

'*Actinomyces provencensis*' sp. nov., '*Corynebacterium bouchesdurhonense*' sp. nov., '*Corynebacterium provencense*' sp. nov. and '*Xanthomonas massiliensis*' sp. nov., 4 new species isolated from fresh stools of obese French patients

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Abstract

We report the main characteristics of '*Actinomyces provencensis*' strain SN12^T sp. nov., '*Corynebacterium bouchesdurhonense*' strain SN14^T sp. nov., '*Corynebacterium provencense*' strain SN15^T sp. nov. and '*Xanthomonas massiliensis*' strain SN8^T sp. nov., which were all isolated from stool samples from obese French patients.

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Keywords: Culturomics, halophilic species, human gut microbiota, new species, taxonogenomics

Original Submission: 5 January 2017; **Revised Submission:** 19 January 2017; **Accepted:** 30 January 2017

Article published online: 4 February 2017

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The study of the human gut microbiota experienced a new revolution with culturomics, a new concept that enables exploring, as comprehensively as possible, the viable population of prokaryotes associated with the human gastrointestinal tract [1,2]. In 2015, using this culturomics approach, we isolated four bacteria which were not identified by matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany) [1,2]. These species were isolated by culture on Columbia agar supplemented with 5% sheep's blood (bioMérieux, Marcy l'Etoile, France) at 37°C under micro-aerophilic conditions using CampyGen (Thermo Scientific, Villebon-sur-Yvette, France) for 48 hours, all from stool samples from obese French patients. All subjects provided written informed consent, and the study was validated by the ethics committee of the IFR48 Federative Research Institute under number 09-022. The strains are anaerobic and facultative, but

growth was better under aerobic conditions. The failure of identification by MALDI-TOF MS of these four strains led us to sequence their 16S rRNA gene using fDI-rP2 primers as described previously using a 3130-XL sequencer (Applied Biosciences, Saint-Aubin, France) [3].

Strain SN12 showed colonies bright grey and nonhaemolytic, with a diameter of 0.5 to 1 mm on 5% sheep's blood–enriched Columbia agar (bioMérieux) after 48 hours of incubation under aerobic conditions. It is a facultative anaerobic bacteria. Cells were Gram-positive, rod-shaped bacilli, spore forming and motile. The strain had positive catalase activity, but oxidase was negative. Strain SN12 had a 16S rRNA gene sequence identity of 95.04% with *Actinomyces odontolyticus* strain JCM 14871 (Genbank accession number NR_114395), the phylogenetically closest species with standing in nomenclature [4] (Fig. 1). This <98.65% similarity leads us to putatively classify SN12 as a member of the *Actinomyces* genus and belonging to *Actinobacteria* phylum [5]. Therefore, we propose the creation of the new species '*Actinomyces provencensis*' (pro.ven.cen'sis, N.L. adj. neut., from Provence, the region in France, where the strain was isolated). SN12^T is the type strain of the species '*Actinomyces provencensis*'.

The colonies of the strain SN14 on 5% sheep's blood–enriched Columbia agar (bioMérieux) at 37°C under aerobic

