**Tessaracoccus massiliensis** sp. nov., a new bacterial species isolated from the human gut

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**Abstract**

A new Actinobacterium, designated *Tessaracoccus massiliensis* type strain SIT-7T (= CSUR P1301 = DSM 29060), have been isolated from a Nigerian child with kwashiorkor. It is a facultative aerobic, Gram positive, rod shaped, non spore-forming, and non motile bacterium. Here, we describe the genomic and phenotypic characteristics of this isolate. Its 3,212,234 bp long genome (1 chromosome, no plasmid) exhibits a G+C content of 67.81% and contains 3,058 protein-coding genes and 49 RNA genes.

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**Introduction**

Kwashiorkor is a nutritional disorder most often seen in low-income and middle-income countries [1]. It is a virulent form of severe acute malnutrition caused by a lack of protein intake. People suffering from kwashiorkor typically have an extremely emaciated appearance in all body parts except their ankles, feet and belly, which swell with fluid. Clinical manifestations include, generalized oedema, anorexia and skin ulcerations [2]. The gastrointestinal microbiota also appears to be involved. To elucidate this hypothesis we have studied by ‘culturomics’ the human digestive microbiota of children suffering from kwashiorkor [3,4]. In this work we isolated a wide range of bacteria, including several new species including our isolate, the strain SIT-7T.

Each newly isolated bacterium in this work is described by using a new and innovative method that we have implemented. Indeed, due to the availability of genomic data through the development of new tools for the sequencing of DNA [5], we introduced a new way of describing the novel bacterial species [6]. This includes, among other features, their genomic [7–11] and proteomic information obtained by matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF-MS) analysis [12]. This concept differs from the usual methods to define a new species, which are based on parameters such as sequencing of the 16S rRNA, phylogeny, G+C content, and DNA-DNA hybridization. These methods are time consuming and involve numerous limitations [13,14].

Here, we present a brief classification and a set of features for strain SIT-7T (= CSUR P1301 = DSM 29060) and we also describe the sequence of the complete genome and its annotation. We named this new isolate *Tessaracoccus massiliensis*.

The genus *Tessaracoccus*, belonging to the family *Propionibacteriaceae*, was first described by Maszenan et al. in 1999 [15] and contains five species with validly published names at the time of writing: *Tessaracoccus bendigonis*, *Tessaracoccus flavescens*, *Tessaracoccus lubricantis*, *Tessaracoccus oleagi* and *Tessaracoccus lapidicaptus* [16–19]. All five species are Gram-positive and non-motile bacteria able to live in various different environments [18]. *Tessaracoccus massiliensis* is the first representative of the genus *Tessaracoccus* to be isolated in humans through ‘culturomics’.
Materials and Methods

Sample informations
The culture sample comes from Niamey (Niger). It was collected from a 2-year-old child with kwashiorkor, a form of acute malnutrition.

Consent was obtained from the child’s parents at the National Hospital of Niamey and the study was approved by the Institut Fédératif de Recherche 48 (Faculty of Medicine, Marseille, France), under agreement number 09-022. The patient did not receive antibiotics at the time of sample collection and the stool sample was collected in sterile plastic containers, divided into aliquots and stored at ~80°C. The pH of the aliquots was also tested by pH meter (ThermoFisher scientific, Saint Aubin, France).

Strain identification and phylogenetic classification
The isolate was cultured in blood culture bottles supplemented with sheep blood, (BioMérieux, Marcy l’Etoile, France) during a 7-day pre-incubation period at 37°C in aerobic atmosphere.

The identification of the strain was carried out by MALDI-TOF mass spectrometric analysis with a Microflex (Bruker Daltonics, Leipzig, Germany), as previously described [19]. Obtained spectra were then compared with the Bruker database and our database, which is continuously updated. If no identification is possible at the genus or species level, sequencing of the 16S rRNA gene is used to achieve a correct identification [20]. DNA extraction was performed using EZ1 DNA Tissue Kit (Qiagen, Courtaboeuf, France). The DNA extract was amplified using PCR technology and universal primers FD1 and RP2 [21] (Eurogentec, Angers, France). The amplifications and sequencing of the amplified products were performed as previously described [22]. Then, 16S rRNA gene sequences were compared with those which are available in GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Identification at the species level was defined by a 16S rDNA gene sequence similarity ≥99% with the sequence of the prototype strain in GenBank. When the percentage of identity is <98.7%, the studied strain is considered as a new species [11].

