Genome sequence and description of *Mobilicoccus massiliensis* sp. nov. isolated from the stool of a Nigerian boy with kwashiorkor

N. Mathlouthi, S. I. Traore, T. Cimmino, S. Khelaifia, T. T. Nguyen, F. Cadoret, C. Couderc, D. Raoult and J.-M. Rolain

Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes (URMITE), UM63 CNRS 7278 IRD 198 INSERM U1905, IHU Méditerranée Infection, Facultés de Médecine et de Pharmacie, Marseille, France

Abstract

Mobilicoccus massiliensis strain SIT2 (= CSUR P1306 = DSM 29065) is a new type strain of *Mobilicoccus* sp. nov. isolated from the stool of a 2-year-old Nigerian boy with kwashiorkor. *M. massiliensis* is Gram positive, facultatively anaerobic, nonsporulating and motile. The 3 842 438 bp long genome contains 3362 protein-coding and 49 RNA genes, including one 5S rRNA gene, one 16S rRNA gene, one 23S rRNA gene and 46 tRNA genes.

Keywords: Culturomics, genome, kwashiorkor, *Mobilicoccus massiliensis*, taxonogenomics

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Corresponding author: J.-M. Rolain, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes (URMITE), UM63 CNRS 7278 IRD 198 INSERM U1905, IHU Méditerranée Infection, Facultés de Médecine et de Pharmacie, Marseille, France.

E-mail: jean-marc.rolain@univ-amu.fr

Mobilicoccus massiliensis strain SIT2 (= CSUR P1306 = DSM 29065) is the type strain of *Mobilicoccus* sp. nov. This bacterium was isolated from the stool of a 2-year-old Nigerian boy with a severe form of acute malnutrition known as kwashiorkor and is part of the culturomics effort, which seeks to cultivate all bacterial species from the human gut [1,2]. It is Gram positive, aerobic or facultatively anaerobic, motile and nonsporulating. The family *Dermatophilaceae* was first proposed by Austwick (1958) and was later emended by Stackebrandt et al. (1997), Stackebrandt and Schumann (2000) and Zhi et al. (2009). This family currently contains two genera: *Dermatophilus* and *Kineosphaera*. The genus *Dermatophilus* was proposed by Gordon (1954) as organisms that form branching mycelia with several transverse and longitudinal divisions, which leads to the formation of packets or clusters of cuboid cells or coccoids. Species of the genus *Dermatophilus* are bacteria isolated from the causative organism of a skin disease [3] and was reported to affect a wide variety of mammalian species. The ruling taxonomic classification of prokaryotes is based on a combination of phenotypic and genotypic criteria [4,5]. However, the three essential criteria that are used, comprising 16S rRNA gene-based phylogeny [4], G+C content and DNA-DNA hybridization [5] have several drawbacks. We recently proposed a new method, taxonogenomics, which uses genomic data in a polyphasic approach to describe new bacterial species [6]. This strategy combines phenotypic characteristics including matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and genomic analyses [7–9].

Here we report for the first time the isolation and characterization of a novel species, *Mobilicoccus massiliensis* sp. nov., with a description of phylogenetic characteristics as well as complete genomic sequencing and annotation to distinguish this species from other species.

The study was approved by the local ethics committee of the Institut Fédératif de Recherche IFR48, Faculty of Medicine, Marseille, France, under agreement 09-022. Strain SIT2 was isolated in March 2014 by cultivation on chocolate agar Poly-ViteX (bioMérieux, Marcy l'Etoile, France) in anaerobic and aerobic condition using GasPak EZ Anaerobe Container System Sachets (Becton Dickinson (BD), San Diego, CA, USA) at 37°C. This strain exhibited a 98% 16S rRNA gene similarity with

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Mobilicoccus pelagius (NZ-BAFF00000000.1), a phylogenetically valid neighbouring *Dermatophilus* species type strain (Fig. 1).

Optimal growth occurred at 37°C after 24 hours of inoculation. Growth was observed under aerobic and anaerobic conditions after 24 hours. Colonies were 0.2–0.5 mm in diameter in gross appearance on blood-enriched Columbia agar. Cells are coccus shaped, Gram positive and non-sporulating (Fig. 2), and the motility test was positive. SIT2 showed catalase activity but was negative for oxidase.

