

Non contiguous-finished genome sequence and description of *Enorma timonensis* sp. nov.

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Keywords: *Enorma timonensis*, genome, culturomics, taxono-genomics

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Enorma timonensis strain GD5^T sp. nov., is the type strain of *E. timonensis* sp. nov., a new member of the genus *Enorma* within the family *Coriobacteriaceae*. This strain, whose genome is described here, was isolated from the fecal flora of a 53-year-old woman hospitalized for 3 months in an intensive care unit. *E. timonensis* is an obligate anaerobic rod. Here we describe the features of this organism, together with the complete genome sequence and annotation. The 2,365,123 bp long genome (1 chromosome but no plasmid) contains 2,060 protein-coding and 52 RNA genes, including 4 rRNA genes.

Introduction

Enorma timonensis strain GD5^T (= CSUR P900 = DSM 26111) is the type strain of *E. timonensis* sp. nov. This bacterium was isolated from the stool of a 53-year-old French woman hospitalized 3 months in an intensive care unit for Guillain-Barre syndrome, as part of a culturomics study aiming at cultivating individually all species within human feces [1-3]. It is a Gram-positive, anaerobic, non-endospore forming, indole-negative, rod-shaped bacillus.

The human gut microbiota consists of billions of microorganisms that outnumber the human cells [4]. Advances in DNA sequence-based technologies and the development of 16S ribosomal RNA sequence-based metagenomic methods have been used to explore the complex gut microbial population, which has a crucial role in human health and disease development [5,6]. The currently used strategy for determining the taxonomic status of a bacterial isolate includes comparing it to its phylogenetically closest neighbors in terms of 16S rRNA gene similarity, G + C content and DNA-DNA hybridization (DDH) [7,8]. However, although considered “gold standards” in bacterial taxonomy, these criteria do not apply to all genera [9,10]. The development of high-throughput sequencing

methods [11] enabled the generation of complete genomic sequences for most bacterial species of medical interest (more than 6,000 bacterial genomes sequenced to date). We recently proposed to describe new bacterial species using a polyphasic approach based on their genome sequence, MALDI-TOF spectrum and main phenotypic characteristics [12-34].

Here, we present a summary classification and a set of features for *E. timonensis* sp. nov. strain GD5^T (= CSUR P900 = DSM 26111) as well as the description of the complete genome sequencing and annotation. These characteristics support the circumscription of the species *E. timonensis*.

The family *Coriobacteriaceae* (Stackebrandt *et al.* 1997) was created in 1997 [35] and presently consists of 13 validated genera [36]: *Adlercreutzia* (Maruo *et al.* 2008) [37], *Asaccharobacter* (Minamida *et al.* 2008) [38], *Atopobium* (Collins and Wallbanks 1993) [39], *Collinsella* (Kageyama *et al.* 1999) [40], *Coriobacterium* (Haas and König 1988) [41], *Cryptobacterium* (Nakazawa *et al.* 1999) [42], *Denitrobacterium* (Anderson *et al.* 2000) [43], *Eggerthella* (Wade *et al.* 1999) [44], *Enterorhabdus* (Clavel *et al.* 2009) [45], *Gordonibacter* (Würdemann *et al.* 2009) [46], *Olsenella* (Dewhirst *et al.* 2001) [47], *Paraeggerthella*

(Würdemann *et al.* 2009) [46], *Slackia* (Wade *et al.* 1999) [44], and the recently described new genus *Enorma* (Mishra *et al.* 2013) [29]. These microorganisms are anaerobic, Gram-positive, rod-shaped bacteria [42]. Members of the family *Coriobacteriaceae* are isolated from the fecal microbiota of humans or animals, and may cause infections such as bacteremia, wound infections and periodontal/endodontic infections. Members of this family also interfere with the metabolism of triglycerides, glucose, and glycogen in humans and animals [35-47].

