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

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Abstract

Strain Marseille-P9525^T is a Gram-positive, obligatory anaerobic and non-motile bacterium isolated from a human faecal micobiota. 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.

Keywords: Culturomics, *Enorma burkinafasonensis*, gut microbiota, new species, taxono-genomics
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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'Éthique 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 (bioMérieux, Marcy l'Étoile, France) and incubated under anaerobic atmosphere generated by AnaeroGen (bioMérieux), 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-P9525^T was derived from a single colony after three subcultures and remained non-identified by MALDI-TOF-MS.

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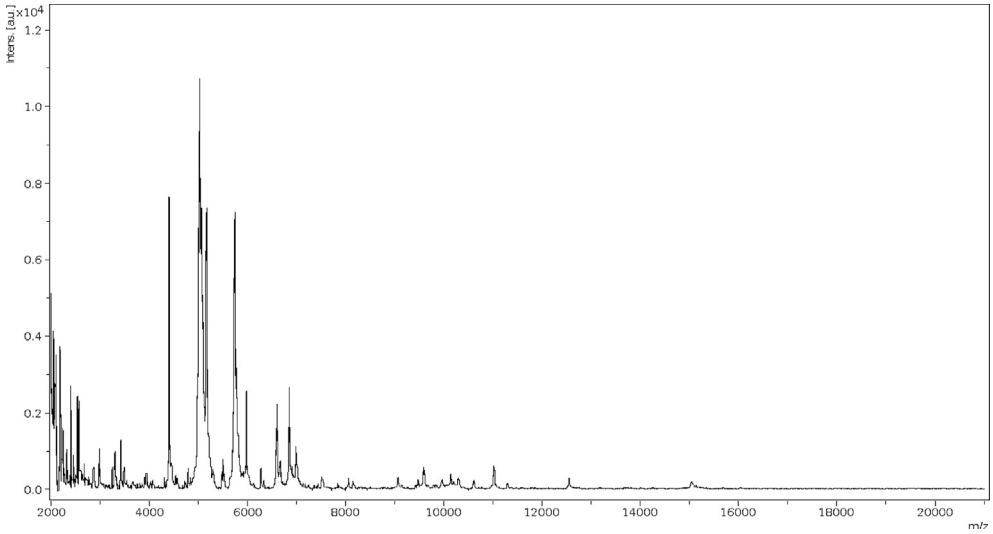
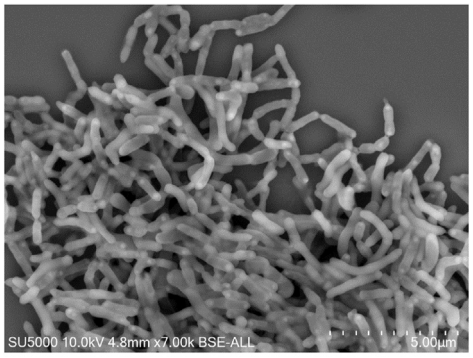


FIG. 1. MALDI-TOF-MS reference mass spectrum of *Enorma burkinafasonensis* sp. nov. strain Marseille-P9525^T obtained by comparing the spectra of 06 individual colonies.

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-βD-xylopyranoside, D-galactose, D-glucose, methyl-αD-glucopyranoside, arbutin and inulin were positive. Negative reactions were obtained for glycerol, erythol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, D-fructose, D-mannose, L-sorbose, L-rhammose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-αD-mannopyranoside, *N*-acetylglucosamine, amygdalin, esculin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentibiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-cetogluconate and potassium 5-cetogluconate.



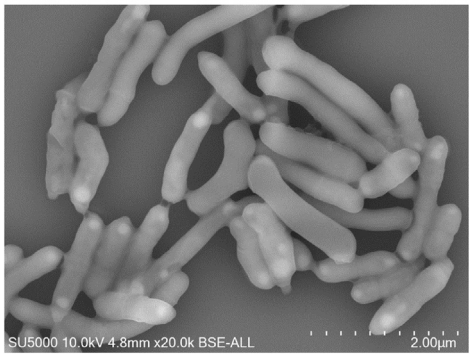


FIG. 2. Scanning electron micrograph (SEM) of *Enorma burkinafasonensis* sp. nov. strain Marseille-P9525^T. Colony was collected from *agar* and immersed in 2.5% glutaraldehyde fixative solution. Drop of suspension was directly deposited on poly-L-lysine-coated microscope slide for 5 min and treated with 1% phosphotungstic acid aqueous solution (pH 2.0) for 2 min to increase SEM image contrast. Slide was gently washed in water, air-dried and examined with tabletop SEM (TM4000; Hitachi, Yokohama, Japan). Scales and acquisition settings are shown.