Commercially available API ZYM and API 50CH strips (bioMérieux) were used to characterize the biochemical properties of the strain according to the manufacturer's instructions. Using an API 50CH strip, *Mobilicoccus massiliensis* SIT2 presented positive reactions for glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl-αD-mannopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-αD-glucopyranoside, *N*-acetylglucosamine, amygdaline, arbutin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, D-melezitose, D-raffinose, amidone, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol and potassium gluconate. Negative reactions were observed for L-sorbose, methyl-αD-mannopyranoside, esculin, inulin, L-arabitol, potassium 2-ketogluconate and potassium 5-ketogluconate. For API ZYM, *Mobilicoccus massiliensis* SIT2 presented positive reaction only for α-galactosidase (Table 1).

Antibiotic susceptibility of our isolates was assessed using the disk diffusion method on Mueller-Hinton agar plates supplemented with 5% blood (BD). The tested antibiotics were ceftriaxone, imipenem, vancomycin, rifampicin, gentamicin, ciprofloxacin, amoxicillin, doxycycline, ciprofloxacin, gentamicin, rifampicin, colistin, meropenem, trimethoprim/sulfonamide, amoxicillin/clavulanic acid, fosfomycin and metronidazole (Sirscan Oxoid, Montpellier, France) (Table 2).

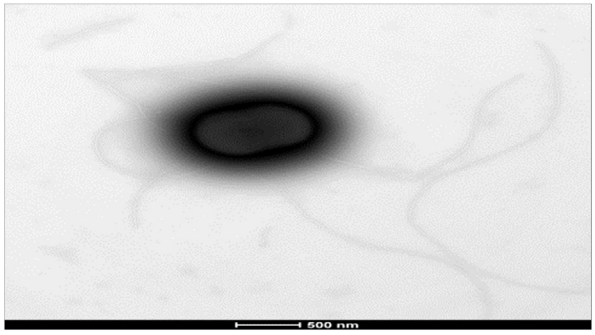

FIG. 2. Transmission electron microscopy of *Mobilicoccus massiliensis* strain SIT2 using Morgani 268D device.

MALDI-TOF MS protein analysis was carried out as previously described [2] using a Microflex spectrometer (Bruker Daltonics, Leipzig, Germany). The resulting score enabled the identification (or not) of the tested species: a score of ≥2 with a validly published species enabled identification at the species level, a score of ≥1.7 but <2 enabled identification at the genus level and a score of <1.7 did not enable any identification. No significant MALDI-TOF MS score was obtained for strain SIT2 against the Bruker database, suggesting that our isolate was not a member of a known species. Consequently, we added the spectrum from strain SIT2 to our database, and the organism was selected for sequencing on the basis of its phylogenetic position and 16S rRNA similarity to members of the genus *Dermatophilus* [2].

The phylogenetic subtree highlighted the phylogenetic position of this bacteria relative to other species. Sequences were recovered by a nucleotide BLAST (Basic Local Alignment

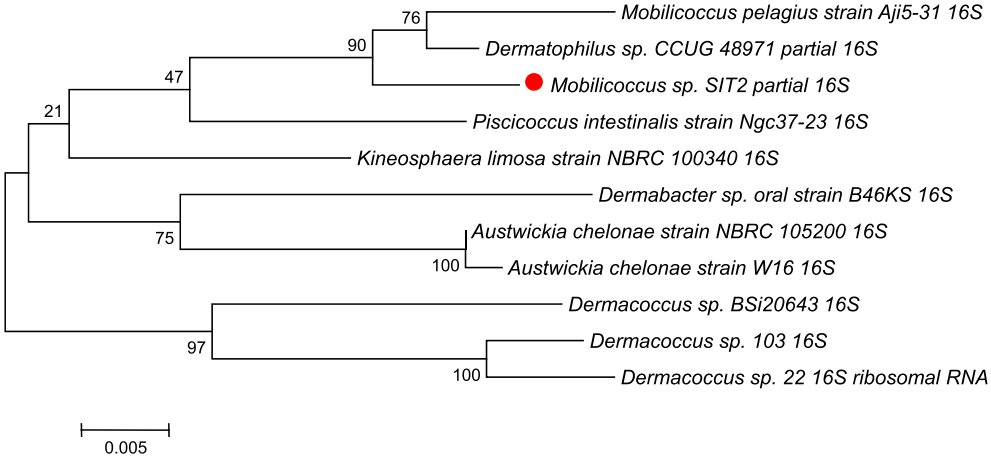

FIG. 1. Phylogenetic tree highlighting position of *Mobilicoccus massiliensis* sp. nov. strain SIT2 (= CSUR P1162 = DSM 29078) relative to other type strains within *Dermatophilus* genus.