**Enorma burkinafasonensis** sp. nov., a new bacterium isolated from a human gut microbiota

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**Abstract**

Strain Marseille-P9525T is a Gram-positive, obligatory anaerobic and non-motile bacterium isolated from a human faecal microbiota. Its phenotypic pattern, including mass spectrometry peptide profile and genome sequence, support the proposal of a new species for which the name *Enorma burkinafasonensis* sp. nov. is proposed. The type strain has been deposited in a public collection.

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**Introduction**

Establishing the repertoire of the gut microbiota contributes to a better understanding of its role in human health and diseases. Culturomics, which comprises the application of high-throughput culture conditions, allows a dramatic extension of the repertoire of bacteria associated with humans [1–3]. Taxono-genomics combining matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), phenotypic characteristics, biochemical properties and the genome sequencing was recently introduced to describe new bacterial species [4–6]. Here we report the characterization of a bacterial strain Marseille-P9525 isolated from human gut microbiota, representative of a new species.

**Isolation and growth conditions**

In September 2018, a fresh stool sample was collected from a 28-year-old Burkinabe woman suspected of having tuberculosis and admitted for diagnostic check-up to the Centre Régional de Lutte Antituberculeuse, Bobo-Dioulasso, Burkina Faso. A stool sample was sent to the collaborative laboratory at IHU in Marseille, France for culturomics as previously described [1]. Informed signed consent was obtained from the patient and study of the strain Marseille-P9525 was approved by the Research Ethics Committee in the Health of Science at Bobo-Dioulasso, Burkina Faso (N/Ref.002-2018-Comité d’Ethique Institutionnel pour la Recherche en Science de la Santé (CEIRS)). Briefly, 1 g of faecal sample was serially diluted in 900 μL of Dulbecco’s phosphate-buffered saline and 50 μL of each dilution was spread on 5% sheep’s blood-enriched Colombia agar (bio-Mérieux, Marcy l’Étoile, France) and incubated under anaerobic atmosphere generated by AnaeroGen (bio-Mérieux), at 37°C and pH 7.5. Several colonies grew 3 days after incubation and each colony was isolated and identified by MALDI-TOF-MS using a Microflex LT spectrometer (Bruker Daltonics, Bremer, Germany) as previously described [6]. The spectra generated were analysed using MALDI Biotyper 3.0 software against the Bruker database, which is constantly updated with the Microbes Evolution, Phylogeny, and Infections (MEPHI) database (Fig. 1). Strain Marseille-P9525T was derived from a single colony after three subcultures and remained non-identified by MALDI-TOF-MS.
Phenotypic characteristics

The colonies were circular and smooth with an average diameter of 0.3 mm. The bacterial cells were Gram-positive, rod-shaped, with length 1.7 μm and width 0.3 μm (Fig. 2). Strain Marseille-P9525^T exhibited catalase-negative and oxidase-negative activities. The biochemical characteristics of strain Marseille-P9525^T are summarized in Table 1. API 50CH (bioMérieux, La Balme-les-grottes, France) and API ZYM (bioMérieux) tests were performed at 37°C under anaerobic conditions and the results are summarized in Table 1. In API ZYM strips, positive enzymatic reactions were observed for acid phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, lipase (C14), naphthol-AS-BI-phosphohydrolase, valine arylamidase, α-glucosidase and β-galactosidase; and negative reactions were observed for alkaline phosphatase, N-acetyl-β-glucosaminidase, trypsin, α-galactosidase, α-chymotrypsin, α-fucosidase, α-mannosidase, β-glucosidase and β-glucuronidase. In API 50CH strips, methyl-β-D-xylopyranoside, D-galactose, D-glucose, methyl-α-D-glucopyranoside, arbutin and inulin were positive. Negative reactions were obtained for glycerol, erythol, D-arabinose, L-arabinose, D-ribose, D-xylene, L-xylene, D-adonitol, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-mannopyranoside, N-acetylglucosamine, amygdalin, esculine, salicine, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-cetogluconate and potassium 5-cetogluconate.
TABLE 1. Differential characteristics of *Enorma burkinafasonensis* strain Marseille-P9525T, *Enorma timonensis* GD5T, *Enorma massiliensis* strain phIT, *Collinsella aerofaciens* strain YIT 10235T, *Collinsella tanakei* strain YIT 12064T and *Coriobacterium glomerans* strain PW2