Phylogenetic analysis based on 16S rRNA of our isolate was performed to identify its phylogenetic affinities with other near isolates, including other members of the genus Tesserococcus. The MEGA 6 (Molecular Evolutionary Genetics Analysis) software allowed us to construct a phylogenetic tree [23]. Sequence alignment of the different species was performed using CLUSTAL W [24,25] and the evolutionary distance was calculated with the Kimura two-parameter model [26].

Physiological and phenotypic characteristics
The phenotypic characteristics of this strain were studied by testing different temperatures and growth atmospheres. With regards to the temperature, we studied the growth at 25, 30, 37, 45 and 56°C. The test was conducted under aerobic conditions in the presence of 5% CO2 and also in anaerobic and microaerophilic atmospheres, created by using AnaeroGen™ (ThermoFisher Scientific, Saint Aubin, France) and Campy-Gen™ (ThermoFisher scientific) respectively. Gram staining and motility were observed by using a light microscope DM1000 (Leica Microsystems, Nanterre, France). Cell morphology was examined after negative staining of bacteria, with a G20 Tecnai transmission electron microscope (FEI Company, Limeil-Brévannes, France). Formation of spores was determined after thermal shock and observed under a microscope.

Biochemical characteristics of our strain were studied using the API 20NE, ZYM and 50 CH strips (BioMérieux) according to manufacturer’s instructions. Antibiotic susceptibility was determined on Mueller–Hinton agar (BioMérieux) using a disc diffusion method (i2A, Montpellier, France). The following antibiotics were tested: doxycycline (30 μg), rifampicin (30 μg), vancomycin (30 μg), erythromycin (15 μg), ampicillin (10 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), gentamicin (500 μg), penicillin (10 μg), trimethoprim/sulfamethoxazole (1, 25 + 23.75 μg), imipenem (10 μg), and metronidazole (4 μg), clindamycin (15 μg), colistin (50 μg) and oxacillin (5 μg).

Genomic sequencing
Genomic DNA (gDNA) of T. massiliensis was sequenced using MiSeq Technology (Illumina Inc, San Diego, CA, USA) with the mate pair strategy. The gDNA was barcoded to be mixed with 11 other projects with the Nextera Mate Pair sample prep kit (Illumina). The mate pair library was prepared with 1 μg of gDNA using the Nextera mate pair Illumina guide. The gDNA sample was simultaneously fragmented and tagged with a mate pair junction adapter. The pattern of fragmentation was validated on an Agilent 2100 BioAnalyzer (Agilent Technologies Inc., Santa Clara, CA, USA) with a DNA 7500 labchip. The DNA fragments ranged in size from 1 kb up to 11 kb with an optimal size of 4.008 kb. No size selection was performed and 388.3 ng of tagged fragments were circularized. The circularized DNA was mechanically sheared to small fragments, with an optimal size of 634 bp, on the Covaris device S2 in microtubes (Covaris, Woburn, MA, USA). The library profile was visualized on a High Sensitivity Bioanalyzer LabChip (Agilent Technologies Inc.) and the final concentration library was measured at 35.59 nmol/L. The libraries were normalized at 2 nM and pooled. After a denaturation step and dilution at 1.5 pM, the pool of libraries was loaded onto the reagent cartridge and then onto the instrument.
along with the flow cell. Automated cluster generation and sequencing run were performed in a single 39-h run in a 2 × 251-bp. Total information of 10.6 Gb was obtained from a 1326 K/mm² cluster density with a cluster passing quality control filters of 99.1% (24 492 260 clusters). Within this run, the index representation for *T. massiliensis* was determined to be 7.06%. The 1 481 197 paired reads were filtered according to the read qualities. These reads were trimmed, and then assembled using the CLC genomics WB4 software.