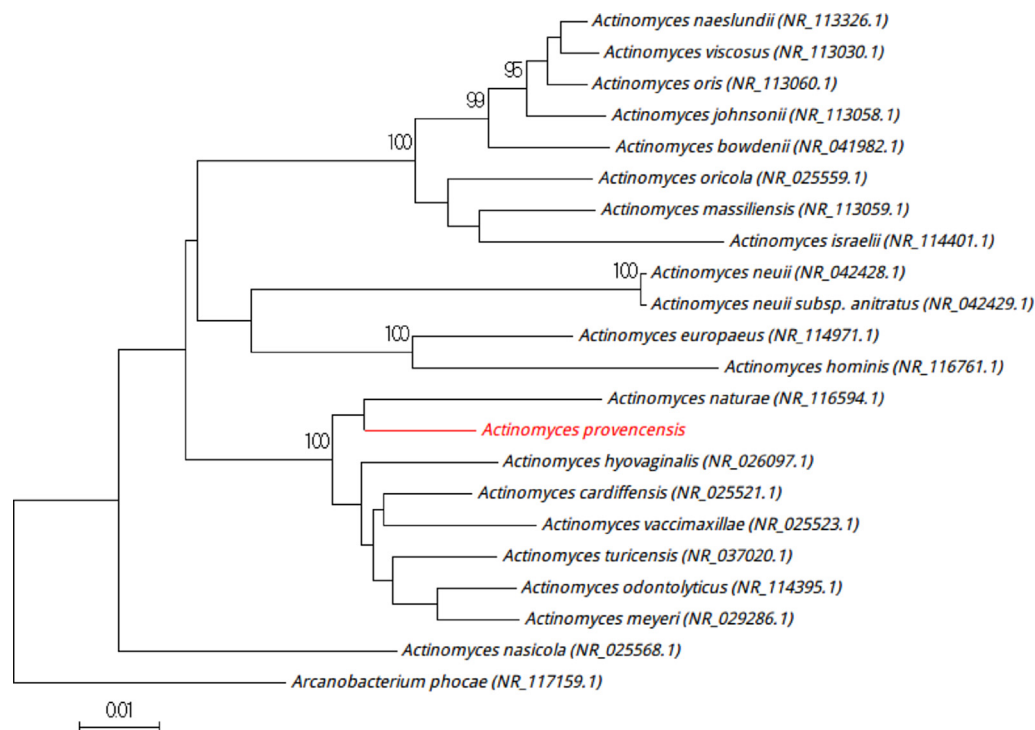


FIG. 1. Phylogenetic tree showing positions of '*Actinomyces provencensis*' strain SN12^T relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained with Kimura two-parameter models using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 1.000 times to generate majority consensus tree. Scale bar indicates 1% nucleotide sequence divergence.

conditions were white, circular, nonhaemolytic and opaque, 1 mm in diameter after 48 hours of incubation. Bacterial cells were Gram positive, rod shaped, facultative anaerobic, spore forming and nonmotile. Under electron microscopy, cells had a mean length and diameter of 0.8 and 1.8–5.0 μm respectively. Catalase and oxidase activities were positive. The strain SN14 exhibited a 97.3% sequence identity with *Corynebacterium tuscaniense* strain ISS-5309 (NR_043093), the phylogenetically closest species with standing in nomenclature (Fig. 2). *Corynebacterium tuscaniense* strain ISS-5309 was isolated from culture of human blood [6]. This <98.65% similarity with its phylogenetically closest species with a validly published name leads us to putatively classify SN14 as a representative strain of a new species within the genus *Corynebacterium* in the *Actinobacteria* phylum [5]. On the basis of the 16S rRNA gene sequence results and its phylogenetical position with the others closest species, we propose the creation of the new species '*Corynebacterium bouchesdurhonense*' (bou.ches.du.rho.nen'se, N.L. adj. neut., from Bouches du Rhône, the county in France where the strain was isolated). SN14^T is the type strain of the species '*Corynebacterium bouchesdurhonense*'.

The colonies of the strain SN15 were milky white, circular, nonhaemolytic and opaque, 1 to 1.5 mm in diameter after 48 hours of incubation on 5% sheep's blood-enriched Columbia agar (bio-Mérieux) under aerobic conditions. Cells were Gram positive, rod shaped (0.6 \times 1.2–1.7 μm), facultative anaerobic, motile and spore forming. Strain SN15 exhibited catalase positivity and oxidase negativity. The strain SN15 had a 16S rRNA gene sequence identity of 97.3% with *Corynebacterium tuscaniense* strain ISS-5309 (NR_043093), the phylogenetically closest species with standing in nomenclature [6] (Fig. 2). This <98.65% similarity leads us to putatively classify SN15 as a new species in the *Actinobacteria* phylum [5]. From these results, we propose the creation of the new species '*Corynebacterium provencense*' (pro.ven.cen'se, N.L. adj. neut., from Procence, the region in France where the strain was isolated). SN15^T is the type strain of the species '*Corynebacterium provencense*'.