| Properties                          | E. burkinafasonensis | E. timonensis | E. massiliensis | C. aerofaciens | C. tanakei | Cor. glomerans |
|-------------------------------------|----------------------|---------------|----------------|---------------|-----------|---------------|
| Cell diameter (μm)                  | 0.3                  | 0.58          | 0.57           | 0.3–0.7       | 0.5       | NA            |
| Oxygen requirement                  | anaerobic            | anaerobic     | anaerobic      | anaerobic     | anaerobic | anaerobic     |
| Gram stain                          | +                    | +             | +              | +             | +         | +             |
| Motility                            | —                    | —             | na             | —             | —         | —             |
| Endospore formation                 | —                    | —             | na             | —             | —         | —             |
| Temperature optimum                 | 37°C                 | 37°C          | 37°C           | 37°C          | 37°C      | 37°C          |
| Habitat                             | human gut            | human gut     | human gut      | human gut     | human gut | na            |
| Catalase                            | —                    | —             | na             | —             | —         | —             |
| Oxidase                             | —                    | —             | na             | —             | —         | —             |
| API ZYM                             | —                    | —             | na             | —             | —         | —             |
| Alkaline phosphatase                | —                    | —             | na             | —             | —         | —             |
| Acid phosphatase                    | +                    | +             | +              | +             | +         | +             |
| α-galactosidase                     | +                    | +             | +              | +             | +         | +             |
| β-galactosidase                     | +                    | +             | +              | +             | +         | +             |
| β-glucuronidase                     | +                    | +             | +              | +             | +         | +             |
| α-glucosidase                       | +                    | +             | +              | +             | +         | +             |
| β-glucosidase                       | +                    | +             | +              | +             | +         | +             |
| Esterase (C4)                       | +                    | —             | na             | —             | —         | —             |
| Esterase lipase (C8)                | +                    | —             | na             | —             | —         | —             |
| Lipase (C14)                        | +                    | —             | na             | —             | —         | —             |
| N-acetyl-β-glucosamidase            | —                    | —             | na             | —             | —         | —             |
| Leucine arylamidase                 | +                    | +             | +              | +             | +         | +             |
| Valine arylamidase                  | +                    | +             | na             | +             | +         | +             |
| Cystine arylamidase                 | +                    | na            | na             | na            | na        | na            |
| Tryptin                             | —                    | na            | na             | na            | na        | na            |
| α-chymotrypsin                      | —                    | na            | na             | na            | na        | na            |
| Naphthol-AS-BI-phosphohydrolase     | +                    | —             | na             | —             | —         | —             |
| d-mannosidase                       | —                    | na            | na             | na            | na        | na            |
| d-fucosidase                        | —                    | na            | na             | na            | na        | na            |
| API 50 CH                           | —                    | —             | na             | na            | na        | na            |
| Glycerol                            | —                    | —             | na             | na            | na        | na            |
| Erythol                             | —                    | —             | na             | na            | na        | na            |
| α-arabinose                          | +                    | +             | +              | +             | +         | +             |
| β-arabinose                          | +                    | +             | +              | +             | +         | +             |
| α-ribose                            | +                    | +             | +              | +             | +         | +             |
| α-xylene                            | +                    | +             | +              | +             | +         | +             |
| α-adonitol                          | +                    | +             | +              | +             | +         | +             |
| Methyl-β-D-xylopyranoside           | +                    | —             | na             | +             | +         | +             |
| Methyl-α-D-glucopyranoside          | +                    | —             | na             | —             | —         | —             |
| Methyl-α-D-glucopyranoside          | +                    | —             | na             | —             | —         | —             |
| N-acetylglucosamine                 | —                    | —             | na             | —             | —         | —             |
| Amygdalin                           | —                    | —             | na             | —             | —         | —             |
| Arbutin                             | +                    | —             | —              | —             | —         | —             |
| Esculin                              | +                    | —             | —              | —             | —         | —             |
| Salicin                             | —                    | —             | na             | na            | na        | na            |
| α-cellubiose                         | —                    | —             | na             | —             | —         | —             |
| α-maltose                            | —                    | —             | na             | —             | —         | —             |
| α-lactose                            | —                    | —             | na             | —             | —         | —             |
| α-melibiose                          | —                    | —             | na             | —             | —         | —             |
| α-saccharose                        | —                    | —             | na             | —             | —         | —             |
| α-trahulose                          | —                    | —             | na             | —             | —         | —             |
| Trehalose                           | —                    | —             | na             | —             | —         | —             |
| Inulin                              | —                    | —             | na             | —             | —         | —             |
| α-melezitose                         | —                    | —             | na             | —             | —         | —             |
| α-raffinose                          | —                    | —             | na             | —             | —         | —             |
| Starch                               | —                    | —             | na             | —             | —         | —             |
| Glycogen                            | —                    | —             | na             | —             | —         | —             |
| Xylose                               | —                    | —             | na             | —             | —         | —             |
| Gentiobiose                          | —                    | —             | na             | —             | —         | —             |
| α-turanose                           | —                    | —             | na             | —             | —         | —             |
| α-lxylose                            | —                    | —             | na             | —             | —         | —             |
| α-tagatose                           | —                    | —             | na             | —             | —         | —             |
| α-fucose                             | —                    | —             | na             | —             | —         | —             |
| α-fucose                             | —                    | —             | na             | —             | —         | —             |
| α-arabitol                           | —                    | —             | na             | —             | —         | —             |
| α-arabitol                           | —                    | —             | na             | —             | —         | —             |
| Potassium gluconate                  | —                    | —             | na             | —             | —         | —             |
| Potassium 2-cetogluconate            | —                    | —             | na             | —             | —         | —             |
| Potassium 5-cetogluconate            | —                    | —             | na             | —             | —         | —             |