### Genome annotation and comparison

Open reading frames (ORFs) were predicted using PRODIGAL [27] with default parameters but the predicted ORFs were excluded if they spanned a sequencing gap region. The predicted bacterial protein sequences were searched against the GenBank database [28] and the Clusters of Orthologous Groups (COG) database using BLASTP (E-value $10^{-3}$, coverage 0.7 and identity percent 30%). If no hit was found, a search against the NR database using BLASTP was performed, with an E-value of $10^{-3}$, coverage 0.7 and identity 30%. If the sequence length was <80 amino acids, we used an E-value of $10^{-5}$. The tRNASCAN-SE tool [29] was used to find tRNA genes, whereas ribosomal RNAs were found using RNAmer [30] and BLASTn against the GenBank database. Lipoprotein signal peptides and the number of transmembrane helices were predicted using PHOBiS [31]. ORFans were identified if their BLASTP E-value was lower than $10^{-3}$ for alignment length >80 amino acids. If alignment lengths were <80 amino acids, we used an E-value of $10^{-5}$. Such parameter thresholds have already been used in previous works to define ORFans. *ARTEMS* [32] was used for data management and DNA PLOTTER [33] for visualization of genomic features. The MAUVE alignment tool (version 2.3.1) was used for multiple genomic sequence alignment [34]. To estimate the mean level of nucleotide sequence similarity at the genome level, we used the MAGI home-made software to calculate the average genomic identity of gene sequences among compared genomes. Briefly, this software combines the PROTEINORTHO software [35] for detecting orthologous proteins in pairwise genomic comparisons, then retrieves the corresponding genes and determines the mean percentage of nucleotide sequence identity among orthologous ORFs using the Needleman–Wunsch global alignment algorithm. Annotation and comparison processes were performed in the MULTI-AGENT software system DAGOBAH [36], that include FIGENIX [37] libraries that provide pipeline analysis. Genomes

![FIG. 1. Phylogenetic tree highlighting the position of relative to other close species. Phylogenetic tree showing the position of *Tessaracoccus massiliensis* strain SIT-7T (underlined) relative to other phylogenetically close members of the family Porphyromonadaceae. GenBank Accession numbers are indicated in parentheses. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using the maximum-likelihood method within the MEGA software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 500 times to generate a majority consensus tree. Only values >90% were displayed. The scale bar indicates a 2% nucleotide sequence divergence](image-url)
from the genus Tessaracoccus and closely related genera were used for the calculation of average genomic identity of gene sequences values. We compared the genome sequence of T. massiliensis strain SIT-7T with those of Propionibacterium propionicum F0230a (CP002734), Aestuariimicrobium kwangyangense DSM 21549 (ATXE01000000), Propionibacterium jensenii DSM 20535 (AUDD00000000), Propionibacterium freudenreichii subsp. shermanii CIRM-BIA1 (FN806773), Propionibacterium avidum 44067 (CP005287), Propionibacterium acnes KPA171202 (AE017283) and Propionibacterium thoenii DSM 20276.

**TABLE 1. Classification and general features of Tessaracoccus massiliensis strain SIT-7**

| Property               | Term |
|-----------------------|------|
| Current classification| Domain: Bacteria |
|                        | Phylum: Actinobacteria |
|                        | Class: Actinobacteria |
|                        | Order: Propionibacterales |
|                        | Family: Propionibacteriaceae |
|                        | Genus: Tessaracoccus |
|                        | Species: Tessaracoccus massiliensis |
| Type strain            | SIT-7T |
| Gram stain             | Positive |
| Cell shape             | Rod-shaped |
| Motility               | Non-motile |
| Sporulation            | Non-endospore forming |
| Temperature range      | Mesophile |
| Optimum temperature    | 37°C |
| pH                     | pH 6 to 9 |
| Optimum pH             | 7.5 |
| Salinity               | 0.0 – 5.0 g/L |
| Optimum salinity       | 0 |
| Oxygen requirement     | Facultative aerobic |

**FIG. 3.** Gram staining of Tessaracoccus massiliensis strain SIT-7T.
The genomic similarity was evaluated among closely studied species from *T. massiliensis* by the DNA–DNA hybridization. *Tessaracoccus massiliensis* was locally aligned 2-by-2 using BLAT algorithm [38,39] against each selected genome previously cited and DNA–DNA hybridization values were estimated from a generalized model [40].

