrichia massiliensis.'*

Strain Marseille-P2818 was isolated on 5% sheep's blood–enriched Columbia agar (bioMérieux) from a sputum sample of a healthy Frenchwoman after 30 days of incubation at 30°C in a blood culture bottle (Becton Dickinson) supplemented with filtered rumen. The strain was able to grow at 28 to 37°C but optimally at 30°C. Colonies had a diameter ranging from 0.5 to 1.5 mm, with a smooth appearance. Bacterial cells were Gram-positive rods, nonmotile, non–spore forming, and catalase and oxidase negative. Strain Marseille-P2818 showed 98.14% sequence identity with *'Actinomyces odontolyticus'* strain F0309 (GenBank accession no. GQ131411), which is the phylogenetically closest species with standing in nomenclature (Fig. 3). Because it had a sequence similarity of <98.65% [6], we propose strain Marseille-P2818 as a new species named

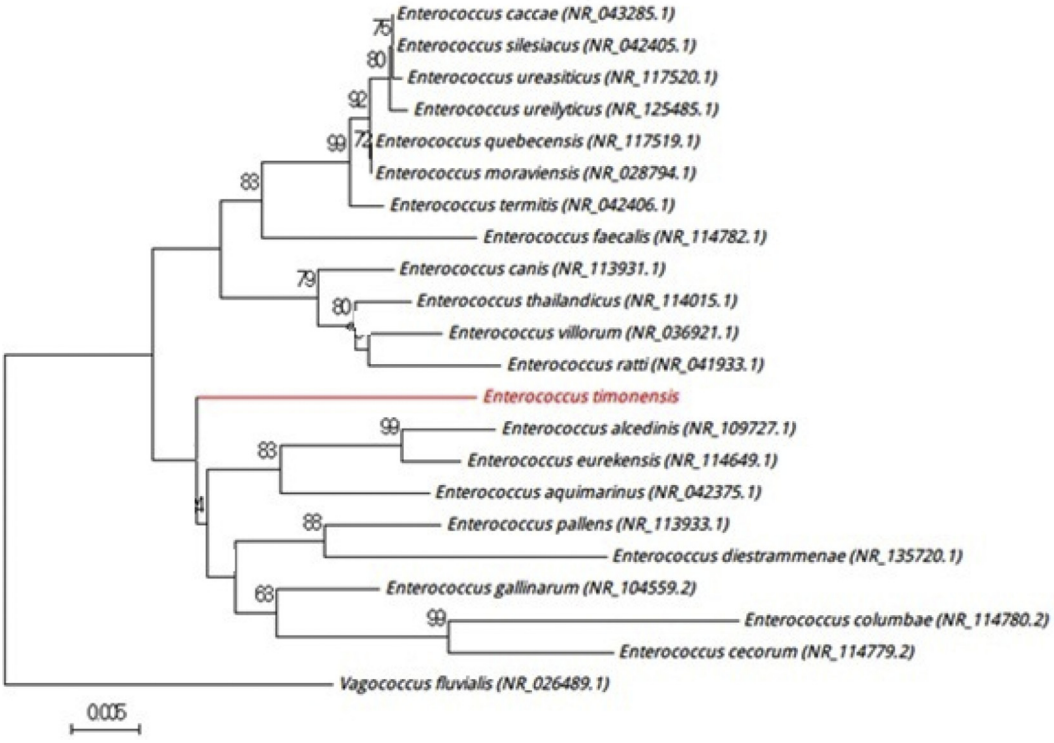



**FIG. 1.** Positioning of *'Enterococcus timonensis'* strain Marseille-P2817 relative to other phylogenetically close neighbours in phylogenetic tree. Sequences of strains involved were aligned by CLUSTALW, and phylogenetic inferences were obtained by MEGA 7.0 software using maximum-likelihood method. Numbers shown at nodes represent percentages of bootstrap values obtained after 500 repeats to generate majority consensus tree. Only bootstrap scores with minimum 90% score were retained. Scale bar indicates 0.5% nucleotide sequence divergence.