+, positive result; –, negative result.
FIG. 3. 16S rRNA-based maximum likelihood phylogenetic tree highlighting the position of *Enorma burkinafasonensis* strain Marseille-P9525\(^T\) relative to other closely related species. The respective GenBank accession numbers for 16S rRNA genes are indicated in parentheses. Sequences were aligned using CLUSTAL W with default parameters, and phylogenies were inferred by the software MEGA 7. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 500 times to generate a majority consensus tree. Only bootstrap values \(\geq 70\%\) were retained. The scale bar indicates a 1% sequence divergence.

TABLE 2. Functional annotation of *Enorma burkinafasonensis* sp. nov. predicted genes according to the COGs (clusters of orthologous groups) database

| Code | Values | Descriptions |
|------|--------|--------------|
| J    | 145    | Translation  |
| A    | 0      | RNA processing and modification |
| K    | 127    | Transcription |
| L    | 103    | Replication, recombination and repair |
| B    | 0      | Chromatin structure and dynamics |
| D    | 22     | Cell cycle control, mitosis and meiosis |
| Y    | 0      | Nuclear structure |
| V    | 40     | Defence mechanisms |
| T    | 49     | Signal transduction mechanisms |
| M    | 93     | Cell wall/membrane biogenesis |
| N    | 3      | Cell motility |
| Z    | 0      | Cytoskeleton |
| W    | 0      | Extracellular structures |
| U    | 18     | Intracellular trafficking and secretion |
| O    | 49     | Post-translational modification, protein turnover, chaperones |
| X    | 0      | Miscellaneous proteins |
| C    | 74     | Energy production and conversion |
| G    | 161    | Carbohydrate transport and metabolism |
| E    | 156    | Amino acid transport and metabolism |
| F    | 47     | Nucleotide transport and metabolism |
| H    | 39     | Coenzyme transport and metabolism |
| I    | 42     | Lipid transport and metabolism |
| P    | 60     | Inorganic ion transport and metabolism |
| Q    | 7      | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 188    | General function prediction only |
| S    | 137    | Function unknown |

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FIG. 4. Distribution of functional classes of predicted genes according to the clusters of orthologous groups of proteins of *Enorma burkinafasonensis* sp. nov. among other bacterial taxa type strains.