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*
	Marseille-P9525T	GD5T	phIT	YIT 10235T	YIT 12064T	PW2
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	—	na
Oxidase	—	—	—	na	—	na
API ZYM						
Alkaline phosphatase	—	—	—	—	+	na
Acid phosphatase	+	—	na	—	+	na
α-galactosidase	—	+	+	—	—	na
β-galactosidase	+	+	+	+	—	na
β-glucuronidase	—	—	—	—	+	na
α-glucosidase	+	+	+	+	—	na
β-glucosidase	—	+	+	—	+	na
Esterase (C4)	+	—	na	—	—	na
Esterase lipase (C8)	+	—	na	—	—	na
Lipase (C14)	+	—	na	na	—	na
N-acetyl-β-glucosaminidase	—	—	—	—	—	na
Leucine arylamidase	+	—	—	+	+	na
Valine arylamidase	+	+	na	+	+	na
Cystine arylamidase	+	na	na	na	na	na
Trypsin	—	na	na	na	na	na
α-chymotrypsin	—	na	na	na	na	na
Naphthol-AS-BI-phosphohydrolase	+	na	na	na	na	na
α-mannosidase	—	na	na	na	na	na
α-fucosidase	—	na	na	na	na	na
API 50 CH						
Glycerol	—	—	—	na	na	na
Erythol	—	—	—	na	na	na
D-arabinose	—	—	—	na	na	na
L-arabinose	—	—	—	na	na	na
D-ribose	—	—	—	na	na	na
D-xylose	—	—	—	na	na	na
L-xylose	—	—	—	na	na	na
D-adonitol	—	—	—	na	na	na
Methyl-βD-xylopyranoside	+	—	—	na	na	na
D-galactose	+	—	—	na	na	na
D-glucose	+	—	—	na	na	na
D-fructose	—	—	—	na	na	na
D-mannose	—	—	—	na	na	na
L-sorbose	—	—	—	na	na	na
L-rhammose	—	—	—	na	na	na
Dulcitol	—	—	—	na	na	na
Inositol	—	—	—	na	na	na
D-mannitol	—	—	—	na	na	na
D-sorbitol	—	—	—	na	na	na
Methyl-αD-mannopyranoside	—	—	—	na	na	na
Methyl-αD-glucopyranoside	+	—	—	na	na	na
N-acetylglucosamine	—	—	—	na	na	na
Amygdalin	—	—	—	na	na	na
Arbutin	+	—	—	na	na	na
Esculin	—	—	—	na	na	na
Salicin	—	—	—	na	na	na
D-cellobiose	—	—	—	na	na	na
D-maltose	—	—	—	na	na	na
D-lactose	—	—	—	na	na	na
D-melibiose	—	—	—	na	na	na
D-saccharose	—	—	—	na	na	na
D-trehalose	—	—	—	na	na	na
Inulin	+	—	—	na	na	na
D-melezitose	—	—	—	na	na	na
D-raffinose	—	—	—	na	na	na
Starch	—	—	—	na	na	na
Glycogen	—	—	—	na	na	na
Xylitol	—	—	—	na	na	na
Gentibiose	—	—	—	na	na	na
D-turanose	—	—	—	na	na	na
D-lyxose	—	—	—	na	na	na
D-tagatose	—	—	—	na	na	na
D-fucose	—	—	—	na	na	na
L-fucose	—	—	—	na	na	na
D-arabitol	—	—	—	na	na	na
L-arabitol	—	—	—	na	na	na
Potassium gluconate	—	—	—	na	na	na
Potassium 2-cetogluconate	—	—	—	na	na	na
Potassium 5-cetogluconate	—	—	–9	na	na	na

+, positive result; –, negative result.

