TAXONOGENOMICS: GENOME OF A NEW ORGANISM

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

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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.

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

**Original Submission:** 16 August 2016; **Revised Submission:** 30 August 2017; **Accepted:** 31 August 2017

**Article published online:** 6 September 2017

*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 PolyViteX (bioMérieux, Marcy l’Étoile, 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...
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-xylene, D-xylene, D-adenitol, methyl-α-D-mannopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-glucopyranoside, N-acetylglucosamine, amygdaline, arbutin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-xylose, D-xylose, D-xylose, D-xylose, D-adonitol, methyl-α-D-mannopyranoside, esculin, inulin, D-xylose, 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).

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 Search):

**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.

**FIG. 2.** Transmission electron microscopy of Mobilicoccus massiliensis strain SIT2 using Morgani 268D device.
Search Tool) against the National Center for Biotechnology Information (NCBI) 16S rRNA Targeted Loci Project database. The bacterium was identified by sequence analysis of the 16S rRNA. Its phylogenetic relationships with closely related species were determined by MEGA v6. The evolutionary history was inferred by using the maximum likelihood method based on the JTT matrix-based model. Strain SIT2 exhibited a 98% 16S rRNA sequence identity with Mobilicoccus pelagius (NZ-BAFF00000000.1), the phylogenetically closest bacterial species with standing in nomenclature (Fig. 1).

Genomic DNA of Mobilicoccus massiliensis was sequenced via MiSeq Technology (Illumina, San Diego, CA, USA) with the two applications: paired end and mate pair. The reads of both applications were trimmed, and the optimal assembly was obtained through SPAdes (St Petersburg genome assembler) software with 245 contigs of coverage in eight scaffolds, which generated a genome size of 3.28 Mb. The GC% was estimated at 29% (Tables 3 and 4).

The genome was annotated by the Rapid Annotation using Subsystem Technology (RAST) bioserver [10]. The resistome

**TABLE 1.** Differential phenotypic characteristics between *Mobilicoccus massiliensis* sp. nov. strain SIT2 and phylogenetically close members of other Dermatophilaceae species.

| Property                  | *Mobilicoccus massiliensis* | *Mobilicoccus pelagius* | *Piscicoccus intestinals* |
|---------------------------|-----------------------------|-------------------------|---------------------------|
| Cell diameter             | 0.2–0.5 mm                  | 0.7–1.2 μm              | 0.7–1 μm                  |
| Oxygen requirement        | Anaerobic                   | Anaerobic               | Anaerobic                 |
| Gram stain                | +                           | +                       | +                         |
| MotilDx                   | +                           | +                       | +                         |
| G + C content (%)         | 70.5                        | 71.6                    | 71.5                      |
| Production of:            |                             |                         |                           |
| Alkaline phosphatase      | –                           | +                       | +                         |
| Acid phosphatase          | –                           | –                       | –                         |
| Catalase                  | +                           | +                       | +                         |
| Oxidase                   | –                           | –                       | –                         |
| α-Glucosidase             | –                           | +                       | +                         |
| β-Glucosidase             | –                           | –                       | +                         |
| α-Galactosidase           | +                           | +                       | +                         |
| β-Galactosidase           | –                           | –                       | +                         |
| Leucine arylamidase       | –                           | +                       | +                         |
| Pyrazinamide              | –                           | –                       | –                         |
| Utilisation of:           |                             |                         |                           |
| Glycerol                  | +                           | –                       | –                         |
| Erythritol                | +                           | –                       | –                         |
| D-Arabinose               | +                           | –                       | –                         |
| L-Arabinose               | +                           | –                       | –                         |
| D-Ribose                  | +                           | –                       | –                         |
| D-Xylose                  | +                           | –                       | –                         |
| L-Xylose                  | +                           | +                       | +                         |
| D-Adonitol                | –                           | +                       | +                         |
| Methyl-α-D-mannopyranoside| +                           | –                       | –                         |
| D-Galactose               | –                           | –                       | +                         |
| D-Glucose                 | –                           | –                       | +                         |
| D-Fructose                | –                           | –                       | +                         |
| D-Mannose                 | –                           | –                       | +                         |
| L-Rhamnose                | +                           | –                       | –                         |
| Dulcitol                  | +                           | –                       | –                         |
| Inositol                  | –                           | –                       | –                         |
| D-Mannitol                | +                           | –                       | –                         |
| D-Sorbitol                | +                           | –                       | –                         |
| Methyl-α-D-glucopyranoside| +                           | –                       | –                         |
| N-Acetylglucosamine       | –                           | –                       | –                         |
| Amygdalin                 | +                           | –                       | –                         |
| Arbutin                   | +                           | –                       | –                         |
| Salicin                   | +                           | –                       | –                         |
| D-Cellulbiose             | +                           | –                       | –                         |
| D-Maltose                 | +                           | –                       | –                         |
| D-Lactose                 | +                           | –                       | –                         |
| D-Melibiose               | +                           | –                       | –                         |
| D-Saccharose              | +                           | –                       | –                         |
| D-Trehalose               | +                           | –                       | +                         |
| D-Melezitose              | +                           | –                       | –                         |
| D-Raffinose               | +                           | –                       | –                         |
| Amidon                    | –                           | –                       | –                         |
| Glycogen                  | –                           | –                       | –                         |
| Xyitol                    | –                           | –                       | –                         |
| Gentibiose                | –                           | –                       | –                         |
| D-Turanose                | –                           | –                       | –                         |
| D-Lyxose                  | –                           | –                       | –                         |
| D-Targtose                | –                           | –                       | –                         |
| D-Fucose                  | –                           | –                       | –                         |
| L-Fucose                  | –                           | –                       | –                         |
| D-Arabinol                | –                           | –                       | –                         |
| Potassium glutonate       | +                           | –                       | –                         |
| Habitat                   | Stool of human boy          | Intestinal tract of fish | Intestinal tract of fish  |