The colonies of the strain SN8 were khaki green, circular, viscous and nonhaemolytic, 1 to 2 mm in diameter on 5% sheep's blood-enriched Columbia agar (bioMérieux) under aerobic conditions after 48 hours of incubation. Bacterial cells were Gram negative, rod shaped, motile and non-spore forming. Observed under electron microscopy, bacterial cells measure 0.6 μm in

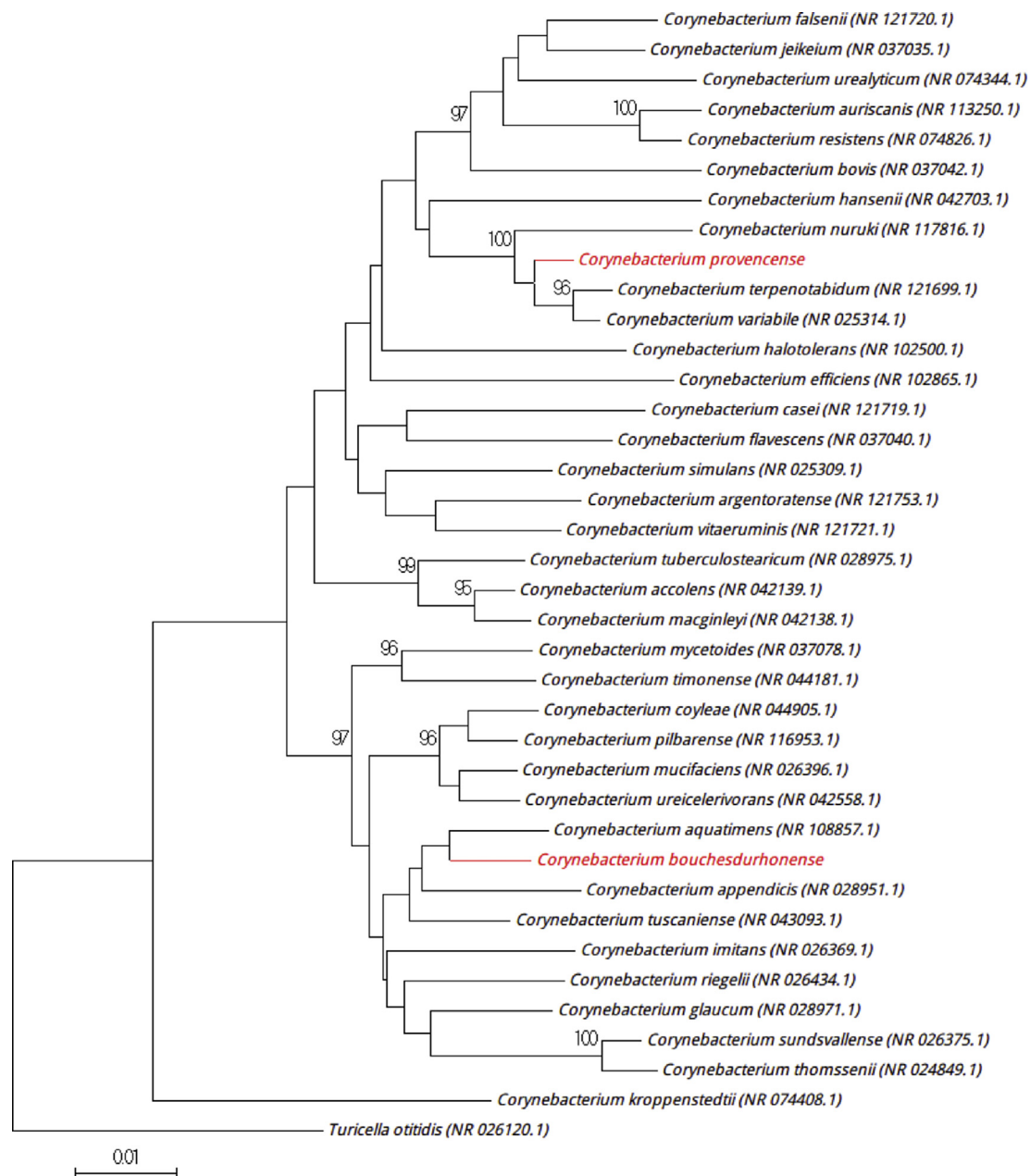


FIG. 2. Phylogenetic tree showing position of '*Corynebacterium bouchesdurhonense*' strain SN14^T and '*Corynebacterium provencense*' strain SN15^T relative to other phylogenetically close neighbours. Alignment and phylogenetic inferences were done as described in Fig. 1.

diameter and 1.8–2.0 μm in length. Catalase activity test was positive. Oxidase activity test was negative. The strain SN8 had a 16S rRNA gene sequence identity of 98.08% with *Xanthomonas campestris* strain ATCC 33913 (NR_074936), the phylogenetically closest species with standing in nomenclature [7] (Fig. 3), which putatively classifies strain SN8 as a member of a new species within the genus *Xanthomonas* in the phylum *Proteobacteria* [5]. Thus, we propose the creation of the new species '*Xanthomonas massiliensis*' (ma.ssi.li.en'sis, N.L. adj. neut., from Massilia, the antic

name of Marseille, France, where the strain was isolated). SN8^T is the type strain of the species '*Xanthomonas massiliensis*'.

