Gorillibacterium timonense sp. nov. and *Vitreoscilla massiliensis* sp. nov., two new bacterial species isolated from stool specimens of obese Amazonian patients

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Abstract

We report the main characteristics of *Gorillibacterium timonense* strain SN4^T sp. nov. and *Vitreoscilla massiliensis* strain SN6^T sp. nov., two new bacterial species isolated from stool specimens of obese Amazonian patients.

Keywords: Culturomics, *Gorillibacterium timonense*, gut microbiota, taxonomy, *Vitreoscilla massiliensis*

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Introduction

In 2015, using the culturomics approach [1], we isolated two bacterial strains that could not be identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Bremen, Germany) [1,2]. These bacteria were cultivated from stool specimens of obese patients from Amazonia, South America. Patients provided signed informed consent, and the study was approved by the ethics committee of the IFR48 under number 09-022.

Strain SN4 was isolated by culture on 5% sheep's blood–enriched Columbia agar (bioMérieux, Marcy l'Etoile, France) at 37°C under microaerophilic conditions. It is a facultative anaerobic bacterium with an optimal growth under aerobic atmosphere on 5% sheep's blood–enriched Columbia agar at 37°C. After 48 hours, colonies were beige and not haemolytic, with a mean diameter of 1 mm. Cells were Gram negative, rod shaped, motile and spore forming. They exhibited a mean diameter of 0.6 µm and a length ranging from 1.8 to 2.5 µm in electron microscopy. No activity was found for catalase or oxidase. Because identification by MALDI-TOF MS failed, the strain's 16S rRNA gene was sequenced using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France) using a previously described protocol [3]. Strain SN4 exhibited a 16S rRNA sequence similarity of 93.13% with *Paenibacillus turicensis* strain MOL722^T, the phylogenetically closest species with standing in nomenclature [4] (Fig. 1). This strain also showed an identity of 95.3% with strain G5^T (= KC_193239 = DSM 27179), the type strain of *Gorillibacterium massiliense* [5]. Therefore, we propose that strain SN4^T is the type strain from a second species within the genus *Gorillibacterium,* for which we propose the name *Gorillibacterium timonense* sp. nov. (ti.m-o.nen'se, N.L. adj. neut., from *timonense,* 'of Timone,' the main hospital of Marseille, France, where strain SN4 was isolated).

Strain SN6 was isolated by culture on 5% sheep's blood–enriched Columbia agar (bioMérieux) after 48 hours' incubation at 37°C in microaerophilic conditions. The colonies of strain SN6 were 0.5 to 1 mm in diameter, grey, smooth and nonhaemolytic. Bacterial cells were Gram-negative bacilli that were not motile and were non–spore forming. Under electron microscopy, bacterial cells measured 0.5 µm in diameter and 1.5 to 2.5 µm in length. No reaction was observed for catalase and oxidase activities. No identification was obtained by MALDI-TOF MS. The 16S rRNA gene sequence from strain SN6 exhibited a similarity of 97.5% with *Vitreoscilla stercoraria* strain Gottingen 1488-6, the phylogenetically closest species with standing in nomenclature (Fig. 2), which putatively classifies strain SN6 as a member of a new species within the genus *Vitreoscilla* in the phylum *Proteobacteria* [6]. Thus, we propose the creation of the new species *Vitreoscilla massiliensis* sp. nov. (ma.si.li.e'n.sis, N.L. gen. fem., *massiliensis,* 'of Massilia,' the Latin name of Marseille, where the strain was first isolated). Strain SN6^T is the type strain of the species *Vitreoscilla massiliensis*.

