Noncontiguous finished genome sequence and description of Bacillus andreraoultii strain SIT1T sp. nov.

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Abstract

Bacillus andreraoultii strain SIT1T (= CSUR P1162 = DSM 29078) is the type strain of B. andreraoultii sp. nov. This bacterium was isolated from the stool of a 2-year-old Nigerian boy with a severe form of kwashiorkor. Bacillus andreraoultii is an aerobic, Gram-positive rod. We describe here the features of this bacterium, together with the complete genome sequencing and annotation. The 4 092 130 bp long genome contains 3718 protein-coding and 116 RNA genes.

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Introduction

Bacillus andreraoultii strain SIT1T (= CSUR P1162 = DSM 29078) is the type strain of B. andreraoultii sp. nov. This bacterium was isolated from the stool of a 2-year-old Nigerian boy with kwashiorkor, a severe form of acute malnutrition, and is part of an effort called culturomics to cultivate all bacterial species from the human gut [1,2]. This is a Gram-positive, aerobic or facultatively anaerobic, motile, indole-negative and rod-shaped bacillus.

The ruling taxonomic classification of prokaryotes is based on a combination of phenotypic and genotypic criteria [3,4]. However, the three essential criteria that are used (16S rRNA gene-based phylogeny [5], G+C content and DNA-DNA hybridization (DDH)) [3,6] exhibit several drawbacks. The number of sequenced bacterial genomes has rapidly increased due to the decrease in cost of sequencing (to date, almost 40 000 bacterial genomes have been sequenced). Therefore, the genomic data, the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) spectrum [7] and phenotypic criteria have been proposed for the description of new bacterial species [8,9].

The genus Bacillus was discovered in 1872 (Cohn, 1872) [10]. The genus is composed of 331 species with validly published names (Bacillus, http://www.doi.namesforlife.com/). Species of the genus Bacillus are ubiquitous bacteria isolated from various environments including soil, gastrointestinal tracts of various insects and animals, vegetation, fresh- and seawater and food [11]. In human beings, the Bacillus species may exist in opportunistic form in immunocompromised patients [12] or in pathogenic form, such as B. cereus (food poisoning) and B. anthracis (anthrax) [13]. Other species may also be found in various human infections, including pneumonia, endocarditis, and ocular, cutaneous, bone or central nervous system infections and bacteraemia [14].
The following is a summary classification and a set of features for *Bacillus andreraoultii* sp. nov. strain SIT1T together with the description of the complete genomic sequencing and annotation. These particularities support the circumscription of the species *Bacillus andreraoultii*.

**Materials and methods**

**Sample collection, strain isolation and culture condition**

A stool sample was obtained from a 2-year-old Nigerian boy with kwashiorkor, a severe form of acute malnutrition, who was admitted to the emergency room in the national hospital in Niamey, the capital city of Niger, in October 2013 [2]. 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. The fecal specimen was preserved at 4°C after collection. Then the stool was sent to Marseille, where it was kept at −80°C until laboratory culture isolation. Strain SIT1T was isolated in March 2014 by cultivation on 5% sheep’s blood–enriched Columbia agar (bioMérieux, Marcy l’Etöile, France) in an anaerobic atmosphere at 37°C after 10 days of stool specimen incubation in a culture bottle containing a blood-enriched Columbia agar medium (bioMérieux). Growth of the strain was tested under anaerobic conditions using GENbag anaer system (bioMérieux), and under aerobic conditions, with or without 5% CO₂. Different growth temperatures (25, 30, 37, 45, 55°C) were also tested.