**Results**

**Phylogenic classification**

Strain SIT-7<sup>T</sup> was first isolated in April 2014 by incubation in a blood-culture bottle with sheep blood and cultivation on 5% sheep blood-enriched Colombia agar in aerobic conditions after a 7-day pre-incubation period at 37°C. MALDI-TOF applied on colonies did not give a reliable identification for the strain SIT-...
Phenotypic description

After 48 h of growth on 5% sheep blood-enriched Colombia agar at 37°C at pH 7.5 in aerobic conditions, the surface colonies were circular, greyish, shiny and smooth with a diameter of 0.7–1.1 mm. Tessaracoccus massiliensis is Gram positive (Fig. 3), non-motile and non-spore-forming. Growth is observed at temperatures ranging from 25 to 45°C, with an optimum at 37°C. Strain SIT-7T grew in an aerobic atmosphere, in the presence of 5% CO₂, and also in anaerobic and microaerophilic atmospheres. Growth was also achieved after 48 h on trypticase soy agar. Under the microscope, cells are rod-shaped and measure 4–5 μm in length and 0.6 μm in diameter (Fig. 4). The strain is catalase-positive and oxidase-negative.

Using the commercially available API 20NE strip, T. massiliensis strain SIT-7T demonstrates positive reactions for potassium nitrate, D-mannose, L-arabinose, D-glucose, but negative for L-arginine, urea, D-maltose, malic acid and L-tryptophan. Using an API ZYM strip, positive reactions were observed for esterase(C4), leucine arylamidase, α-chymotrypsin, alkaline phosphatase, cystine arylamidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and β-galactosidase.

Substrate oxidation and assimilation were examined with an API 50CH strip at 37°C. Positive reactions were obtained for D-mannose, D-arabinose, D-galactose, D-ribose, D-sucrose, D-fructose and D-glucose. We compared some of these phenotypic characteristics with the most closely related species (Table 2). By comparison with T. lubricantis, its phylogenetically closest neighbour, T. massiliensis differed in β-galactosidase, acid formation from L-arabinose, D-maltose and D-lactose.

Our strain SIT-7T was susceptible to doxycycline, rifampicin, vancomycin, nitrofurantoin, amoxicillin, erythromycin, ampicillin, ceftriaxone, ciprofloxacin, gentamicin, penicillin, trimethoprim/sulfamethoxazole and imipenem, but resistant to metronidazole.

### TABLE 2. Differential characteristics of Tessaracoccus massiliensis strain SIT-7T and other strains

| Properties                  | T. massiliensis | T. lubricantis | T. bendigoensis | T. oleagi | T. flavescent | T. lopicatus | P. avidum | P. propionicum | P. acnes |
|-----------------------------|----------------|----------------|-----------------|-----------|---------------|-------------|-----------|----------------|----------|
| Cell diameter (μm)          | 0.6–0.9        | 0.5–2          | 0.5–1.1         | 0.48–1    | 0.6–1.2       | 0.45–1      | 0.25–0.75 | 0.2–0.8        | 0.4–0.5  |
| Indole (tryptophanase)      | −              | −              | −               | −         | −             | −           | +         | +              | +        |
| Oxidase                     | +              | +              | +               | +         | −             | −           | −         | −              | −        |
| Nitrate reductase           | +              | +              | +               | +         | −             | −           | −         | −              | −        |
| Urease                      | −              | −              | −               | −         | −             | −           | −         | −              | −        |
| β-galactosidase             | −              | −              | −               | −         | −             | −           | −         | −              | −        |
| N-acetyl-glucosamine        | +              | −              | −               | −         | −             | −           | −         | +              | +        |
| Acid from                   | −              | −              | −               | −         | −             | −           | −         | +              | +        |
| D-Arabinose                 | +              | −              | +               | +         | −             | −           | −         | −              | −        |
| D-Mannose                   | +              | −              | +               | +         | −             | −           | −         | −              | −        |
| D-Glucose                   | +              | +              | +               | +         | −             | +           | +         | −              | −        |
| D-Fructose                  | −              | −              | −               | −         | −             | −           | −         | +              | +        |
| D-Maltose                   | −              | −              | −               | −         | −             | −           | −         | +              | +        |
| D-Lactose                   | −              | −              | −               | −         | −             | −           | −         | −              | −        |
| Habitat                     | human gut      | metal-working fluid | activated sludge biomass | saline soil | saline soil | Iberian pyrite belt | chlorosolvent | human mouth | skin     |