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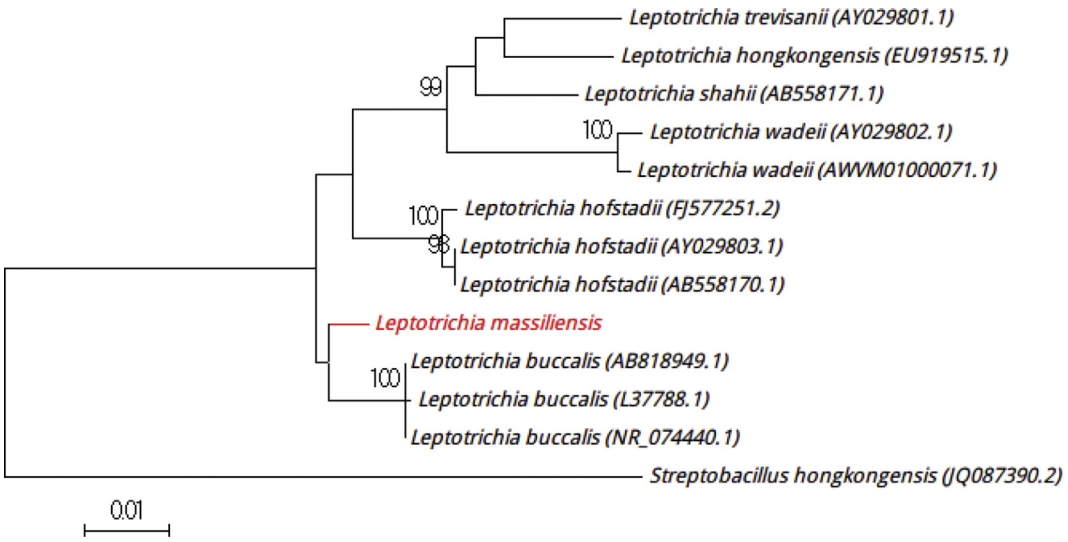



**FIG. 2.** Positioning of *'Leptotrichia massiliensis'* strain Marseille-P3007 relative to other phylogenetically close strains in phylogenetic tree. CLUSTALW was used to align sequences, and phylogenetic inferences were generated by MEGA 7.0 software with maximum-likelihood method. Scale bar indicates 1% nucleotide sequence divergence, and numbers at nodes are percentages of 500 bootstrap values obtained in order to generate consensus tree. Only bootstrap scores with minimum 90% score were retained.

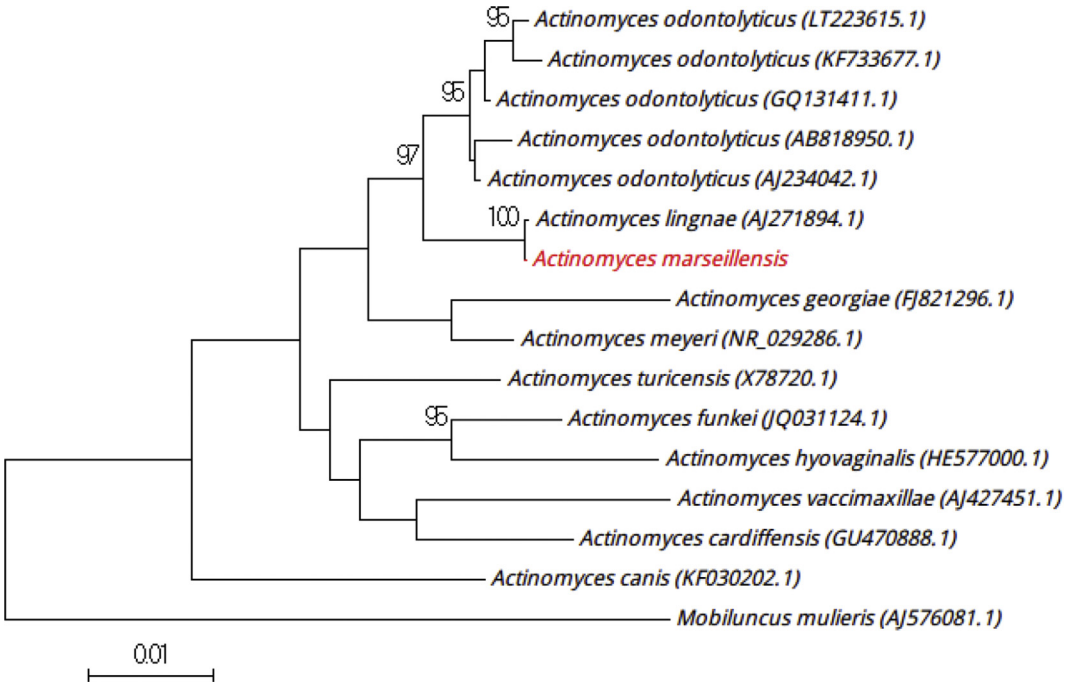



**FIG. 3.** Positioning of *'Actinomyces marseillensis'* strain Marseille-P2818 relative to other phylogenetically close strains in phylogenetic tree. CLUSTALW was used to align sequences, and phylogenetic inferences were generated by MEGA 7.0 software with maximum-likelihood method. Scale bar indicates 1% nucleotide sequence divergence, and numbers at nodes are percentages of 500 bootstrap values obtained in order to generate consensus tree. Only bootstrap scores with minimum 90% score were retained.