**MALDI-TOF and 16S sequencing**

MALDI-TOF protein analysis was carried out as previously described [15] using a Microflex spectrometer (Bruker Daltonics, Leipzig, Germany). In brief, a pipette tip was used to pick one isolated bacterial colony from a culture agar plate and spread it as a thin film on an MSP 96 MALDI-TOF target plate (Bruker). Twenty distinct deposits from 20 isolated colonies were tested for strain SIT1T. Each smear was overlaid with 2 μL of matrix solution (saturated solution of alpha-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) and allowed to dry for 5 minutes. Spectra were recorded in the positive linear mode for the mass range of 2000 to 20,000 Da (parameter settings: ion source 1 (IS1), 20 kV; IS2, 18.5 kV; lens, 7 kV). A spectrum was obtained after 240 shots with variable laser power. The time of acquisition was between 30 seconds and 1 minute per spot. The 20 SIT1T spectra were imported into MALDI BioTyper 3.0 software (Bruker) and analysed by standard pattern matching (with default parameter settings) against the main spectra of 7379 bacteria. The method of identification included the m/z from 3000 to 15,000 Da. For every spectrum, a maximum of 100 peaks were compared with spectra in the database. The resulting score enabled the identification of species whether tested or not: 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.

Identification of bacteria continued with a 16S rRNA standard PCR coupled with sequencing. That was performed using GeneAmp PCR System 2720 thermal cyclers (Applied Biosystems, Bedford, MA, USA) and ABI Prism 3130xl Genetic Analyser capillary sequencer (Applied Biosystems) respectively [16]. The 16S rRNA nucleotides sequence was corrected using Chromas Pro 1.34 software (Technelysium, Tewantin, Australia), and the BLASTn searches were performed in the online PubMed National Center for Biotechnology Information (NCBI) database (http://blast.ncbi.nlm.nih.gov/gate1.inist.fr/Blast.cgi).

**Morphologic, biochemical and antibiotic susceptibility tests**

*Bacillus andreraoultii* strain SIT1T was observed, after negative colouration, using a Morgani 268D (Philips, Amsterdam, The Netherlands) transmission electron microscope at an operating voltage of 60 kV. The Gram colouration was performed using Color Gram 2 Kit (bioMérieux) and observed by using the DM1000 photonic microscope (Leica Microsystems, Wetzlar, Germany) with a 100× oil-immersion objective lens. The sporulation test was done doing a thermic shock (80°C during 30 minutes). To evaluate the motility of *Bacillus andreraoultii*, fresh colonies were observed between blades and slats using a DM1000 photonic microscope (Leica) with a 40× objective lens.

API ZYM, API 20 NE and API 50 CH (bioMérieux) gallery systems were used to perform biochemical assays. Oxidase (Becton Dickinson, Franklin Lakes, NJ, USA) and catalase assays (bioMérieux) were done separately. The antibiotic susceptibility was tested using SirScan Discs antibiotics (i2a, Montpellier, France).

**Genome sequencing**

Genomic DNA extraction of *Bacillus andreraoultii* strain SIT1T was performed according to the method previously described [17]. The DNA was resuspended in 205 μL TE buffer. The DNA concentration was 401.63 ng/μL as measured by a Qubit fluorometer using the high-sensitivity kit (Life Technologies, Carlsbad, CA, USA).

Genomic DNA of *B. andreraoultii* was sequenced on the MiSeq Technology (Illumina, San Diego, CA, USA) with the
mate pair strategy. The gDNA was barcoded in order to be mixed with 11 other projects with the Nextera Mate Pair sample prep kit (Illumina).

The gDNA was quantified by a Qubit assay with the high-sensitivity kit (Life Technologies) to 87.67 ng/μL. The mate pair library was prepared with 1 μg of genomic DNA using the Nextera mate pair Illumina guide. The genomic DNA 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, Santa Clara, CA, USA) with a DNA 7500 lab chip. The DNA fragments ranged in size from 1 to 11 kb, with an optimal size of 4.851 kb. No size selection was performed, and 432.1 ng of tagmented fragments were circularized. The circularized DNA was mechanically sheared into small fragments with an optimal size of 684 bp on the Covaris device S2 in microtubes (Covaris, Woburn, MA, USA). The library profile was visualized on a High Sensitivity Bioanalyzer LabChip (Agilent), and the final concentration library was measured at 64.49 nmol/L.