Differential characteristics compared with Tessaracoccus bendegensis strain Ban 104T, Tessaracoccus flavescent strain SIT-739T, Tessaracoccus lopicatus strain IPBSL-7(T), Tessaracoccus lubricantis strain KSS-17Se(T), Tessaracoccus oleiagri strain SL014B-20A1(T), Propionibacterium avidum strain ATCC 25577, Propionibacterium propionicum strain ATCC 14157, Propionibacterium acnes strain ATCC 6919.

+ positive; − negative; na, not available.

### TABLE 3. Nucleotide content and gene count levels of the genome

| Attribute            | Value | % of total |
|----------------------|-------|------------|
| Size (bp)            | 3 212 234 | 100 |
| G+C content (bp)     | 2 177 721 | 67.81 |
| Coding region (bp)   | 3 129 675 | 83.34 |
| Total genes          | 3 310 7 | 100 |
| RNA genes            | 49     | 1.57 |
| Protein-coding genes | 3058   | 98.46 |
| Genes with function prediction | 384 | 12.55 |
| Genes assigned to clusters of orthologous groups | 1770 | 57.88 |
| Genes with peptide signals | 409 | 13.57 |
| Gene associated to resistance genes | 4 | 0.13 |
| Gene associated to bacteriocin genes | 13 | 0.42 |
| Protein without homologue (orfan) | 405 | 13.24 |

*The total is based on either the size of the genome in base pairs or the total number of protein-coding genes in the annotated genome.*
Genome properties
The genome is 3,212,234 bp long with 67.81% G+C content (Table 3 and Fig. 5). It is composed of 17 scaffolds (composed of 20 contigs). On the 3,107 predicted genes, 3,058 were protein-coding genes, and 49 were RNAs (one gene is 5S rRNA, one gene is 16S rRNA, one gene is 23S rRNA, 46 genes are tRNA genes). A total of 2,042 genes (66.78%) were assigned as putative function (by COGs or by NR BLAST). In all, 405 genes were identified as ORFans (13.24%). The remaining genes (499 genes; 16.32%) were annotated as hypothetical proteins. The details of the statistics of the genome are presented in Table 3. Table 4 and Fig. 6 shows the proportion of genes divided in the different COGs functional categories. The genome sequence has been deposited in EMBL-EBI under accession number CCYJ00000000.

Genome comparison
The draft genome sequence of T. massiliensis is smaller than those of P. propionicum F0230a (CP002734), (3.21 and 3.45 Mb respectively), but larger than P. avidum 44067 (CP005287), P. acnes KPA171202 (AE017283), P. freudenreichii subsp. shermanii CIRM-BIA1 (FN806773), P. thoenii DSM 20276 (KE384018), A. kwangyangense DSM 21549 (ATXE01000000), P. jensenii DSM 20535 (AUDD00000000) and T. oleagri CGMCC 1.9159 (PRJNA303532) (2.52; 2.56; 2.61; 2.93, 2.99; 3.02; 3.15 MB, respectively).