Classification and features

A stool sample was collected from a 53-year-old woman living in Marseille, France and hospitalized for 3 months in an intensive care unit for Guillain-Barre syndrome. She received antibiotics at the time of stool sample collection. The patient gave an informed and signed consent, and the agreement of the local ethics committee of the Institut Federatif de Recherche 48 (Marseille, France) was obtained under agreement 09-022. The fecal specimen was preserved at -80°C after collection. Strain GD5^T (Table 1) was isolated in 2012 by anaerobic cultivation at 37°C on 5% sheep blood-enriched Columbia agar (BioMerieux, Marcy l'Etoile, France), after 3 weeks of preincubation of the stool sample with clarified and sterile sheep rumen in an anaerobic blood culture bottle.

The 16S rDNA sequence (GenBank accession number JX424767) of *E. timonensis* strain GD5^T exhibited the highest similarity (95.0%) with its phylogenetically closest published species, *Enorma massiliensis* (Figure 1). By comparison with the type species of genera from the family *Coriobacteriaceae*, *E. timonensis* exhibited a 16S rDNA sequence similarity ranging from 84 to 95%. This value was lower than the 98.7% 16S rDNA gene sequence threshold recommended by Stackebrandt and Ebers to delineate a new species without carrying out DNA-DNA hybridization [8].

Growth at different temperatures (25, 30, 37, 45°C) was tested. No growth was observed at 25°C or 30°C. Growth occurred at both 37 and 45°C, but optimal growth was observed at 37°C after 48 hours of incubation. Colonies were translucent grey and approximately 0.4 mm in diameter on 5% sheep blood-enriched Columbia agar (BioMerieux). Growth of the strain was tested in

blood-enriched Columbia agar under anaerobic and microaerophilic conditions using GENbag anaer and GENbag microaer systems, respectively (BioMerieux), and under aerobic conditions, with or without 5% CO₂. Growth was achieved only anaerobically. Gram staining showed Gram-positive and non-sporulated rods (Figure 2). A motility test was negative. Cells grown on agar have a mean diameter of 0.58 µm and a mean length of 1.32 µm, and are mostly grouped in short chains or small clumps (Figure 3).

Strain GD5^T exhibited neither catalase nor oxidase activities (Table 2). Using an API ZYM strip (BioMerieux), positive reactions were observed for leucine arylamidase, valine arylamidase, cystine arylamidase, naphthol-AS-BI-phospho-hydrolase, β-galactosidase, β-glucuronidase, α-glucosidase and β-glucosidase. Negative reactions were observed for acid phosphatase, nitrate reduction, urease alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), trypsin, α-chemotrypsin, acid phosphatase, α-galactosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase. Using an API Rapid ID 32A strip (BioMerieux), positive reactions were observed for proline arylamidase, phenylalanine arylamidase, histidin arylamidase, serine arylamidase. Negative reactions were observed for urease, arginine dihydrolase, tyrosin arylamidase, leucyl-glycyl arylamidase, alanine arylamidase, glycine arylamidase and arginine arylamidase. Using an API 50 CH strip (BioMerieux), negative reactions were recorded for fermentation of glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl-βD-xylopranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-xylopyranoside, methyl-α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, esculin ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, inulin, D-melezitose, D-raffinose, amidon, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate, potassium-5-ketogluconate.

E. timonensis is susceptible to amoxicillin-clavulanic acid, metronidazole, imipenem, vancomycin, rifampicin, gentamicin and resistant to penicillin G, amoxicillin, ceftriaxon, erythromycin, and trimethoprim/sulfamethoxazole.

Table 1. Classification and general features of *Enorma timonensis* strain GD5^T according to the MIGS recommendations [48]