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*'Actinomyces marseillensis'* (mar.sei.ll.en'sis, L. gen. neut. adj., from *marseillensis,* the Latin name for Marseille, where the strain was isolated). Strain Marseille-P2818$^T$ is the type strain of the new species *'Actinomyces marseillensis.'*

Strain Marseille-P2985 was isolated from the sputum of a healthy Frenchman on 5% sheep's blood–enriched Columbia agar (bioMérieux) at 37°C after incubation in a blood culture bottle (Becton Dickinson) supplemented with 4 mL filtered rumen at 37°C under anaerobic conditions. Colonies had a diameter ranging from 1 to 5 mm, and were smooth and white. Bacterial cells were spore-forming Gram-positive rods, nonmotile, and catalase positive and oxidase negative. 16S rRNA gene sequence–based identification of strain Marseille-P2985 showed 97.91% sequence identity with *Actinomyces georgiae* strain AGU5 (GenBank accession no. GU561319), which is the phylogenetically closest species with standing in nomenclature (Fig. 4). Because it had a similarity value of <98.65% [6], we propose strain Marseille-P2985 to be a new species named *'Actinomyces pacaensis'* (pa.ca'en.sis, L. gen. masc. n., from *pacaensis,* 'of PACA,' the acronym of Provence Alpes Côte d'Azur, the region where the strain was isolated). Strain Marseille-P2985$^T$ is the type strain of the new species *'Actinomyces pacaensis.'*

Strain Marseille-P3109 was isolated from the sputum of healthy Frenchman on 5% sheep's blood–enriched Columbia agar (bioMérieux) after 15 days of incubation in a blood culture bottle (Becton Dickinson) supplemented with filtered rumen under anaerobic conditions at 37°C. Colonies had a diameter ranging from 0.8 to 2 mm with a smooth and grey appearance. Bacterial cells were Gram-positive rods, nonmotile and not spore-forming, and catalase and oxidase negative. 16S rRNA gene sequence–based identification of strain Marseille-P3109 showed 98.49% sequence identity with *Actinomyces naeslundii* strain JCM 8349 (GenBank accession no. NR_113326), which is the phylogenetically closest species with standing in nomenclature (Fig. 5). Having a similarity value of <98.65% [6], we propose that strain Marseille-P3109 be a new species named *'Actinomyces oralis'* (o.ra'lis, N.L. neut. adj., *oralis,* 'of the mouth,' the source from which the strain was isolated). Strain Marseille-P3109$^T$ is the type strain of the new species *'Actinomyces oralis.'*

Strain Marseille-P3561 was isolated from the sputum of healthy Frenchman on 5% sheep's blood–enriched Columbia