*+, positive result; -, negative result.*
was analysed with the Antibiotic Resistance Gene-ANNOTation (ARG-ANNOT) database and BLASTp in GenBank [11]. The functional annotation of protein sequences was performed using BLASTp against the GenBank and Clusters of Orthologous Groups (COGs) databases [11]. The exhaustive bacteriocin database available in our laboratories (Bacteriocins of the Unité des Maladies Infectieuses et Tropicales Emergentes (URMITE) database; http://drissifatima.wix.com/bacteriocins) was performed by collecting all currently available sequences from the databases and from NCBI. Protein sequences from this database allowed putative bacteriocins from human gut microbiota to be identified using BLASTp methodology [11]. The genome of *Mobilicoccus massiliensis* SIT2 has been deposited in GenBank with accession number CDGT01000000 and 16S rRNA accession number LK985391. The genome is 3,842,438 bp long with 70.47% G+C content. It is composed of 21 scaffolds (composed of 24 contigs). Of the 3,681 predicted genes, 3,362 were protein-coding genes and 49 were RNAs (one 5S rRNA gene, one 16S rRNA gene, one 23S rRNA gene and 46 tRNA genes). A total of 2,437 genes were assigned as putative function (by COGs or by NR BLAST). The remaining genes were annotated as hypothetical proteins (683 genes, 20.32%) (Fig. 3).

The draft genome sequence of *Mobilicoccus massiliensis* is larger than those of *Mobilicoccus pelagius* Aji5-31, *Dermatophilus congolensis* DSM 44180, *Dermacoccus nishinomiyaensis*, *Arsenicicoccus* spp. and *Austwickia chelonea* NBRC 105200 (3.54, 2.62, 3.03, 3.53 and 3.54 MB respectively) but smaller than those of *Kineosphaera limosa* NBC 100340 (4.5 MB) (Table 5).

The G+C content of *Mobilicoccus massiliensis* is smaller than those of *Mobilicoccus pelagius* Aji5-31 and *Arsenicicoccus* spp. (71.9 and 72.7% respectively), but larger than those of *Dermatophilus congolensis* DSM 44180, *Kineosphaera limosa* NBC 100340, *Dermacoccus nishinomiyaensis* and *Austwickia chelonea* NBRC 105200 (59.4, 70.4, 69.1 and 66.1% respectively). The gene content of *Mobilicoccus massiliensis* is larger than those of *Mobilicoccus pelagius* Aji5-31, *Dermatophilus congolensis* DSM 44180, *Kineosphaera limosa* NBC 100340, *Dermacoccus nishinomiyaensis* and *Austwickia chelonea* NBRC 105200 (3090, 2340, 4375, 2745, 3271 and 3046 respectively) (Table 5).

The comparison of amino acid sequence homology of the predicted genes, as shown in Fig. 4, by bidirectional BLAST takes hits from the RAST annotation [10] is a useful way to evaluate the protein similarity using BLAST between NBRC 104925 and the fully sequenced SIT2. Fig. 5 provides the distribution of functional classes of predicted genes of *M. massiliensis* and *M. pelagius*.

Antimicrobial susceptibility testing demonstrate that the strain *M. massiliensis* SIT2 was susceptible to ceftriaxone,
imipenem, vancomycin, rifampicin, gentamicin, ciprofloxacin, amoxicillin, doxycycline, ciprofloxacin, gentamicin, rifampicin and colistin but resistant to trimethoprim/sulfamethoxazole, fosfomycin and metronidazole. 

In silico analysis of resistome revealed the presence of resistance genes (Table 2).

