'Selenomonas massiliensis,' a new anaerobic bacterial species isolated from human oral microbiota

S. Belkacemi, N. Cassir, J. Delerce, F. Cadoret and B. La Scola

Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, CNRS (UMR 7278), IRD (198), INSERM (U1095), AMU (UM63), Institut Hospitalo-Universitaire Méditerranée-Infection, Faculté de médecine, Aix-Marseille Université, Marseille, France

Abstract

We report the main characteristics of *'Selenomonas massiliensis'* sp. nov., strain Marseille-P4036^T (= CSUR P4036). The culturomic combined with taxonogenomic methods were used to identify and characterize this new anaerobic bacterial species, which was isolated from an oral sample of a 25-year-old healthy woman.

Keywords: Culturomics, oral microbiota, *'Selenomonas massiliensis'*, taxonogenomics
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Corresponding author. N. Cassir, Pôle des Maladies Infectieuses, Aix-Marseille Université, IRD, Assistance Publique—Hôpitaux de Marseille (AP-HM), Microbes, Evolution, Phylogeny and Infection (MEΦI), Institut Hospitalo-Universitaire (IHU)—Méditerranée Infection, France, 19-21 Boulevard Jean Moulin, 13385 Marseille Cedex 05, France.
E-mail: cassirnadim@gmail.com

In March 2017, as part of the culturomics study to assess the microbial diversity of the human microbiota [1,2], a new bacterial species was isolated from an oral sample of a 25-year-old healthy woman living in Marseille, France. We isolated a bacterial strain 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]. The study was approved by the Institut Fédératif de Recherche 48 (agreement 09-022, Marseille, France), and the patient's consent was obtained.

Strain Marseille-P4036^T was first isolated on Columbia medium supplemented with 5% sheep's blood (bioMérieux, Marcy l'Etoile, France) at 37°C in anaerobic atmosphere (AnaeroGen Compact; Oxoid, Thermo Scientific, Dardilly, France) after a 10-day preincubation in an anaerobic bottle of blood culture (Becton Dickinson, Le Pont-de-Claix, France) containing 5% sheep's blood supplemented with 5% rumen fluid previously filter sterilized through a 0.2 µm pore filter (Thermo Fisher Scientific, Villebon-sur-Yvette, France). Colonies were colorless with a diameter ranging from 0.5 to 2 mm. Bacterial cells were Gram-negative, rod-shaped bacilli 0.5 µm wide by 2 to 4 µm long and motile by means of long flagella. The strain Marseille-P4036^T grew after 48 hours of incubation under anaerobic conditions at a temperature ranging from 28°C to 37°C, but optimally at 37°C. Growth was impossible in microaerophilic (campyGEN; Oxoid) conditions. The bacterial cells tolerated a pH of 6.5 to 7 and an NaCl concentration less than 50 mg/L. After 20 minutes of thermal shock at 80°C, this bacterium did not grow at 37°C on Columbia agar enriched with 5% sheep's blood, confirming the spore search result by microscopy, which was negative. Biochemical analysis of strain Marseille-P4036^T was carried out using API 50CH, API 20NE and API ZYM strips according to the manufacturer's instructions (bioMérieux). The production of catalase, urease and oxidase for strain Marseille-P4036^T was negative. However, the production of phosphatase alkaline, esterase (C4), α-chymotrypsin, phosphatase acid, naphthol phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase and α-fucosidase was positive. We found no utilization of glucose, arabinose, mannose, maltose and raffinose, while the reaction with lactose was positive.

After three failed identifications by systematic MALDI-TOF MS screening on a Bruker Microflex spectrometer [3], the

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16S rRNA gene was sequenced using universal primers FD1 and RP2 (Eurogentec, Angers, France) as previously described [4] and a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France). Strain Marseille-P4036^T exhibited a 98.1% sequence identity with the type strains *Centipeda periodontii*/*Selenomonas flueggei,* the phylogenetically closest species with standing in nomenclature (Fig. 1). Therefore, strain Marseille-P4036^T was classified as a member of the genus *Selenomonas* in the order of *Clostridiales* within the *Firmicutes* phylum. Because the sequence identity with the phylogenetically closest validated species was <98.7%, which is the threshold recommended to define a species according to the nomenclature [5,6], we propose that it may be a representative strain of a new species within the genus *Selenomonas*. We thus suggest the creation of the new species named *'Selenomonas massiliensis'* sp. nov., strain Marseille-P4036^T (mas.si.li.en'sis, L. adj. fem., *massiliensis* from 'Massilia,' the Latin name for Marseille, France, where the strain was isolated).