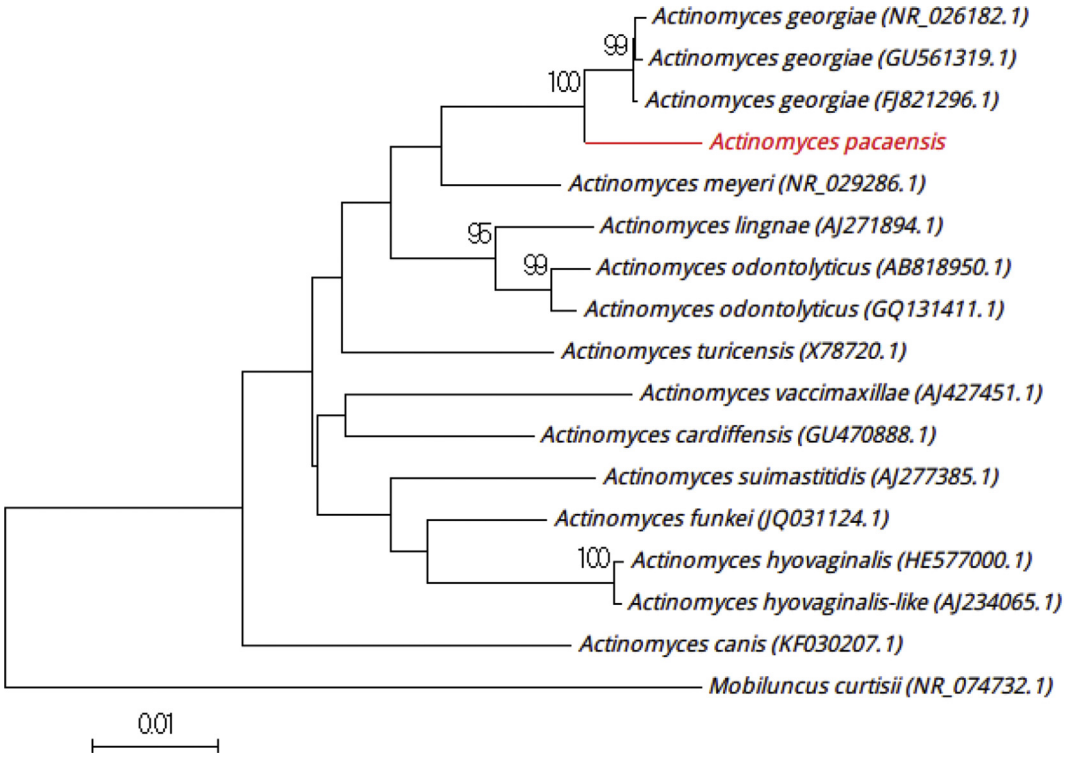



**FIG. 4.** Positioning of *'Actinomyces pacaensis'* strain Marseille-P2985 relative to other phylogenetically close strains in phylogenetic tree. CLUSTALW was used to align sequences, and phylogenetic inferences were generated by MEGA 7.0 software with maximum-likelihood method. Scale bar indicates 1% nucleotide sequence divergence, and numbers at nodes are percentages of 500 bootstrap values obtained in order to generate consensus tree. Only bootstrap scores with minimum 90% score were retained.

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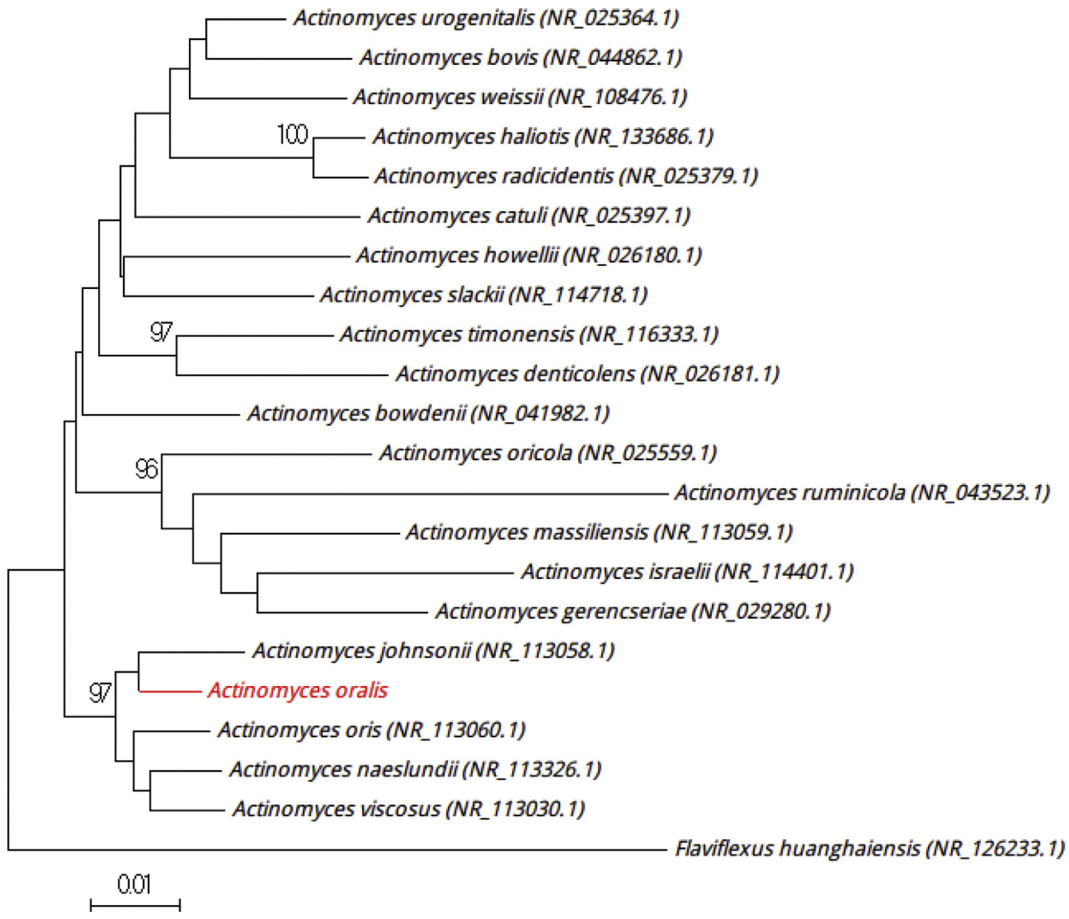