FIG. 5. Heatmap generated with ORTHOANI values calculated using the OAT software between *Enorma burkinafasonensis* strain Marseille-P9525^T^ and other closely related species with standing in nomenclature.
**Strain identification**

The 16S rRNA gene was sequenced using the Big Dye Terminator v1.1 Cycle Sequencing Kit and 3500xL Genetic Analyzer capillary sequencer (Thermo-Fisher, Saint-Aubin, France), to classify this bacterium as previously described [7]. Amplification was carried out using the primer pair fD1 and rP2 (Eurogentec, Angers, France). The 16S rRNA nucleotide sequence was assembled and corrected using CODONCODE ALIGNER software (http://www.codoncode.com). Strain Marseille-P9525 exhibited a 98.30% sequence identity with Enorma phocaeensis strain Marseille-P3242 (GenBank accession number NR_147403.1), the phylogenetically closest species with standing in nomenclature (Fig. 3). As a consequence, we classify this strain as a member of a new species within the Enorma genus in the Actinobacteria phylum.

**Genome sequencing**

Genomic DNA was extracted using the EZ1 biorobot with the EZ1 DNA tissue kit (Qiagen, Hilden, Germany) and then sequenced on a MiSeq sequencer (Illumina, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit and NexteraXTPairedEnd (Illumina) as previously described [8]. The assembly was performed using a pipeline containing several software (VELVET [9], SPades [10] and SOAPDENOVO [11]), on trimmed data (MiSEQ and TRIMMOMATIC [12] software) or on untrimmed data (only MiSEQ software). GAPCLOSER was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value < 25% of the mean depth were removed. The best assembly was selected using different criteria (number of scaffolds, N50, number of N).

The genome sequence of strain Marseille-P9525T and its genomic content has been reported previously [13]. Briefly, the genome of strain Marseille-P9525T has a total size of 2.23411 Mb, with a G-C content of 66.8%. It is composed of 49 contigs and 48 scaffolds. The genome presents one repeat region, 1951 predicted genes, with 1902 protein-coding genes and 49 tRNA genes. The distribution of genes in functional categories (clusters of orthologous groups) is shown within Table 2. The distribution of genes into clusters of orthologous groups categories was similar in all nine compared genomes (Fig. 4). The degree of genomic similarity of strain Marseille-P9525T with closely related species was estimated using ORTHOANI version 0.93.1 [14] (https://www.ezbiocloud.net/tools/orthoani) and yielded 84.20% sequence similarity with Enorma phocaeensis,

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**Table 3. DNA-DNA hybridization (DDH) values obtained by sequence comparison of all studied genomes using GGDC, formula 2 (DDH Estimates Based on Identities/HSP length)**