The libraries were normalized at 2 nM and pooled. After denaturation and dilution at 15 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 a single 39-hour sequencing run were performed at a 2 × 251 bp read length.

All 8.2 Gb of information was obtained from a 947K/mm² cluster density, with a cluster passing quality control filters with 99% (18 111 784 clusters). Within this run, the index representation for Bacillus andreraoultii was determined to 9.27%. The 1 513 908 paired reads were filtered according to the read qualities. The reads obtained from applications were trimmed, and an optimal assembly of 14 scaffolds and 58 contigs was obtained through the SOAPdenovo software, which generated a genome size of 4.09 Mb.

**Genome annotation and comparison**

Open reading frames (ORFs) were predicted using Prodigal [18] with default parameters, but the predicted ORFs were excluded if they spanned a sequencing gap region (contain N). The predicted bacterial protein sequences were searched against the Clusters of Orthologous Groups of Proteins (COGs) database using BLASTP (e-value 1e-03, coverage of 0.7, and an identity percentage of 30%). If no hit was found, it search against the NR database using BLASTP with an e-value of 1e-03, coverage of 0.7 and an identity percentage of 30%. If sequence lengths were smaller than 80 amino acids, we used an e-value of 1e-05. The rRNAScanSE tool [19] was used to find tRNA genes, whereas ribosomal RNAs were found by using RNAmmer [20]. Lipoprotein signal peptides and the number of transmembrane helices were predicted using Phobius [21]. ORFans were identified if all the BLASTP performed did not retrieve positive results. Such parameter thresholds have already been used in previous works to define ORFans. Genomes were automatically retrieved from the 16s RNA tree using Phylogenetic software [22]. For each selected genome, complete genome sequences, proteome genome sequences and Orfeome genome sequences were retrieved from the FTP site of NCBI. All proteomes were analysed with Proteinortho [23]. Then for each couple of genomes a similarity score was computed. This score is the mean value of nucleotide similarity between all couple of orthologies in the two genomes studied (average genomic identity of orthologous gene sequences, AGIOS) [9]. The resistome was analysed with the ARG-ANNOT (Antibiotic Resistance Gene-ANNOTation) database and BLASTp in GenBank [24]. The exhaustive bacteriocin database available in our laboratories (Bacteriocins from the URMITE database) (http://drissifatima.wik.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 [25]. PHAST (PHAge search tool) was used to identify phage sequences [26].

An annotation of the entire proteome was performed to define the distribution of functional classes of predicted genes according to the clusters of orthologous groups of proteins using the same method as for the genome annotation.

**Results**

**Strain identification and phylogenetic analyses**

No significant MALDI-TOF score was obtained for strain SIT1 T against the Bruker database, suggesting that our isolate was not a member of a known species. We added the spectrum from strain SIT1 T to our database (Figure 1). Then a gel view was performed to show the spectral differences with other members of the genus Bacillus (Figure 2).

Using 16S rRNA phylogeny analyses, we demonstrated that strain SIT1 T exhibited a 96% 16S rRNA sequence identity with Bacillus thermoamylolovorans (GenBank accession no. HM030742), the phylogenetically closest bacterial species with standing in nomenclature (Figure 3). Its 16S rRNA sequence was deposited in GenBank under accession no. LK021120. This value was lower than the 98.7% 16S rRNA gene sequence threshold recommended by Stackebrandt and Ebers [5] to delineate a new species without carrying out DNA-DNA hybridization. Thus, this bacterium was considered as a new species called Bacillus andreraoultii strain SIT1 T belonging family Bacillaceae (Table 1).
FIG. 1. Reference mass spectrum from *Bacillus andreraoultii* strain SIT1<sup>T</sup>. Spectra from 20 individual colonies were compared and reference spectrum generated.