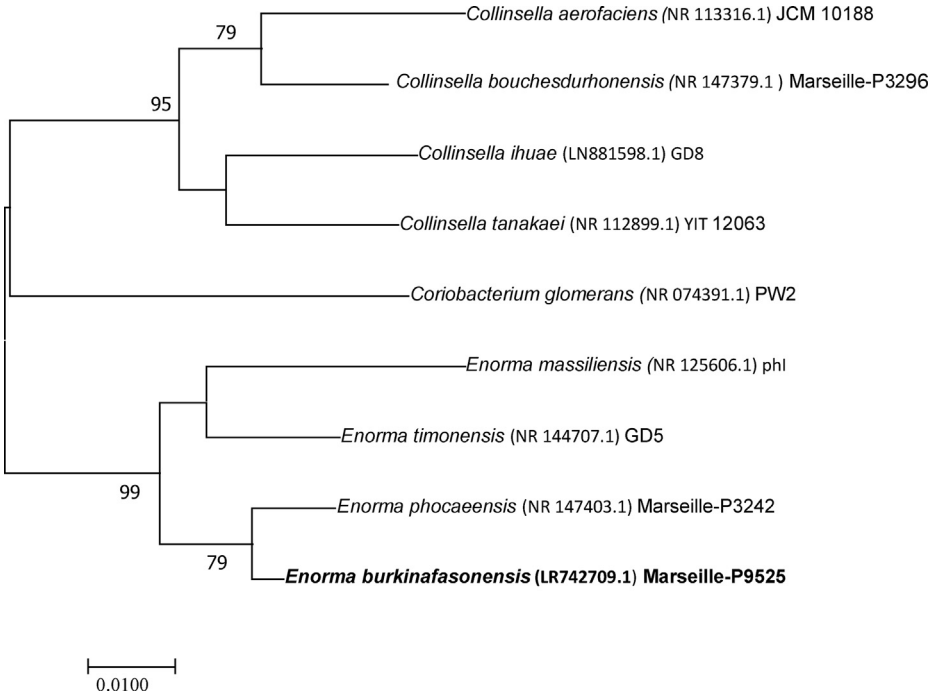


FIG. 3. 16S rRNA-based maximum likelihood phylogenetic tree highlighting the position of *Enorma burkinafasonensis* strain Marseille-P9525^T relative to other closely related species. The respective GenBank accession numbers for 16S rRNA genes are indicated in parentheses. Sequences were aligned using 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 bare 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	Mobilome: prophages, transposons
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