The analysis of the genome did not demonstrate the presence of bacteriocin and nonreducing polyketide synthases. SIT2 is equipped with an intact flagellar system of 41 Coding DNA Sequence (CDS) encoding six cytoplasmic signal transduction proteins, the products of the che genes (cheA, cheB, cheR, cheW, cheY and cheZ), transmembrane proteins with receptor functions termed methyl-accepting chemotaxis proteins or MCPs, flagellar assembly proteins (FlIP, FlIQ, FlIR, flhA, flhB), chemotaxis protein (motA, motB), flagellar motor switch protein (FlIG, FlIM, FlIN, FlIY), rod, hook and filament (FlgC, FlgG, FlgK, FlgL, flID, flIC) and regulation (RNA polymerase sigma factor for flagellar operon FlIA).

On the basis of phenotypic, phylogenetic and genomic analyses (taxonogenomics), we propose that strain SIT2 represents a novel species of the genus Mobilicoccus for which the name

| TABLE 5. Genome features of Mobilicoccus SIT2 genome compared to other Dermatophilaceae species. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Strain                          | Accession No.   | Size (Mb)       | GC%             | Gene            | Protein         |
| Mobilicoccus SIT2               | NZ_CDGT00000000.1 | 3.84           | 70.5            | 3377            | 3182            |
| Mobilicoccus pelagius           | NZ_BAFE00000000.1 | 3.54           | 71.9            | 3090            | 2895            |
| Dermatophilus congolensis       | NZ_AUCS00000000.1 | 2.62           | 59.4            | 2340            | 2204            |
| Kineosphaera limosa             | NZ_BAHG00000000.1 | 4.80           | 70.4            | 4375            | 4033            |
| Mobilicoccus massiliensis       | NZ_CP008889.1    | 3.03           | 69.1            | 2745            | 2619            |
| Arsenicoccus spp.               | NZ_CP0012070.1   | 3.53           | 72.7            | 3271            | 3052            |
| Austwickia chelonae             | NZ_BAGZ00000000.1 | 3.54           | 66.1            | 3046            | 2903            |

FIG. 3. Graphical circular map of genome.

FIG. 4. Proteomic comparison and in silico DNA-DNA hybridization between Mobilicoccus massiliensis SIT2 and Mobilicoccus pelagius NBRC 104925.
Mobilicoccus massiliensis is proposed. The genome sequences are deposited in GenBank under accession numbers CDGT01000000 and 16S LK985391 respectively.

Conflict of interest
None declared.

Acknowledgements
We are grateful to L. Hadjadj (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, Aix- Marseille Université, Marseille, France) for technical assistance. Supported in part by the Centre National de la Recherche Scientifique (CNRS) and Infectiopôle Sud Fondation.

References
[1] Dubourg G, Lagier JC, Robert C, Armougom F, Hugon P, Metidji S, et al. Culturomics and pyrosequencing evidence of the reduction in gut microbiota diversity in patients with broad-spectrum antibiotics. Int J Antimicrob Agents 2014;44:117–24.
[2] Lagier JC, Armougom F, Million M, Hugon P, Pagnier I, Robert C, et al. Microbial culturomics: paradigm shift in the human gut microbiome study. Clin Microbiol Infect 2012;18:1185–93.
[3] Hamada M, Iino T, Iwami T, Harayama S, Tamura T, Suzuki K. Mobilicoccus pelagius gen. nov., sp. nov. and Piscicoccus intestinalis gen. nov., sp. nov., two new members of the family Dermatophilaceae, and reclassification of Dermatophilus chelonae (Masters et al. 1995) as Austwickia chelonae gen. nov., comb. nov. J Gen Appl Microbiol 2010;56:427–36.
[4] Stackebrandt E, Frederiksen W, Garrity GM, Grimont PA, Kämpfer P. Notes on the characterization of prokaryote strains for taxonomic purposes. Int J Syst Evol Microbiol 2002;52:1043–7.
[5] Tindall BJ, Rosselló-Móra R, Busse HJ, Ludwig W, Kämpfer P, Maiden MC, et al. Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. Int J Syst Evol Microbiol 2010;60:249–66.
[6] Ramasamy D, Mishra AK, Lagier JC, Padmanabhan R, Rossi M, Sentausa E, et al. A polyphasic strategy incorporating genomic data for...
the taxonomic description of novel bacterial species. Int J Syst Evol Microbiol 2014;64:384–91.

[7] Kokcha S, Mishra AK, Lagier JC, Million M, Leroy Q, Raoult D. Non contiguous – finished genome sequence and description of Bacillus timonensis sp. nov. Stand Genom Sci 2012;6:346–55.

[8] Lagier JC, El Karkouri K, Nguyen TT, Armougom F, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Anaerococcus senegalensis sp. nov. Stand Genom Sci 2012;6:116–25.

[9] Mishra AK, Lagier JC, Robert C, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Clostridium senegalense sp. nov. Stand Genom Sci 2012;6:386–95.

[10] Aziz RK, Bartels D, Best AA, DeJongh M, Díaz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genom 2008;9:75.

[11] Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, et al. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob Agents Chemother 2014;58:212–20.