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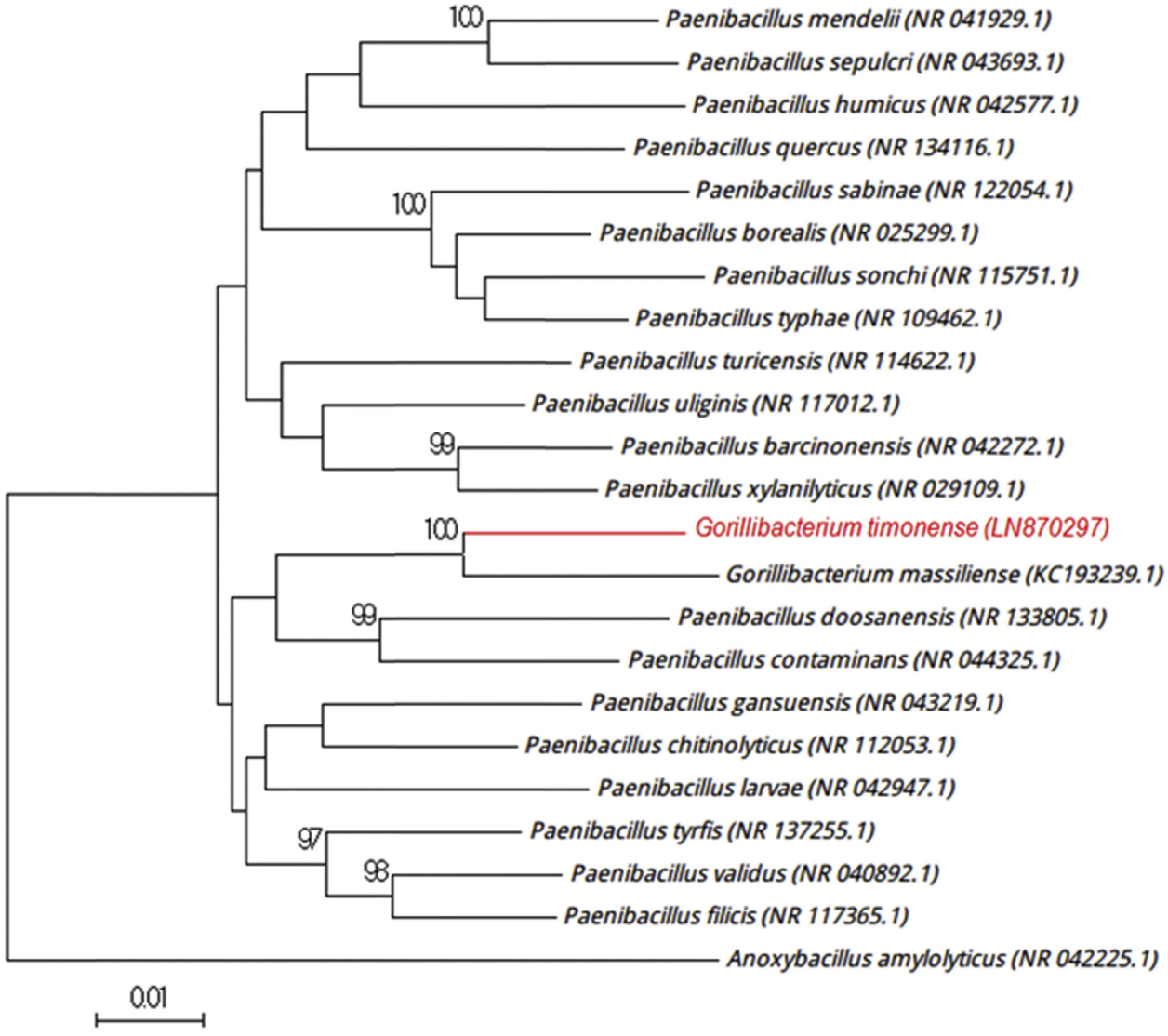

FIG. 1. Phylogenetic tree showing position of *Gorillibacterium timonense* strain SN4^{T} (red) relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using maximum-likelihood method with Kimura 2 parameter within MEGA software. GenBank accession numbers are indicated in parentheses. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 1000 times to generate majority consensus tree. Scale bar indicates 1% nucleotide sequence divergence.