FIG. 5. Positioning of *'Actinomyces oralis'* strain Marseille-P3109 relative to other phylogenetically close strains in phylogenetic tree. CLUSTALW was used to align sequences, and phylogenetic inferences were generated by MEGA 7.0 software with maximum-likelihood method. Scale bar indicates 1% nucleotide sequence divergence, and numbers at nodes are percentages of 500 bootstrap values obtained in order to generate consensus tree. Only bootstrap scores with minimum 90% score were retained.

agar (bioMérieux) after 10 days of incubation in a blood culture bottle (Becton Dickinson) supplemented with filtered rumen under aerobic conditions at 37°C. Colonies had a diameter ranging from 0.6 to 1.4 mm and had a smooth appearance. Bacterial cells were Gram-positive rods, nonmotile, non–spore forming, and catalase and oxidase negative. Strain Marseille-P3561 16S rRNA gene sequence showed a 96.12% sequence similarity with *Actinomyces hyovaginalis* strain BM 1192/5 (GenBank accession no. NR_026097), which is the phylogenetically closest species with standing in nomenclature (Fig. 6). Having a similarity value of the <98.65% threshold recommended to define a new species [6], we propose that strain Marseille-P3561$^T$ be a new species named *'Actinomyces culturomici'* (cul.tu.ro.mi.ci, L. gen. neut. n., from *culturomici,* 'of culturomics,' to refer to the strategy used to isolated the strain). Strain Marseille-P3561$^T$ is the type strain of the new species *'Actinomyces culturomici.'*

Finally, strain Marseille-P3249 was isolated from the sputum of a healthy Frenchman on 5% sheep's blood–enriched Columbia agar (bioMérieux) after 20 days of incubation in a blood culture bottle (Becton Dickinson) supplemented with filtered rumen, under aerobic conditions at 37°C. Colonies had a diameter ranging from 0.5 to 1.2 mm and a smooth appearance. Bacterial cells were nonmotile and non–spore forming, Gram positive and coccus shaped, and catalase and oxidase negative. 16S rRNA gene sequence–based identification of strain Marseille-P3249 showed a 98.3% sequence identity with *Gemella bergeri* strain 617-93 (GenBank accession no. NR_026420.1), which is the phylogenetically closest species with standing in nomenclature (Fig. 7). Having a sequence similarity of <98.65% [6], we propose strain Marseille-P3249 be a new species named *'Gemella massiliensis'* (mas.il.i.en'sis, L. gen. neut. n., from *massiliensis,* 'of Massilia,' the Latin name of Marseille, the place where the strain was isolated). Strain Marseille-P3249$^T$ is the type strain of the new species *'Gemella massiliensis.'*