MIGS ID	Property	Term	Evidence code ^a
		Domain <i>Bacteria</i>	TAS [49]
		Phylum <i>Actinobacteria</i>	TAS [50,51]
		Class <i>Actinobacteria</i>	TAS [35]
	Current classification	Order <i>Coriobacteriales</i>	TAS [29,52,53]
		Family <i>Coriobacteriaceae</i>	TAS [52,53]
		Genus <i>Enorma</i>	TAS [53]
		Species <i>Enorma timonensis</i>	IDA
		Type strain GD5 ^T	IDA
	Gram stain	positive	IDA
	Cell shape	rod	IDA
	Motility	non motile	IDA
	Sporulation	non sporulating	IDA
	Temperature range	mesophile	IDA
	Optimum temperature	37°C	IDA
MIGS-6.3	Salinity	unknown	IDA
MIGS-22	Oxygen requirement	anaerobic	IDA
	Carbon source	unknown	NAS
	Energy source	unknown	NAS
MIGS-6	Habitat	human gut	IDA
MIGS-15	Biotic relationship	free living	IDA
MIGS-14	Pathogenicity	Unknown	
	Biosafety level	2	
	Isolation	human feces	
MIGS-4	Geographic location	France	IDA
MIGS-5	Sample collection time	January 2012	IDA
MIGS-4.1	Latitude	43.296482	IDA
MIGS-4.1	Longitude	5.36978	IDA
MIGS-4.3	Depth	Surface	IDA
MIGS-4.4	Altitude	0 m above sea level	IDA

^aEvidence codes - IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable 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 [54]. If the evidence is IDA, then the property was directly observed for a live isolate by one of the authors or an expert mentioned in the acknowledgements.