FIG. 2. Gel view comparing *Bacillus andreraoultii* SIT1<sup>T</sup> to other *Bacillus* species. Gel view displays raw spectra of loaded spectrum files arranged in pseudo-gel-like look. x-axis records m/z value. Left y-axis displays running spectrum number originating from subsequent spectra loading. Peak intensity is expressed in arbitrary units by greyscale scheme code, as indicated on right y-axis. Displayed species are indicated at left.
Phenotypic and biochemical characteristics

*B. andreraoultii* growth occurred at all temperatures tested; however, optimal growth was observed between 37 and 45°C after 24 hours of incubation. Colonies were 0.1 to 0.3 μm diameter on blood-enriched Columbia agar. Growth was achieved aerobically and weak growth anaerobically. Gram staining showed rod-shaped, Gram-positive bacilli (Figure 4). Cells were grown on agar sporulate. A motility test was positive. Cells grown on agar are smooth and greyish after 24 hours of incubation, and they have an average width and length of 0.5 μm and 3 μm, respectively, and exhibited flagella (Figure 5).

Strain SIT1T showed catalase activity but was negative for oxidase. Using an API ZYM strip, positive reactions were observed for alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase. Negative reactions were observed for lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Using an API 20 NE strip, the nitrate reductase—hydrolysis reaction (β-glucosidase, esculine), and β-galactosidase and assimilation reaction (potassium gluconate) were also positive. Negative reactions were found for urease, indole, arginine dihydrolase and fermentation (glucose).

Using an API 50 CH strip, positive reactions were recorded for L-arabinose, D-ribose, D-xylose, D-glucose, D-fructose, D-mannose, N-acetylglucosamine, arbutine, esculine, salicine, D-cellobiose, D-maltose, D-saccharose, D-trehalose and amidon.

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**TABLE 1.** Classification and general features of *Bacillus andreraoultii* strain SIT1T according to MIGS recommendations [27].

| MIGS ID | Property | Term | Evidence code |
|---------|----------|------|---------------|
| **Current classification** | | | |
| Domain | Bacteria | TAS [28] |
| Phylum | Firmicutes | TAS [29–31] |
| Class | Bacilli | TAS [32–33] |
| Order | Bacillales | TAS [34,35] |
| Family | Bacillaceae | TAS [35,36] |
| Genus | Bacillus | TAS [10,35] |
| Species | *Bacillus andreraoultii* | IDA |
| Type strain | SIT1T | IDA |
| **Gram stain** | | | IDA |
| **Cell shape** | | | IDA |
| **Motility** | | | IDA |
| **Sporulation** | | | IDA |
| **Temperature range** | | | IDA |
| **Optimum temperature** | 37–45°C | IDA |
| **Salinity** | | | IDA |
| **Carbon source** | Unknown | IDA |
| **Energy source** | Unknown | IDA |
| **Habitat** | Human gut | IDA |
| **Biological relationship** | Free-living | IDA |
| **Pathogenicity** | Unknown | IDA |
| **Bioavailability level** | 2 | IDA |
| **Isolation** | Human faeces | IDA |
| **Sample collection time** | March 2013 | IDA |
| **Latitude** | 43.296482 | IDA |
| **Longitude** | 5.36978 | IDA |
| **Depth** | Surface | IDA |
| **Altitude** | 0 m above sea level | IDA |

MIGS, minimum information about a genome sequence.

*E*vidence codes are as follows: IDA, inferred from direct assay; TAS, traceable author statement (i.e. a direct report exists in the literature); NAS, nontraceable author statement (i.e. not directly observed for the living, isolated sample, but based on a generally accepted property for the species or anecdotal evidence). These evidence codes are from the Gene Ontology project (http://www.geneontology.org/GO.evidence.shtml). If the evidence code is IDA, then the property should have been directly observed, for the purpose of this specific publication, for a live isolate by one of the authors, or by an expert or reputable institution mentioned in the acknowledgements.