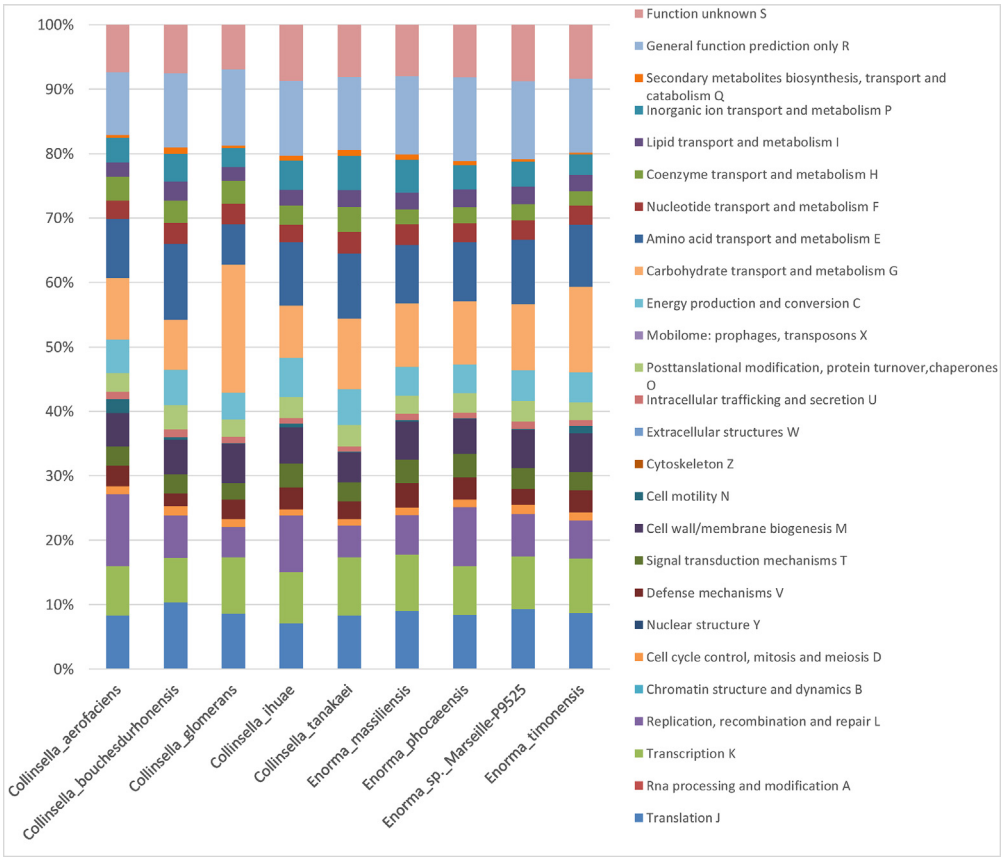


FIG. 4. Distribution of functional classes of predicted genes according to the clusters of orthologous groups of proteins of *Enorma burkinafasonensis* sp. nov. among other bacterial taxa type strains.

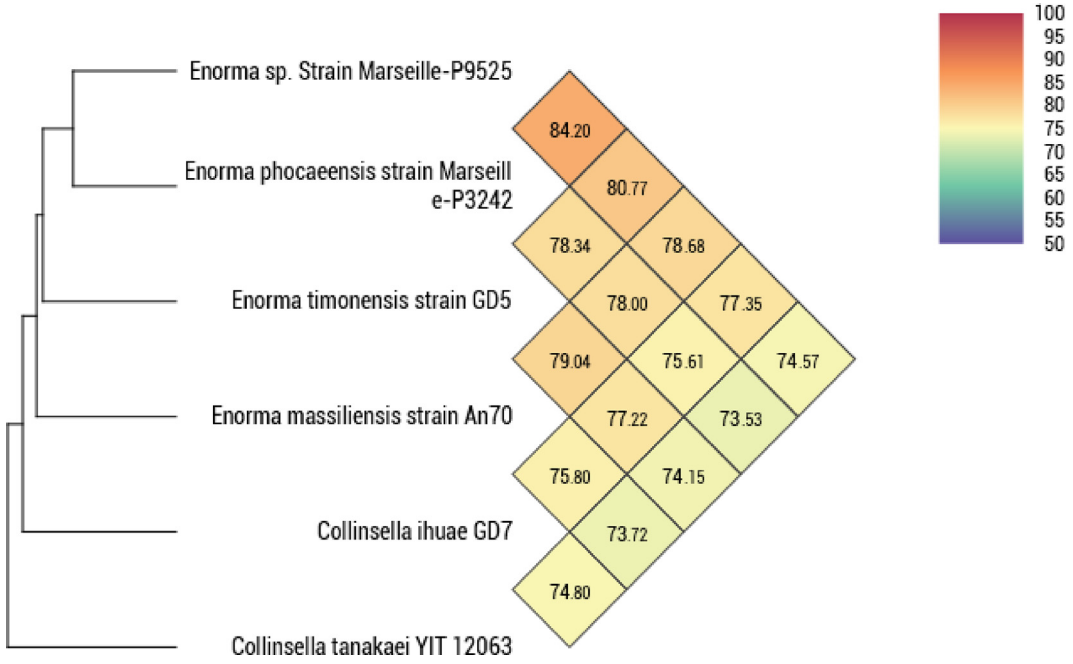


FIG. 5. Heatmap generated with OrthoANI values calculated using the OAT software between *Enorma burkinafasonensis* strain Marseille-P9525^T and other closely related species with standing in nomenclature.