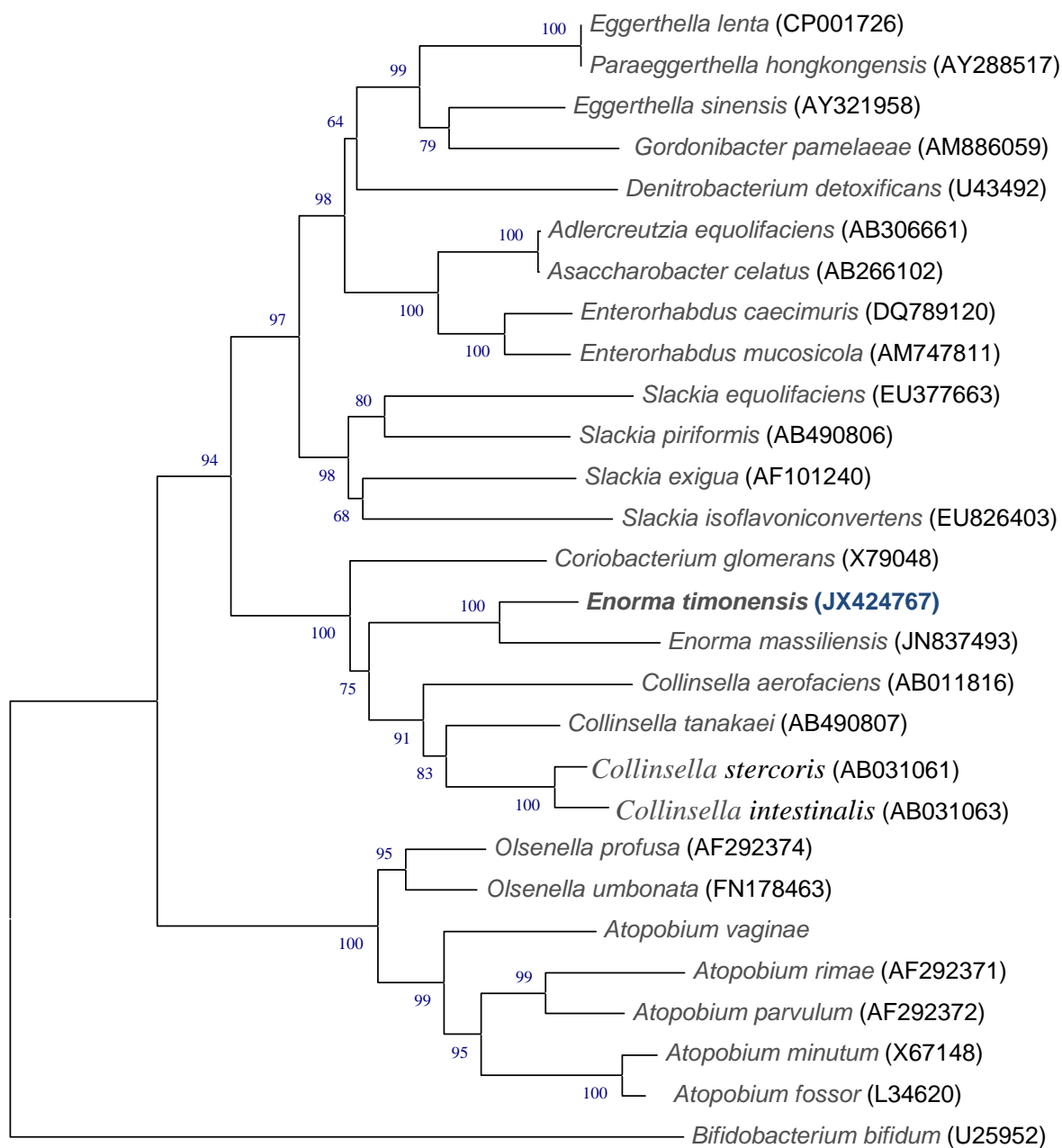


Figure 1. Phylogenetic tree highlighting the position of *Enorma timonensis* strain GD5^T relative to other type strains within the *Coriobacteriaceae* family. GenBank accession numbers are indicated in parentheses. Sequences were aligned using CLUSTALW, and phylogenetic inferences obtained using the maximum-likelihood method within the MEGA software. Numbers at the nodes are percentages of 500 bootstrap replicates supporting that node. The tree is a majority consensus tree. *Bifidobacterium bifidum* was used as outgroup. The scale bar represents a 2% nucleotide sequence divergence.

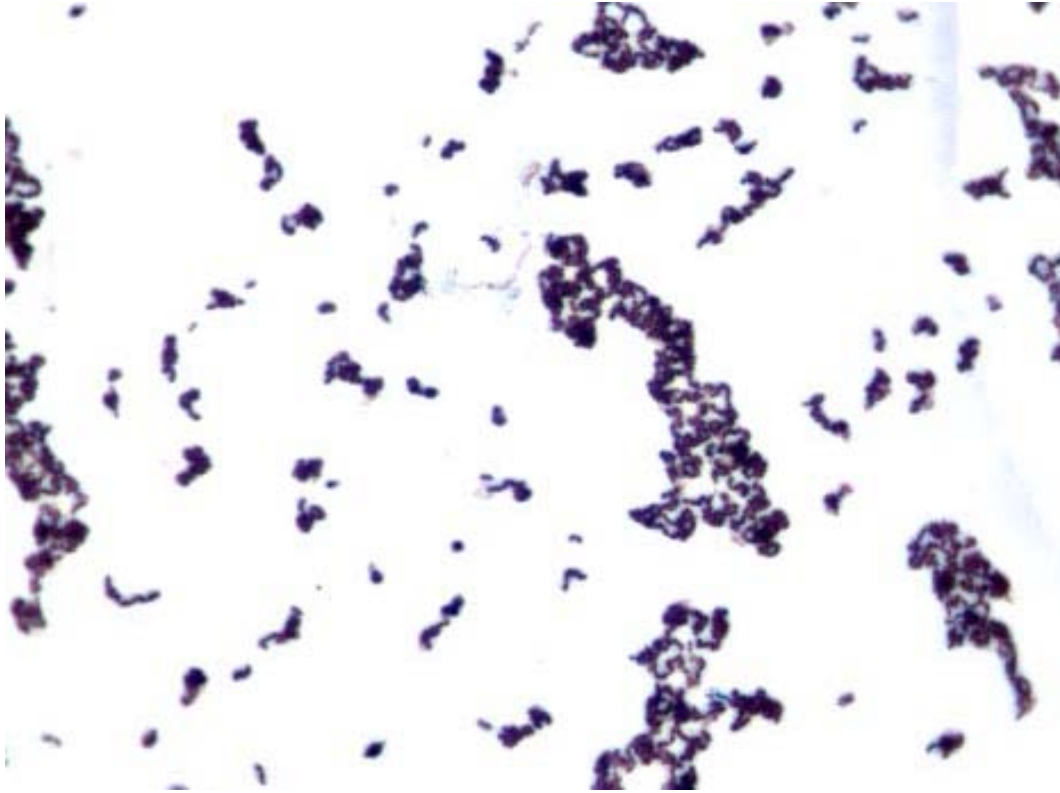


Figure 2. Gram staining of *E. timonensis* strain GD5^T.