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**FIG. 3.** Phylogenetic tree highlighting position of *Bacillus andreraoultii* sp. nov. strain SIT1T (= CSUR P1162 = DSM 29078) relative to other type strains within *Bacillus* genus. Strains and their corresponding GenBank accession numbers for 16S rRNA genes are (type = T): *B. infantis* strain NRRL B-14911, NR121756; *B. firmus* strain 5695m-D2, AJ309007; *B. subterraneus* strain COO13B, NR104749; *B. niacini* strain Et9/1, KJ722425; *B. fumarioli* strain R-14705, AJ581126; *B. thermolactis* strain R-33520, AM910339; *B. thermoamylovorans* strain N12-2, HM037042; *B. circulans* strain WSBC20059, Y13063; *B. coagulans* strain 36D1, DQ299726; *B. oleronius* strain ATCC70005, NR043325; *Flavifex huanghaiensis* strain H5, JN815236. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using maximum likelihood method within MEGA6. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 1000 times to generate majority consensus tree. *Flavifex huanghaiensis* strain H5 (JN815236) was used as outgroup. Scale bar = 2% nucleotide sequence divergence.

**FIG. 4.** Gram staining of *B. andreraoultii* strain SIT1T.
Negative reactions were recorded for glycerol, erythritol, D-arabinose, L-xylose, L-adonitol, methyl-β-D-xylopyranoside, D-galactose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, amygdalin, D-lactose, D-melibiose, inulin, D-melezitose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate.

*Bacillus andreraoultii* SIT1 was resistant to trimethoprim-sulphamethoxazole, macrolide (erythromycin), vancomycin and the third generation of cephalosporin (ceftriaxone) but was susceptible to fosfomycin, imipenem, penicillin, amoxicillin, gentamicin, ciprofloxacin, doxycycline and rifampicin. Five species with validly published names in the *Bacillus* genus were selected to make a phenotypic comparison with *B. andreraoultii* (Table 2).

**Genomic characteristics and genome comparison**

*Bacillus andreraoultii* SIT1 was selected for sequencing on the basis of its phenotypic differences, phylogenetic position and

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**TABLE 2. Differential characteristics of Bacillus andreraoultii SIT1<sup>T</sup>; B. thermoamylovorans strain LMG 18084<sup>T</sup>; B. thermolactis strain R-6488<sup>T</sup>; B. circulans strain LMG 13261<sup>T</sup>; B. subterraneus strain COO13B<sup>T</sup>; and B. niacin 2923<sup>T</sup>**