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)

Digital DNA-DNA hybridization										
	Enorma_sp._Marseille-P9525	*Enorma_timonensis_GD5*	*Enorma_phocaeensis_Marseille-P3242*	*Enorma_massiliensis_phI*	*Collinsella_aerofaciens_ATCC_25986*	*Collinsella_bouchesdurhonensis_Marseille-P3296*	*Collinsella_lhuae_GD8*	*Collinsella_tanakaei_YIT_12063*	*Coriobacterium_glomerans_PW2*
Enorma_sp._Marseille-P9525									
Enorma_timonensis_GD5	25.30% (23%–27.8%)								
Enorma_phocaeensis_Marseille-P3242	28.40% (26%–30.9%)	24.60% (22.3%–27.1%)							
Enorma_massiliensis_phI	24.60% (22.3%–27.1%)	25.20% (22.9%–27.7%)	26.60% (24.2%–29.1%)						
Collinsella_aerofaciens_ATCC_25986	23.50% (21.2%–26%)	22.10% (19.9%–24.6%)	22.60% (20.3%–25%)	21.50% (19.3%–24%)					
Collinsella_bouchesdurhonensis_Marseille-P3296	23.40% (21.1%–25.8%)	21.90% (19.6%–24.3%)	21.90% (19.6%–24.3%)	21.70% (19.5%–24.2%)	25.00% (22.7%–27.5%)				
Collinsella_lhuae_GD8	23.70% (21.4%–26.1%)	24.00% (21.7%–26.5%)	23.90% (21.6%–26.3%)	24.00% (21.7%–26.5%)	22.10% (19.8%–24.5%)	21.40% (19.2%–23.9%)	22.70% (20.4%–25.2%)		
Collinsella_tanakaei_YIT_12063	22.20% (19.9%–24.7%)	22.20% (19.9%–24.6%)	22.30% (20%–24.7%)	22.90% (20.6%–25.3%)	22.50% (20.3%–25%)	22.00% (19.7%–24.4%)	20.40% (18.2%–22.8%)	21.60% (19.3%–24%)	
Coriobacterium_glomerans_PW2	20.50% (18.3%–22.9%)	20.50% (18.2%–22.9%)	21.40% (19.2%–23.9%)	21.00% (18.8%–23.5%)	21.00% (18.8%–23.4%)	20.50% (18.3%–22.9%)			

Strain identification

The 16S rRNA gene was sequenced using the Big Dye Terminator v1.1 Cycle Sequencing Kit and 3500xLGenetic 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 Nextera XT Paired End (Illumina) as previously described [8]. The assembly was performed using a pipeline containing several software (VELVET [9], SPADES [10] and SOAP DENOVO [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-P9525^T and its genomic content has been reported previously [13]. Briefly, the genome of strain Marseille-P9525^T 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-P9525^T 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*,

TABLE 4. Description of *Enorma burkinafasonensis* sp. nov. strain Marseille-P9525^T

Type of description	New description
Species name	Burkinafasonensis
Genus name	*Enorma*
Specific epithet	Burkinafasonensis
Species status	sp. nov.
Species etymology	*Enorma burkinafasonensis* (bur.ki.na.fa.so.nen'sis, L. masc. adj. burkinafasonensis related to Burkina Faso, the name of the c ountry where the sample was collected).
Authors	Nina GOUBA, Maxime Descartes MBOGNING FONKOU, Yasmine HASSANI, Jamal SAAD, Michel DRANCOURT, Mustapha FELLAG
Designation of the type strain	Marseille-P9525
Strain collection number	CSURP9525
16S rRNA gene accession number	LR742709.1
Genome accession number	GCA_902150035.1
Genome status	Whole genome
Genome size	2.23411 Mb
GC%	66.8
Country of origin	Bobo-Dioulasso, Burkina Faso
Date of isolation	01/02/2019
Source of isolation	Human stool sample
Growth medium, incubation conditions used for standard cultivation	Columbia agar supplemented with 5% sheep's blood, 37°C for 72 hours of incubation under anaerobic atmosphere generated by AnaeroGen (bioMérieux), at 37°C and pH 7.5.
Gram strain	Positive
Cell shape	Rod
Cell size	Mean diameter 0.3 µm and length 1.7 µm
Motility	Non-motile
Sporulation	No
Colony morphology	Circular and smooth with an average diameter of 0.3 mm
Temperature range	37°–45°C
Lowest temperature for growth	37°C
Temperature optimum	37°C
Relationship to O_2	Strictly anaerobe
O_2 for strain testing	Anaerobiosis, microaerophilic, aerobiosis
Oxidase	Negative
Catalase	Negative