| Strain                           | Marseille-P9525T | Marseille-P3242 | Timonensis | Massiliensis | Aerofaciens | Bouches | Ihuae | Tanakaei | Glomerans |
|----------------------------------|-----------------|----------------|------------|--------------|-------------|---------|-------|----------|-----------|
| | 25.30% (23%–27.8%) | 28.40% (26%–30.9%) | 24.60% (22.3%–27.1%) | 21.50% (19.3%–24.9%) | 27.00% (24.6%–29.5%) | 21.00% (18.8%–23.4%) | 21.00% (18.8%–23.4%) | 20.00% (18.3%–21.9%) | 24.20% (22.5%–26.5%) | 24.50% (22.7%–26.6%) |
| | 25.20% (22.9%–27.7%) | 22.60% (20.8%–23.5%) | 21.70% (19.5%–24.2%) | 21.00% (18.8%–23.4%) | 26.60% (24.2%–29.5%) | 21.00% (18.8%–23.4%) | 21.00% (18.8%–23.4%) | 20.00% (18.3%–21.9%) | 24.20% (22.5%–26.6%) | 24.50% (22.7%–26.6%) |
| | 26.60% (24.2%–29.5%) | 21.70% (19.5%–24.2%) | 21.00% (18.8%–23.4%) | 21.00% (18.8%–23.4%) | 24.00% (21.7%–26.5%) | 21.00% (18.8%–23.4%) | 21.00% (18.8%–23.4%) | 20.00% (18.3%–21.9%) | 24.20% (22.5%–26.6%) | 24.50% (22.7%–26.6%) |
80.77% with *Enorma timonensis*, 78.68% with *Enorma massiliensis*, 77.35% with *Collinsella ihuae* and 74.57% with *Collinsella tanakaei* (Fig. 5), which is lower than the 95% threshold used to discriminate bacterial species. *In silico* DNA–DNA hybridization values obtained using the GGDC version 2.0 online tool (http://ggdc.dsmz.de/ggdc.php#) are reported in Table 3. For strain Marseille-P9525T, these values ranged from 20.50% with *Coriobacterium glomerans* strain PW2 to 28.40% with *Enorma phocaeensis* strain Marseille-P3242. Such values were lower than the 70% threshold recognized to delineate distinct species (Table 4).

### Conclusion

On the basis of unique phenotypic features, including the MALDI-TOF spectrum, a 16S rRNA sequence divergence of >1.3%, DNA–DNA hybridization values < 70% and an
**OrthoANI value of <95% of the phylogenetically closest species with standing in nomenclature, we formally propose strain Marseille-P9525T as the type strain of Enorma burkinafasonensis sp. nov., a new species within the genus Enorma (Fig. 6).**

**Description of Enorma burkinafasonensis sp. nov.**

*Enorma burkinafasonensis* (bur.ki.na.fa.so.nen’sis, L. masc. adj. *burkinafasonensis* related to Burkina Faso, the name of the country where the sample was collected). The bacterium belongs to the family Coriobacteriaceae within the phylum Actinobacteria. The type strain Marseille-P9525T (CSUR P9525) was isolated after 3 days at 37°C and pH 7.5 in an anaerobic atmosphere from a fresh stool sample collected from a 28-year-old Burkinabe woman suspected of having tuberculosis. Colonies are circular and smooth. Bacterial cells are Gram-positive, rod-shaped, non-motile and non-spore-forming bacteria with negative catalase and oxidase activities. Using an APIZYM strip, train shaped, non-motile and non-spore-forming bacteria with negative reactions for acid phosphatase and oxidase activities. Using an API 50CH strip, positive reactions for acid phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, lipase (C14), naphthol-AS-BI-phosphohydrolase, valine arylamidase, α-glucosidase and β-glucosidase, but negative reaction for alkaline phosphatase, N-acetyl-β-D-glucosaminidase, trypsin, α-galactosidase, α-chymotrypsin, α-fucosidase, α-mannosidase, β-glucosidase and β-galactosidase. Using an API 50CH strip, positive reactions were obtained for methyl-β-D-xylopyranoside, d-galactose, d-glucose, methyl-α-D-glucopyranoside, arbutin and inulin.

The genome of Marseille-P9525T is 2.23411 Mb long, with a G-C content of 66.8%.

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LR742709 and GCA_902150035.1, respectively.

**Nucleotide sequence accession number**

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LR742709 and GCA_902150035.1, respectively.

**Deposit in culture collections**

Strain Marseille-P9525 T was deposited in the CSUR (Collection de Souches de l’Unité des Rickettsies) under number CSURP9525.

**Conflict of interest**

None to declare.

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