| Property                        | B. andreraoultii | B. thermoamylovorans | B. thermolactis | B. circulans | B. subterraneus | B. niacin |
|---------------------------------|------------------|----------------------|-----------------|--------------|----------------|----------|
| Cell diameter (μm)              | 0.1–0.3          | 0.5–4                | 1–4             | 1–3          | 0.5–12         | 3–5      |
| Mean length (μm)                | 3                | 5                    | 10              | 4.2          | 25             | 5.6      |
| Oxygen requirement              | Flanaerobic      | Flanaerobic          | Flanaerobic     | Flanaerobic  | Flanaerobic    | Aerobic  |
| Gram stain                      | +                | +                    | +               | –            | +              | +        |
| Motility                        | +                | –                    | –               | –            | +              | –        |
| Flagella                        | +                | NA                   | +               | –            | +              | –        |
| Endospore formation             | +                | +                    | +               | –            | +              | –        |
| Production of:                  |                  |                      |                 |              |                |          |
| Alkaline phosphatase            | +                | NA                   | NA              | NA           | NA             | NA       |
| Acid phosphatase                | +                | NA                   | NA              | NA           | NA             | NA       |
| Catalase                        | –                | +                    | +               | –            | +              | –        |
| Oxidase                         | –                | +                    | +               | –            | –              | +        |
| Nitrate reductase               | +                | +                    | +               | –            | +              | –        |
| Indole                          | –                | –                    | –               | –            | –              | –        |
| Urease                          | –                | –                    | –               | –            | –              | –        |
| α-Galactosidase                 | +                | NA                   | NA              | NA           | NA             | NA       |
| β-Galactosidase                 | +                | NA                   | NA              | NA           | NA             | NA       |
| α-Glucosidase                   | +                | NA                   | NA              | NA           | +              | NA       |
| β-Glucosidase                   | +                | NA                   | NA              | NA           | –              | –        |
| Esterase                        | +                | NA                   | NA              | NA           | NA             | NA       |
| Esterase lipase                 | +                | NA                   | NA              | NA           | NA             | NA       |
| Naphthol-AS-BI-phosphohydrolase | +                | NA                   | NA              | NA           | NA             | NA       |
| N-acetyl-β-glucosaminidase      | –                | NA                   | NA              | NA           | NA             | NA       |
| Pyrazinamidase                  | NA               | NA                   | NA              | NA           | NA             | NA       |
| α-Mannosidase                   | –                | NA                   | NA              | NA           | NA             | NA       |
| α-Fucosidase                    | –                | NA                   | NA              | NA           | NA             | NA       |
| Leucine arylamidase             | –                | NA                   | NA              | NA           | NA             | NA       |
| Valine arylamidase              | –                | NA                   | NA              | NA           | NA             | NA       |
| Cystine arylamidase             | –                | NA                   | NA              | NA           | NA             | NA       |
| α-Chemotrypsin                  | –                | NA                   | NA              | NA           | NA             | NA       |
| Trypsin                         | –                | NA                   | NA              | NA           | NA             | NA       |
| Utilization of:                 |                  |                      |                 |              |                |          |
| 5-Keto-gluconate                | –                | –                    | –               | v            | NA             | NA       |
| D-Xylose                        | +                | +                    | +               | –            | +              | –        |
| D-Fructose                      | +                | +                    | +               | –            | –              | +        |
| D-Glucose                       | +                | +                    | +               | –            | +              | –        |
| α-Mannose                       | +                | +                    | +               | –            | +              | –        |
| Habitat                         | Human gut        | Wine, grass . . .     | Raw milk        | Bee larvae   | Subterranean water | Soil    |

*, positive result; –, negative result; v, variable result; NA, data not available.
16S rRNA sequence similarity to other members of the *Bacillus* genus. It was part of a culturomics study aimed at isolating all bacterial species from human digestive flora in patients with kwashiorkor, an acute form of malnutrition. It is the first sequenced genome from *B. andreraoultii* sp. nov. The European Molecular Biology Laboratory (EMBL) accession number of *B. andreraoultii* genome is CCFJ00000000 and consists of 14 scaffolds and 58 contigs (Figure 6). Table 3 shows the project information and its association with minimum information about a genome sequence (MIGS) version 2.0 compliance [27].

The genome is 4 092 130 bp long with 35.42% G+C content. On the 3843 predicted genes, 3718 were protein-coding genes and 116 were RNAs genes. The remaining genes were annotated as hypothetical proteins (712 genes, >19.15%). The properties and statistics of the genome are summarized in Table 4. The distribution of genes into functional COGs categories is presented in Table 5. The draft genome sequence of *Bacillus andreraoultii* was smaller than those of *Bacillus halodurans* C-125, *Bacillus pseudofirmus* OF4 and *Lysinibacillus sphaericus*.
Bacillus andraeraoultii contained a bacteriocin (colicin) consisting of 175 amino acids harboured with the bacteriocins of Paenibacillus and Lactobacillus (Figure 8) and sharing 62% of the homology of Bacillus vireti. The results did not indicate the presence of nonribosomal peptide synthetases and polyketide synthases and phage. We performed the analysis of the resistance of Bacillus andraeraoultii SITI\(^T\) antibiotic classes of the macrolide–lincosamide–streptogramin B (MLSB) antibiotics, such as ATP-binding transporters (ABC) isoB and mrsD, major facilitator transporters mepA, transferases (vetA) and two-component system vancomycin resistance vanS/vanR, norA (Table 7).