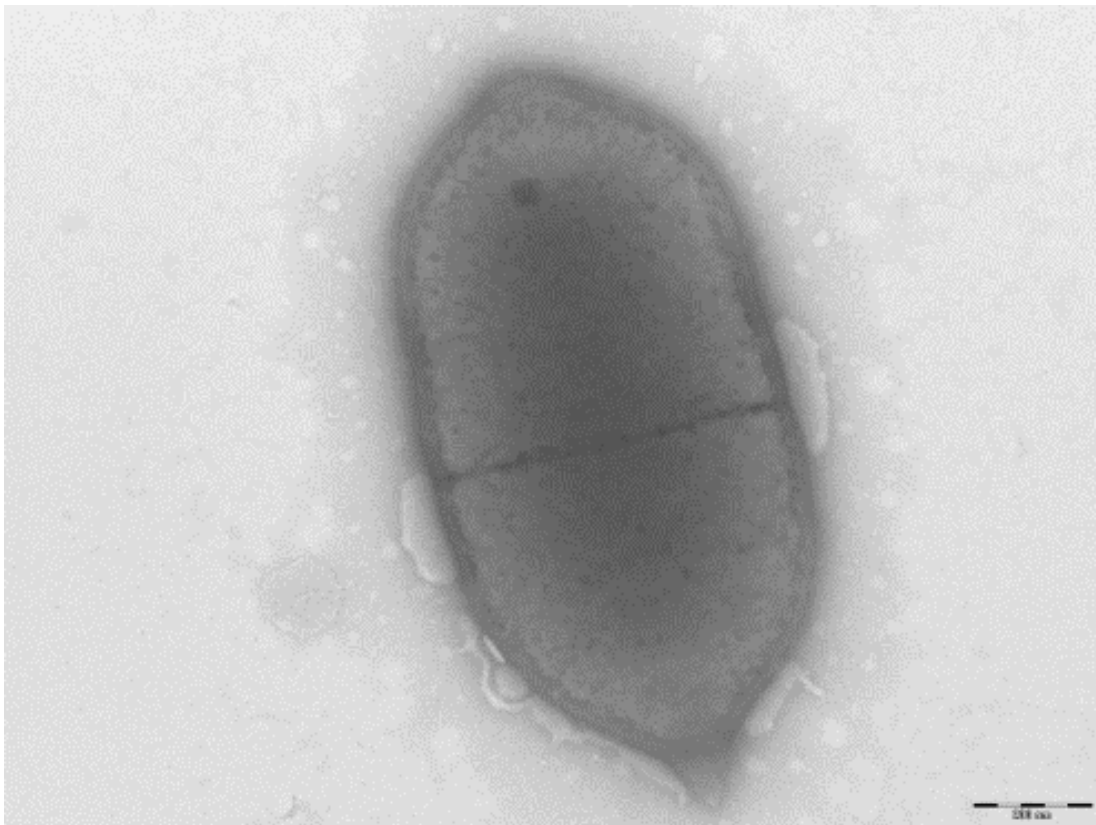


Figure 3. Transmission electron microscopy of *E. timonensis* strain GD5^T using a Morgani 268D (Philips) at an operating voltage of 60kV. The scale bar represents 200μm.

Table 2. Differential characteristics of *Enorma timonensis* GD5^T, *Enorma massiliensis* strain phi^T, *Collinsella aerofaciens* strain YIT 10235^T, *Collinsella tanakei* strain YIT 12064^T and *Coriobacterium glomerans* strain PW2.