MALDI-TOF MS spectrum

The MALDI-TOF MS spectra of these species are available online (<http://mediterranean-infection.com/article.php?leref=256&titre=urms-database>).

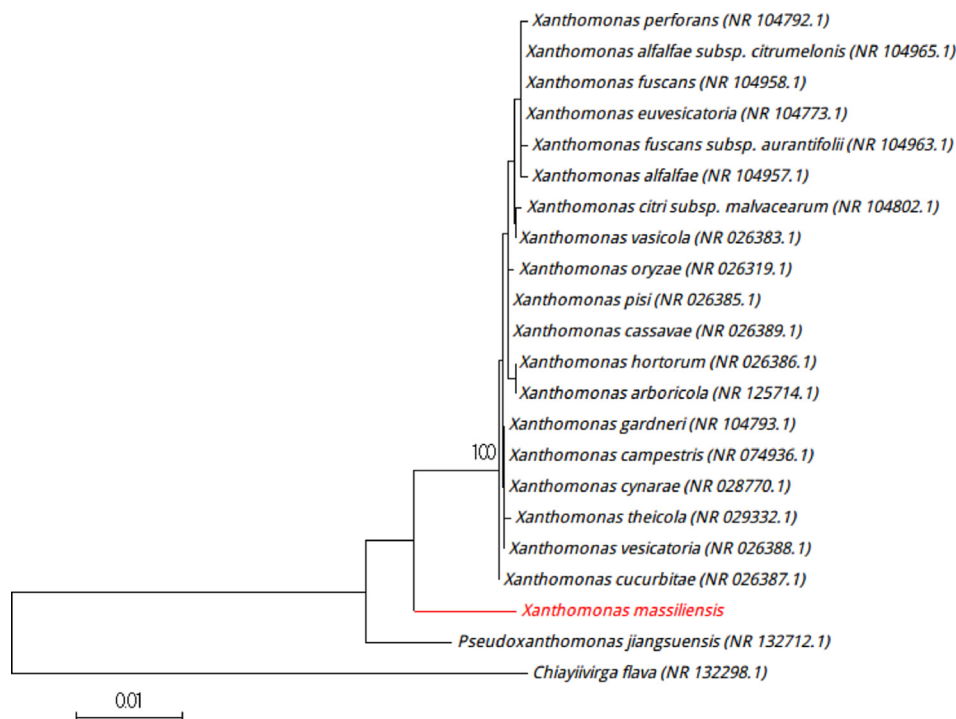


FIG. 3. Phylogenetic tree showing position of ‘*Xanthomonas massiliensis*’ strain SN8^T relative to other phylogenetically close neighbours. Alignment and phylogenetic inferences were done as described in Fig. 1.

Nucleotide sequence accession number

The 16S rRNA gene sequence was deposited in GenBank under the following accession numbers: ‘*Actinomyces provencensis*’ strain SN12^T (LN881591), ‘*Corynebacterium bouchesdurhonense*’ strain SN14^T (LN881599), ‘*Corynebacterium provencense*’ strain SN15^T (LN890283) and ‘*Xanthomonas massiliensis*’ strain SN8^T (LN881611).

Deposit in a culture collection

The strains were deposited in the Collection de Souches de l’Unité des Rickettsies (CSUR, WDCM 875) under numbers P2166 (‘*Actinomyces provencensis*’ strain SN12^T), P2067 (‘*Corynebacterium bouchesdurhonense*’ strain SN14^T), P2161 (‘*Corynebacterium provencense*’ strain SN15^T) and P2129 (‘*Xanthomonas massiliensis*’ strain SN8^T).

Acknowledgement

This study was funded by the Fondation Méditerranée Infection.

Conflict of Interest

None declared.

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