**Conclusion**

On the basis of phenotypic, phylogenetic and genomic analyses (taxonogenomics), we formally propose the creation of Bacillus andraeraoultii sp. nov. that contains the strain SITI\(^T\). This bacterium was isolated from the stool of a 2-year-old Nigerian boy with kwashiorkor, a severe form of acute malnutrition.

**Description of Bacillus andraeraoultii sp. nov.**

The Bacillus andraeraoultii name come from André Raoult, who was a military doctor who worked with malnutrition and kwashiorkor in Senegal and who described its specific features [37].

Strain SITI\(^T\) is aerobic, Gram positive, endospore forming, motile and rod shaped. Growth was achieved aerobically between 25 and 55°C (optimum 37 to 45°C). After 24 hours of growth on 5% sheep’s blood–enriched Columbia agar at 37°C, bacterial colonies were smooth and greyish with a diameter of 0.1 to 0.3 mm. The cells had a mean width and length of 0.5 \(\mu\)m and 3 \(\mu\)m, respectively, and exhibited flagella. They were catalase positive and oxidase negative.

Bacillus andraeraoultii SITI\(^T\) was resistant to trimethoprim-sulphamethoxazole, macrolide (erythromycin), vancomycin and ofloxacin, but not to ampicillin, gentamicin, chloramphenicol, ciprofloxacin and nalidixic acid. The strain was positive for catalase and oxidase, negative for indole and Voges–Proskauer reactions, and was motile and rod shaped. Growth was achieved aerobically between 25 and 55°C (optimum 37 to 45°C). After 24 hours of growth on 5% sheep’s blood–enriched Columbia agar at 37°C, bacterial colonies were smooth and greyish with a diameter of 0.1 to 0.3 mm. The cells had a mean width and length of 0.5 \(\mu\)m and 3 \(\mu\)m, respectively, and exhibited flagella. They were catalase positive and oxidase negative.

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Strain SITI\(^T\) is aerobic, Gram positive, endospore forming, motile and rod shaped. Growth was achieved aerobically between 25 and 55°C (optimum 37 to 45°C). After 24 hours of growth on 5% sheep’s blood–enriched Columbia agar at 37°C, bacterial colonies were smooth and greyish with a diameter of 0.1 to 0.3 mm. The cells had a mean width and length of 0.5 \(\mu\)m and 3 \(\mu\)m, respectively, and exhibited flagella. They were catalase positive and oxidase negative.

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**FIG. 7.** Distribution of functional classes of predicted genes in genomes from *Bacillus andreraoultii*, *Anoxybacillus flavithermus*, *Bacillus atrophaeus*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus halodurans*, *Bacillus pseudofirmus*, *Bacillus pumilus*, *Lysinibacillus sphaericus* and *Solibacillus silvestris* chromosomes according to clusters of orthologous groups of proteins.

**FIG. 8.** Molecular phylogenetic analysis by maximum likelihood method of representatives of genus *Bacillus andreraoultii* *SIT* inferred from 16S rRNA gene sequence. Tree with highest log likelihood ($-2930.4905$) is shown. Percentage of trees in which associated taxa clustered together is shown next to branches. Initial trees for heuristic search were obtained automatically by applying neighbour-joining and BioNJ algorithms to matrix of pairwise distances estimated using JTT model, then selecting topology with superior log likelihood value. Tree is drawn to scale, with branch lengths measured in number of substitutions per site. Analysis involved 14 amino acid sequences. There 155 positions in final data set. Evolutionary analyses were conducted in MEGA5.
and the third generation of cephalosporin (ceftriaxone). It contained a bacteriocin.

The genome is 4 092 130 bp long, and the G+C content is 35.42%. The 16S rRNA gene sequence and whole-genome shotgun sequence of B. andraeaulitii strain SIT1T are deposited in GenBank under accession nos. LK021120 and CCFJ00000000, respectively. The type strain SIT1T (═ CSUR P1162 = DSM 29078) was isolated from the stool of a 2-year-old Nigerien boy with kwashiorkor, a severe form of acute malnutrition.

Conflict of Interest

None declared.

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