The G+C content of T. massiliensis is smaller than those of P. thoenii, A. kwangyangense, P. jensenii, T. oleagri (68%, 68.5%, 68.7%, 69.7%, respectively), but larger than those of P. freudenreichii subsp. shermanii, P. avidum, P. propionicum and P. acnes (67.27%, 63.48%, 66.06% and 60.01%, respectively).

TABLE 4. Number of genes associated with the 25 general clusters of orthologous group functional categories

| Code | Value | % value | Description |
|------|-------|---------|-------------|
| J    | 144   | 4.7     | Translation |
| A    | 126   | 4.12    | RNA processing and modification |
| K    | 184   | 6.01    | Transcription |
| L    | 0     | 0.03    | Replication, recombination and repair |
| B    | 17    | 0.55    | Chromatin structure and dynamics |
| Y    | 0     | 0       | Cell cycle control, mitosis and meiosis |
| V    | 46    | 1.50    | Nuclear structure |
| T    | 62    | 2.02    | Signal transduction mechanisms |
| M    | 81    | 2.64    | Cell wall/membrane biogenesis |
| N    | 0     | 0       | Cell mobility |
| Z    | 0     | 0       | Cytoskeleton |
| W    | 0     | 0       | Extracellular structures |
| U    | 22    | 0.71    | Intracellular trafficking and secretion |
| O    | 69    | 2.25    | Post-translational modification, protein turnover, chaperones |
| C    | 133   | 4.34    | Energy production and conversion |
| G    | 205   | 6.70    | Carbohydrate transport and metabolism |
| E    | 169   | 5.52    | Amino acid transport and metabolism |
| F    | 65    | 2.12    | Nucleotide transport and metabolism |
| H    | 87    | 2.84    | Coenzyme transport and metabolism |
| I    | 1    | 0.03    | Lipid transport and metabolism |
| P    | 113   | 3.69    | Inorganic ion transport and metabolism |
| Q    | 37    | 1.20    | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 238   | 7.78    | General function prediction only |
| S    | 112   | 3.66    | Function unknown |
|     | 1288  | 42.11   | Not in clusters of orthologous groups |

*aThe total is based on the total number of protein-coding genes in the annotated genome.*
### TABLE 5. The numbers of orthologous proteins shared between genomes (upper right)*

|                     | Aestuariimicrobium kwangyangense | Tessaracoccus massiliensis | Propionibacterium freudenreichii | Propionibacterium acnes | Propionibacterium propionicum | Propionibacterium avidum | Propionibacterium oleaigri | Propionibacterium jensenii |
|---------------------|----------------------------------|----------------------------|----------------------------------|-------------------------|-------------------------------|--------------------------|---------------------------|---------------------------|
| A. kwangyangense    | 2633                             | 1231                       | 1007                             | 1196                    | 1123                          | 1082                     | 1155                      | 1252                      | 1197                      |
| T. massiliensis     | 69.97                            | 3052                       | 1030                             | 1180                    | 1111                          | 1249                     | 1133                      | 1382                      | 1170                      |
| P. freudenreichii   | 69.129                           | 68.65                      | 2318                             | 1075                    | 1027                          | 1013                     | 1041                      | 1003                      | 1090                      |
| P. thoenii          | 69.47                            | 68.54                      | 69.4                             | 2629                    | 1280                          | 1113                     | 1326                      | 1163                      | 1497                      |
| P. acnes            | 66.09                            | 65.63                      | 65.96                            | 70.18                   | 2373                          | 1084                     | 1411                      | 1092                      | 1305                      |
| P. propionicum      | 69.4                             | 73.2                       | 67.93                            | 68.2                    | 65.35                         | 3064                     | 1087                      | 1193                      | 1122                      |
| P. avidum           | 68.19                            | 67.68                      | 67.77                            | 72.49                   | 81.27                         | 67.06                    | 2302                      | 1108                      | 1348                      |
| T. oleaigri         | 70.13                            | 74.56                      | 68.73                            | 68.62                   | 65.57                         | 72.01                    | 67.63                     | 2916                      | 1155                      |
| T. jensenii         | 69.94                            | 68.96                      | 69.53                            | 83.34                   | 70.4                          | 68.57                     | 72.92                     | 69.21                      | 2700                      |

*Average percentage similarity of nucleotides corresponding to orthologous proteins shared between genomes (lower left) and numbers of proteins per genome (bold).