Properties	<i>E. timonensis</i>	<i>E. massiliensis</i>	<i>C. aerofaciens</i>	<i>C. tanakei</i>	<i>C. glomerans</i>
Cell diameter (µm)	0.58	0.57	0.3 – 0.7	0.5	NA
Oxygen requirement	anaerobic	anaerobic	anaerobic	anaerobic	anaerobic
Gram stain	+	+	+	+	+
Salt requirement	na	na	na	na	na
Motility	-	-	na	-	-
Endospore formation	-	-	-	na	-
Production of					
Alkaline phosphatase	-	-	-	+	na
Acid phosphatase	-	na	-	+	na
Catalase	-	-	na	-	na
Oxidase	-	-	na	-	na
Nitrate reductase	-	-	na	-	na
Urease	-	-	-	-	na
α-galactosidase	+	+	-	-	na
β-galactosidase	+	+	+	-	na
β-glucuronidase	-	-	-	+	na
α-glucosidase	+	+	+	-	na
β-glucosidase	+	+	-	+	na
Esterase	-	na	-	-	na
Esterase lipase	-	na	-	-	na
Indole	-	-	na	-	na
N-acetyl-β-glucosaminidase	-	-	-	-	na
6-Phospho-β-galactosidase	-	-	-	-	na
Arginine arylamidase	+	+	+	+	na
glutamic acid decarboxylase	-	-	-	-	na
Leucyl glycine arylamidase	-	-	+	+	na
Alanine arylamidase	-	-	-	-	na
Proline arylamidase	+	+	+	+	na
Serine arylamidase	+	-	-	-	na
Tyrosine arylamidase	-	-	-	-	na
Glycine arylamidase	-	-	+	+	na
Utilization of					
D-mannose	-	+	+	+	+
Habitat	human gut	human gut	human gut	human gut	na

na: data not available

+/-: depending on tests used

Matrix-assisted laser-desorption/ionization time-of-flight (MALDI-TOF) MS protein analysis was carried out as previously described [55] using a Microflex spectrometer (Bruker Daltonics, Leipzig, Germany). Twelve distinct deposits were done for strain GD5^T from twelve isolated colonies. The twelve GD5^T spectra were imported into the MALDI BioTyper software (version 2.0, Bruker)

and analyzed by standard pattern matching (with default parameter settings) against the main spectra of 4,706 bacteria, which were used as reference data, in the BioTyper database. For strain GD5^T, no significant score was obtained, thus suggesting that our isolate was not a member of a known species. We added the spectrum from strain GD5^T to our database (Figure 4, Figure 5).