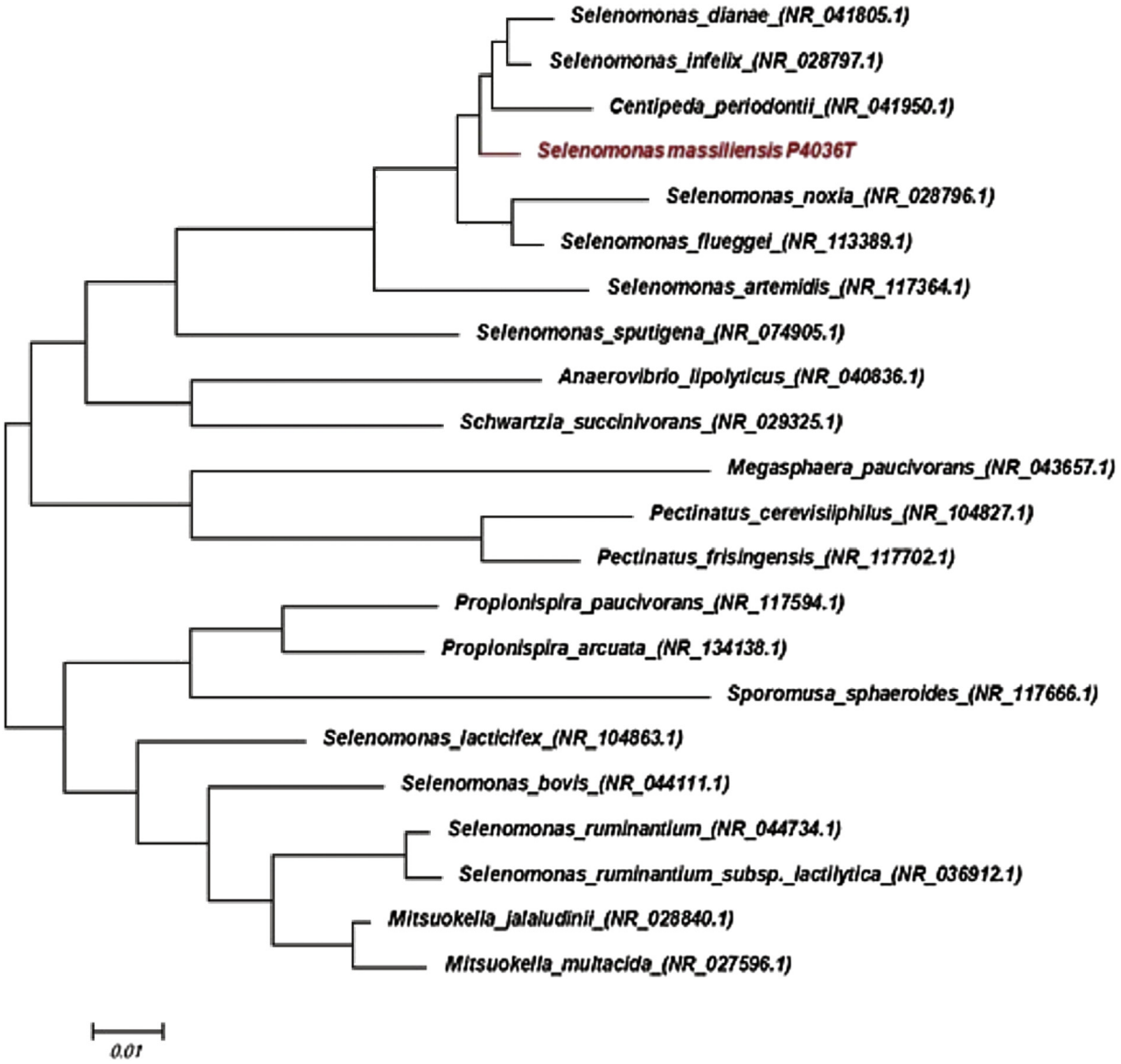

FIG. 1. Phylogenetic tree showing position of *'Selenomonas massiliensis'* strain Marseille-P4036^T (red) relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTAL W, and phylogenetic inferences were obtained using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 1000 times to generate majority consensus tree. Only bootstraps scores of at least 95% were retained. Scale bar indicates a 2% nucleotide sequence divergence.

MALDI-TOF MS spectrum

The MALDI-TOF MS spectrum of *'Selenomonas massiliensis'* Marseille-P4036 ^{T} is available online (http://www.mediterranee-infection.com/article.php?laref=937&titre=r-s-t).

Nucleotide sequence accession number

The 16S rRNA gene sequence was deposited in GenBank under accession number LT970915 (https://www.ncbi.nlm.nih.gov/nuccore/LT970915.1).

Deposit in a culture collection

Strain Marseille-P4036^{T} was deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR) under number P4036^{T}.

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Conflict of interest

None declared.

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