### TABLE 6. Pairwise comparison of Tessaracoccus massiliensis with other species using GGDC, formula 2 (DNA–DNA hybridization estimates based on identities / HSP length)*

|                     | Tessaracoccus massiliensis | Propionibacterium avidum | Tessaracoccus oleaigri | Propionibacterium freudenreichii | Propionibacterium acnes | Propionibacterium propionicum | Propionibacterium avidum | Propionibacterium oleaigri | Tessaracoccus jensenii |
|---------------------|---------------------------|--------------------------|------------------------|---------------------------------|-------------------------|-------------------------------|--------------------------|---------------------------|------------------------|
| Tessaracoccus massiliensis | 100.00% [100%–100%]    | 13.50% [11.2%–16.3%]    | 19.30% [17.1%–21.7%]   | 19.20% [17%–21.6%]             | 20.20% [18%–22.6%]      | 19.10% [16.9%–21.8%]          | 19.40% [17.2%–21.6%]   | 20.30% [18.1%–22.7%]   | 14.00% [11.2%–17.3%] |
| Propionibacterium avidum     | 100.00% [100%–100%]    | 21.40% [19.1%–23.8%]    | 15.50% [12.6%–18.9%]    | 19.70% [17.5%–22.1%]          | 13.00% [10.3%–16.3%]    | 23.50% [21.2%–25.9%]         | 19.80% [17.6%–22.2%]   | 18.90% [16.8%–21.3%]   | 19.60% [17.4%–22%]    |
| Tessaracoccus oleaigri      | 100.00% [100%–100%]    | 19.40% [17.2%–21.8%]    | 19.30% [17.3%–21.9%]    | 20.40% [18.2%–22.8%]         | 19.60% [17.4%–22%]      | 18.90% [16.8%–21.3%]         | 19.60% [17.4%–22%]    | 18.90% [16.8%–21.3%]   | 19.60% [17.4%–22%]    |
| Propionibacterium jensenii  | 100.00% [100%–100%]    | 28.70% [25.4%–30.3%]    | 21.40% [19.1%–23.8%]    | 20.10% [17.9%–22.5%]         | 18.90% [16.8%–21.3%]    | 19.70% [17.5%–22.1%]         | 19.70% [17.5%–22.1%]   | 18.90% [16.8%–21.3%]   | 19.70% [17.5%–22.1%]   |
| Propionibacterium thoenii   | 100.00% [100%–100%]    | 14.10% [11.3%–17.4%]    | 21.30% [19.1%–23.8%]    | 20.10% [17.9%–22.5%]         | 18.90% [16.8%–21.3%]    | 19.70% [17.5%–22.1%]         | 19.70% [17.5%–22.1%]   | 18.90% [16.8%–21.3%]   | 19.70% [17.5%–22.1%]   |
| Propionibacterium freudenreichii | 100.00% [100%–100%] | 19.30% [17.1%–21.7%] | 20.90% [18.7%–23.4%] | 19.70% [17.5%–22.1%] | 19.70% [17.5%–22.1%] | 19.70% [17.5%–22.1%] | 19.70% [17.5%–22.1%] | 18.90% [16.8%–21.3%] | 19.70% [17.5%–22.1%] |
| Propionibacterium propionicum | 100.00% [100%–100%] | 12.80% [10.1%–16%] | 19.60% [17.4%–22%] | 19.70% [17.5%–22.1%] | 19.70% [17.5%–22.1%] | 19.70% [17.5%–22.1%] | 19.70% [17.5%–22.1%] | 18.90% [16.8%–21.3%] | 19.70% [17.5%–22.1%] |
| Propionibacterium acnes    | 100.00% [100%–100%]    | 12.90% [10.2%–16.2%]    | 19.60% [17.4%–22%]      | 19.70% [17.5%–22.1%]         | 19.70% [17.5%–22.1%]    | 19.70% [17.5%–22.1%]         | 19.70% [17.5%–22.1%]   | 18.90% [16.8%–21.3%]   | 19.70% [17.5%–22.1%]   |