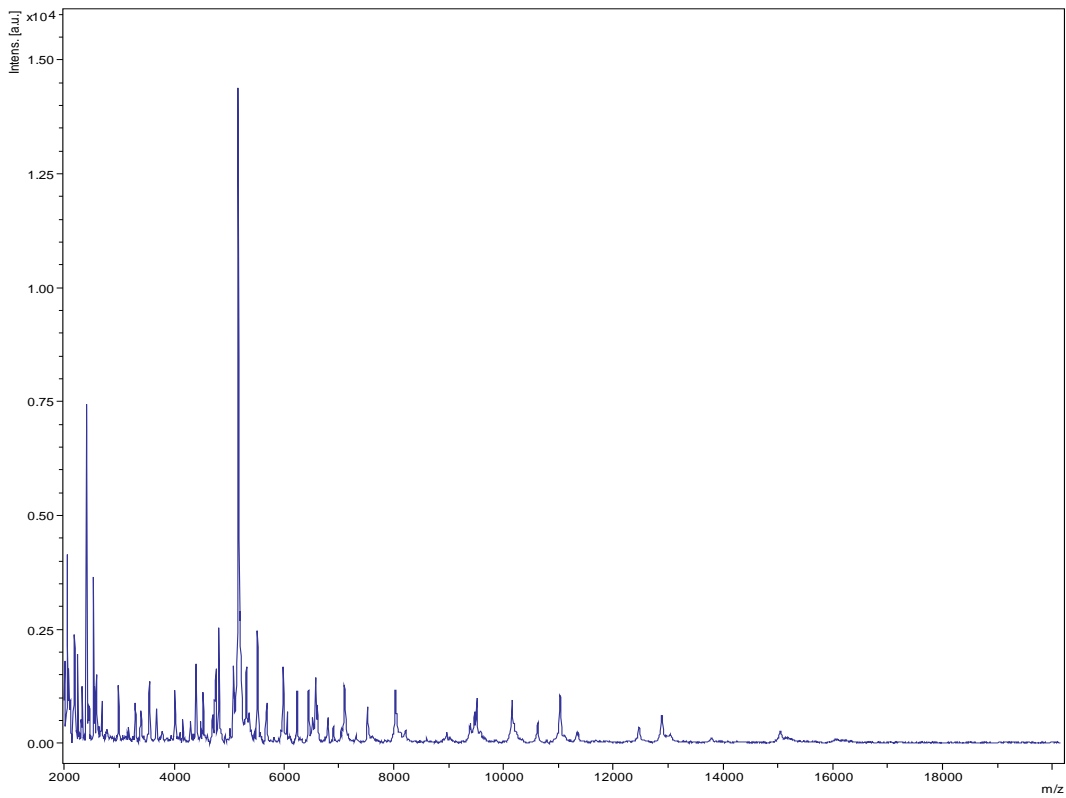


Figure 4. Reference mass spectrum from *E. timonensis* strain GD5^T. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

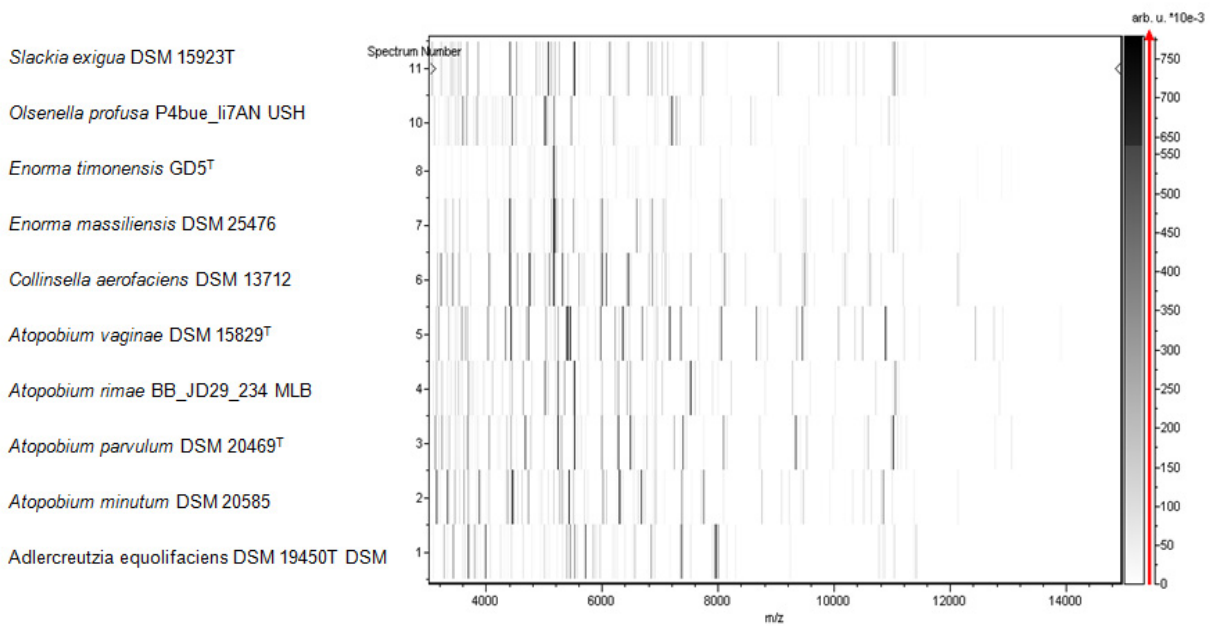


Figure 5. Gel view comparing *E. timonensis* sp. nov strain GD5^T and other members of the *Coriobacteriaceae* family. The gel view displays the raw spectra of all loaded spectrum files arranged in a pseudo-gel like look. The x-axis records the m/z value. The left y-axis displays the running spectrum number originating from subsequent spectra loading. The peak intensity is expressed by a gray scale scheme code. The color bar and the right y-axis indicate the relation between the color a peak is displayed with and the peak intensity in arbitrary units. Displayed species are indicated on the left.

Genome sequencing information

Genome project history

The organism was selected for sequencing on the basis of its phylogenetic position and 16S rDNA similarity to *E. massiliensis* and other members of the family *Coriobacteriaceae* and is part of a study of the human digestive flora aiming at isolating all

bacterial species within human feces [1-3]. It was the 2nd genome of an *Enorma* species and the first genome of *E. timonensis* sp. nov. The GenBank accession number is CAPF00000000 and consists of 105 contigs. Table 3 shows the project information and its association with MIGS version 2.0 compliance [48].