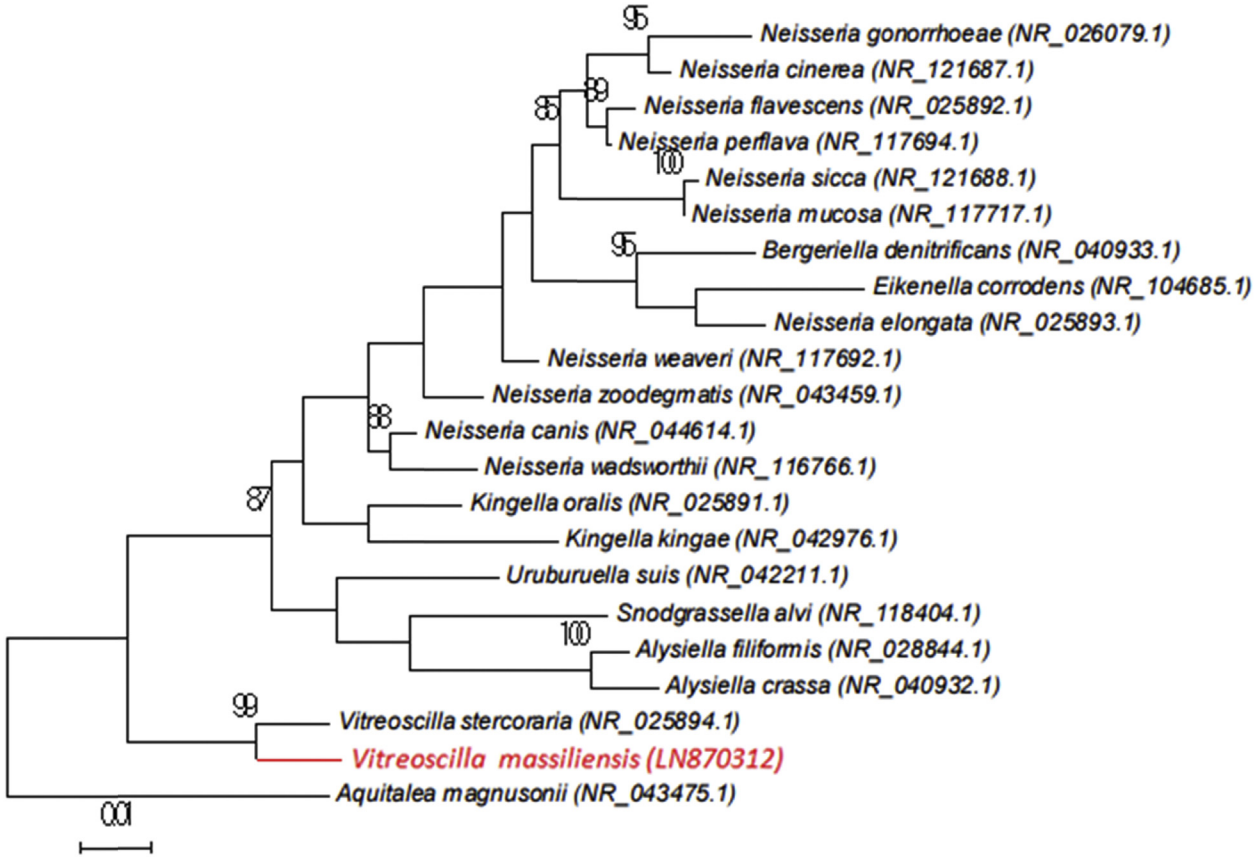

FIG. 2. Phylogenetic tree showing position of *Vitreoscilla massiliensis* SN6^T (red) relative to other phylogenetically close neighbours. Alignment and phylogenetic inferences were performed as described for *Gorillibacterium timonense* strain SN4^T. GenBank accession numbers are indicated in parentheses. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 1000 times to generate majority consensus tree. Scale bar indicates 1% nucleotide sequence divergence.

MALDI-TOF MS spectra

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

Nucleotide sequence accession number

The 16S RNA gene sequences from *Gorillibacterium timonense* strain SN4^T and *Vitreoscilla massiliensis* SN6^T were deposited in GenBank under accession numbers LN870297 and LN870312, respectively.

Deposit in a culture collection

Gorillibacterium timonense strain SN4^T and *Vitreoscilla massiliensis* strain SN6^T were deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under numbers P2011 and P2036, respectively.

Conflict of interest

None declared.

Acknowledgement

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