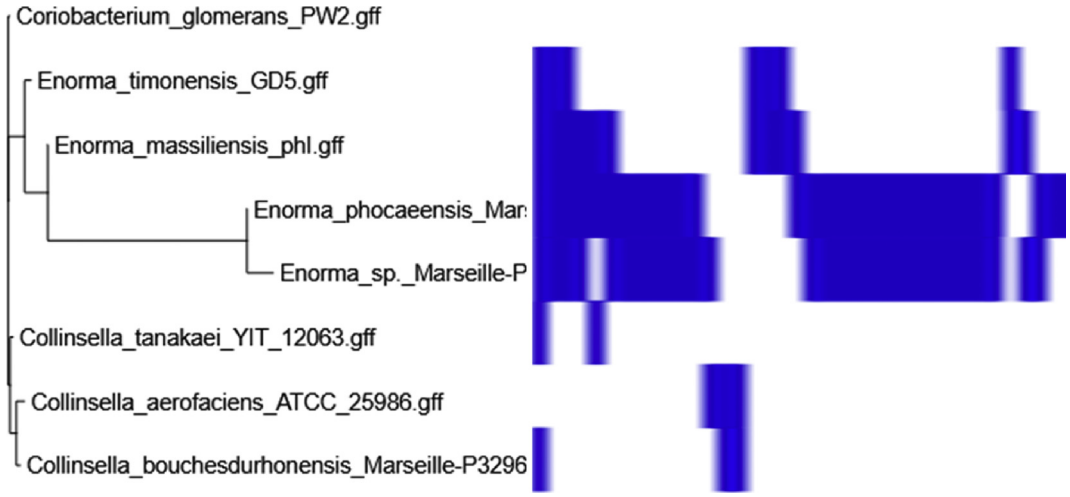


FIG. 6. Pangenome.

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-P9525^T, 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-P9525^T 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-P9525^T (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 Marseille-P9525^T exhibits 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-galactosidase, but negative reaction for alkaline phosphatase, *N*-acetyl- β-glucosaminidase, trypsin, α-galactosidase, α-chymotrypsin, α-fucosidase, α-mannosidase, β-glucosidase and β-glucuronidase. Using an API 50CH strip, positive reactions were obtained for methyl-βD-xylopyranoside, D-galactose, D-glucose, methyl-αD-glucopyranoside, arbutin and inulin.

The genome of Marseille-P9525^T 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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References

[1] Lagier J-C, 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.
[2] Lagier J-C, Hugon P, Khelaifia S, Fournier P-E, Scola BL, Raoult D. The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. Clin Microbiol Rev 2015;28:237–64.
[3] Lagier J-C, Khelaifia S, Alou MT, Ndongo S, Dione N, Hugon P, et al. Culture of previously uncultured members of the human gut microbiota by culturomics. Nat Microbiol 2016;1:1–8.
[4] Fournier P-E, Lagier J-C, Dubourg G, Raoult D. From culturomics to taxonomogenomics: a need to change the taxonomy of prokaryotes in clinical microbiology. Anaerobe 2015;36:73–8.
[5] Ramasamy D, Mishra AK, Lagier J-C, Padhmanabhan 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.
[6] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier P-E, Rolain JM, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 2009;49:543–51.
[7] Morel AS, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta JP. Complementarity between targeted real-time specific PCR and conventional broad-range 16S rDNA PCR in the syndrome-driven diagnosis of infectious diseases. Eur J Clin Microbiol Infect Dis 2015;34:561–70.
[8] Diop A, Khelaifia S, Armstrong N, Labas N, Fournier PE, Raoult D. Microbial culturomics unravels the halophilic microbiota repertoire of table salt: description of *Gracilibacillus massiliensis* sp. nov. Microb Ecol Health Dis 2016;27.
[9] Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 2008;18:821–9.

[10] Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19:455–77.
[11] Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J. SOAPdenov02: an empirically improved memory-efficient short-read de novo assembler. Gigascience 2012;1:18.
[12] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114–20.
[13] Gouba N, Saad J, Drancourt M, Fellag M. Genome sequence of *Enorma* sp. strain Marseille-P9525T, a member of a human gut microbiome. Microb Resour Announc 2019;8.
[14] Lee I, Ouk Kim Y, Park S-C, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–3.