*The confidence intervals indicate the inherent uncertainty in estimating DNA–DNA hybridization values from intergenomic distances based on models derived from empirical test data sets (which are always limited in size). These results are in accordance with the 16S rRNA (Fig. 3) and phylogenomic analyses as well as the GGDC results.
The gene content of T. massiliensis is smaller than those of P. propionicum, but larger than those of P. freudenreichii subsp. shermanii, P. avidum, P. acnes, A. kwangyangense, P. thoennii, P. jenseni and T. oleiagri (2439, 2242, 2938, 2966, 2684, 2687 and 2754, respectively).

Tessaracoccus massiliensis also shared 69.97, 1133, 1170, 1180, 1249, 1382, orthologous genes with, P. freudenreichii, P. acnes, P. avidum, P. jenseni, P. thoennii, P. propionicum and T. oleiagri, respectively (Table 5).

The DNA–DNA hybridization was of 13.50% (11.2%–16.3%) with P. avidum, 14.00% (11.2%–17.3%) with A. kwangyangense, 19.10% (16.9%–21.5%) with P. freudenreichii, 19.20% (17%–21.6%) with P. jenseni, 19.30% (17.1%–21.7%) with T. oleiagri, 19.40% (17.2%–21.8%) with P. propionicum, 20.20% (18%–22.6%) with P. jenseni and 20.30% (18.1%–22.7%) with P. acnes (Table 6). These data confirm T. massiliensis as a unique species.

Finally, we observed that all compared genomes have nearly the same number of genes in each of the COG categories (Table 4, Fig. 6).

Conclusions

Based on phenotypic, genomic and phylogenetic analyses, we formally propose the creation of Tessaracoccus massiliensis sp. nov., represented here by the strain SIT-7T. The strain was isolated from a stool sample of a Nigerian child suffering from kwashiorkor, and represents the first Tessaracoccus species isolated in human, as in culturomics studies.

Description of Tessaracoccus massiliensis sp. nov.

Tessaracoccus massiliensis (mas.si.li.en’sis. L. masc. adj. massi- liensis of Massilia, the old Roman name for Marseille, where the strain was isolated).

Facultative anaerobic, Gram-positive, oxidase-negative, catalase-positive, non-endospore-forming, non-motile rods. Colonies are circular, greyish, shiny and smooth with a 0.7–1.1 mm diameter on Columbia agar + 5% sheep blood. Good growth occurs at 25–45°C (optimum 37°C) and with a pH between 6 and 9 (optimum 7.5). Cells measure 4–5 μm in length and 0.6 μm in diameter.

Using API 50CH and ZYM strip, positive reactions were observed for D-mannose, D-arabinose, D-galactose, D-ribose, D-sucrose, D-fructose and D-glucose, esterase(C4), leucine arylamidase, α-chymotrypsin, alkaline phosphatase, cystine arylamidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and β-galactosidase. API 20NE strip showed that T. massiliensis assimilates D-mannose, D-glucose and L-arabinose. Potassium nitrate is reduced. SIT-7T was susceptible to doxycycline, rifampicin, vancomycin, nitrofurantoin, amoxicillin, erythromycin, ampicillin, ceftriaxone, ciprofloxacin, gentamicin, penicillin, trimethoprim/sulfamethoxazole and imipenem.

The G+C content of the genome is 67.81%. Accession numbers of the sequences of 16S rRNA and genome deposited in EMBL-EBI are LK985394 and CCYJ00000000, respectively.

The habitat of the microorganism is the human digestive tract. The type strain SIT-7T (= CSUR P1301, = DSM29060) was isolated from a stool specimen of a Nigerian child suffering from kwashiorkor.

Conflict of Interest

None declared.

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