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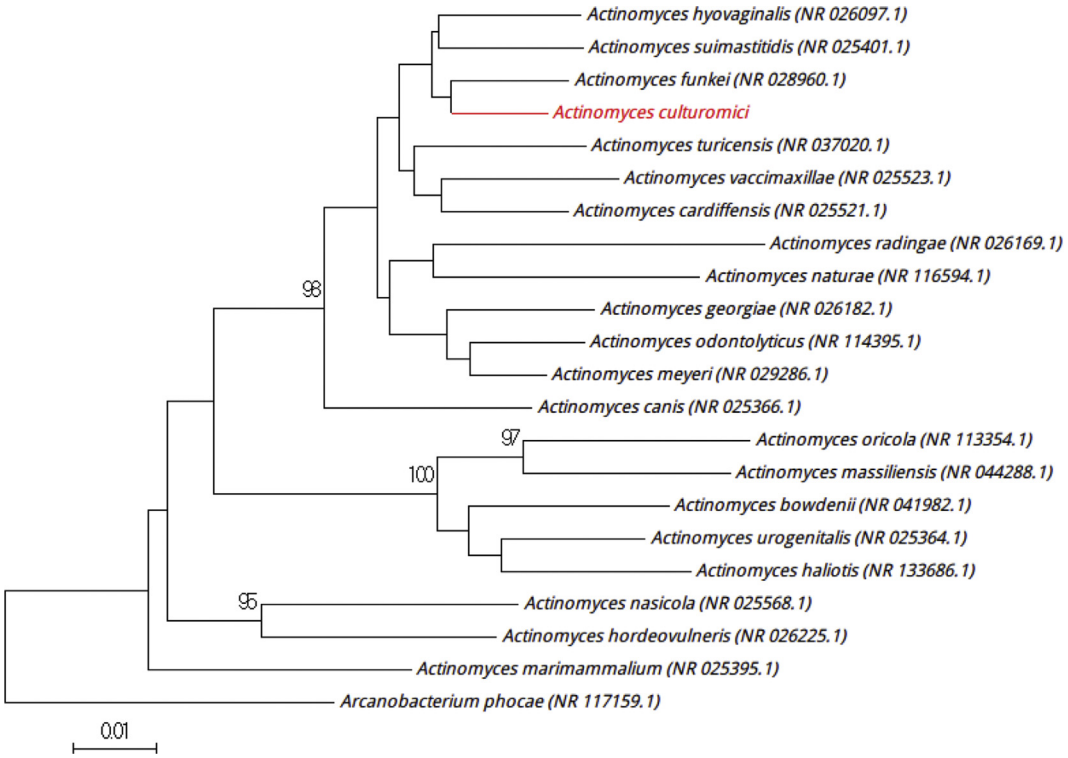



**FIG. 6.** Positioning of *'Actinomyces culturomici'* strain Marseille-P3561 relative to other phylogenetically close strains in phylogenetic tree. CLUSTALW was used to align sequences, and phylogenetic inferences were generated by MEGA 7.0 software with maximum-likelihood method. Scale bar indicates 1% nucleotide sequence divergence, and numbers at nodes are percentages of 500 bootstrap values obtained in order to generate consensus tree. Only bootstrap scores with minimum 90% score were retained.

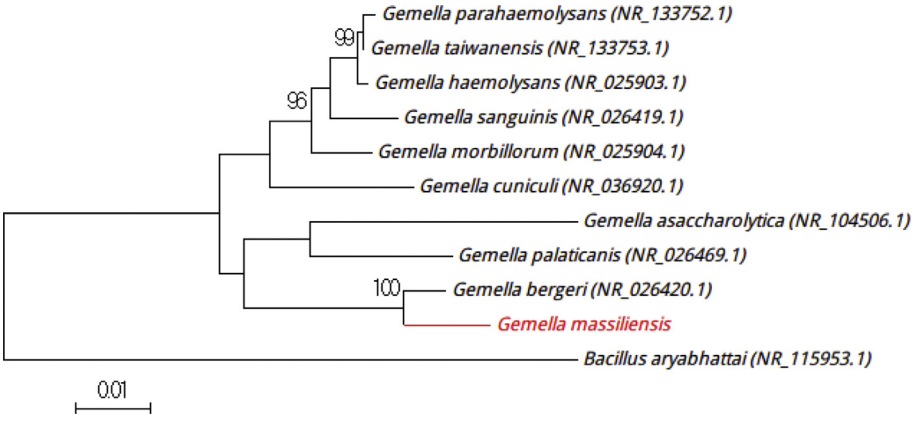



**FIG. 7.** Positioning of *'Gemella massiliensis'* strain Marseille-P3249 relative to other phylogenetically close strains in phylogenetic tree. CLUSTALW was used to align sequences, and phylogenetic inferences were generated by MEGA 7.0 software with maximum-likelihood method. Scale bar indicates 1% nucleotide sequence divergence, and numbers at nodes are percentages of 500 bootstrap values obtained in order to generate consensus tree. Only bootstrap scores with minimum 90% score were retained.

## Nucleotide sequence accession numbers

The 16S rRNA gene sequences of *'Enterococcus timonensis,' 'Actinomyces marseillensis,' 'Leptotrichia massiliensis,' 'Actinomyces pacaensis,' 'Actinomyces oralis,' 'Actinomyces culturomici'* and *'Gemella massiliensis'* were deposited in GenBank under the following accession numbers, respectively: LT576388, LT576412, LT576400, LT576401, LT627670 and LT628479.

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## Deposit in a culture collection

Strains Marseille-P2817, P3007, P2818, P3109, P3561 and P3249 were deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under the following numbers, respectively: P2817, P3007, P3109, P3561 and P3249.

## Conflict of interest

None declared.

## Acknowledgement

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