Table 3. Project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	High-quality draft
MIGS-28	Libraries used	One paired-end 454 3-kb library
MIGS-29	Sequencing platforms	454 GS FLX Titanium
MIGS-31.2	Fold coverage	43.5
MIGS-30	Assemblers	Newbler version 2.5.3
MIGS-32	Gene calling method	Prodigal
	INSDC ID	PRJEB543
	GenBank ID	CAPF00000000
	GenBank Date of Release	April 25, 2013
MIGS-13	Project relevance	Study of the human gut microbiome

Growth conditions and DNA isolation

Enorma timonensis sp. nov., strain GD5^T (= CSUR P900 = DSM 26111), was grown anaerobically on 5% sheep blood-enriched Columbia agar (BioMerieux) at 37°C. Four Petri dishes were spread and resuspended in 1ml TE buffer prior to being treated with 2.5 µg/µL lysozyme for 30 minutes at 37°C, and then with Proteinase K overnight at 37°C. The DNA was then purified by 3 successive phenol-chloroform extractions followed by an ethanol precipitation at -20°C overnight. Following centrifugation, the DNA was then resuspended in 305 µL TE buffer. The DNA was then concentrated and purified using a QIAamp kit (Qiagen). The yield and concentration was measured by the Quant-it Picogreen kit (Invitrogen) on the Genios Tecan fluorometer at 66.5 ng/µL.

Genome sequencing and assembly

DNA (5 µg) was mechanically fragmented on a Hydroshear device (Digilab, Holliston, MA, USA) with an enrichment size at 3-4kb. The DNA fragmentation was visualized through the Agilent 2100 BioAnalyzer on a DNA labchip 7500 with an optimal size of 4.4kb. A 3kb paired-end library was constructed according to the 454 GS FLX Titanium paired-end protocol (Roche). Circularization and nebulization were performed and generated a pattern with an optimal at 470 bp. After

PCR amplification through 17 cycles followed by double size selection, the single stranded paired-end library was then quantified on the Agilent 2100 BioAnalyzer on a RNA pico 6000 LabChip at 136 pg/µL. The library concentration equivalence was calculated as 5.31E+08 molecules/µL. The library was stored at -20°C until further use.

The paired-end library was clonally amplified with 0.5cpb and 2cbp in 2 SV-emPCR with the GS Titanium SV-emPCR Kit (Lib-L) v2 (Roche). The yields of the emPCRs were 9.37 and 14.09%, respectively, in the range of 5 to 20% from the Roche procedure.

Approximately 790,000 beads were loaded on 1/4 region of a GS Titanium PicoTiterPlate PTP Kit 70x75 and sequenced with the GS-FLX Titanium Sequencing Kit XLR70 (Roche). The run was performed overnight and then analyzed on the cluster through the gsRunBrowser and gsAssembler (Roche). A total of 282,633 passed filter wells were obtained and generated 102.68Mb with a length average of 363 bp. The globally passed filter sequences were assembled using Newbler with 90% identity and 40bp as overlap. The final assembly identified 5 scaffolds and 105 large contigs (>1,500 bp) generating a genome size of 2.36 Mb which corresponds to a coverage of 43.5 genome equivalents.

Genome annotation

Open Reading Frames (ORFs) were predicted using Prodigal [56] with default parameters. However, the predicted ORFs were excluded if they spanned a sequencing gap region. The predicted bacterial protein sequences were searched against the GenBank [57] and Clusters of Orthologous Groups (COG) databases using BLASTP. The tRNAs and rRNAs were predicted using the tRNAScanSE [58] and RNAmmer [59] tools, respectively. Lipoprotein signal peptides and numbers of transmembrane helices were predicted using SignalP [60] and TMHMM [61], respectively. ORFans were identified if their BLASTP *E*-value was lower than 1e-03 for alignment length greater than 80 amino acids. If alignment lengths were smaller than 80 amino acids, we used an *E*-value of 1e-05. Such parameter thresholds have already been used in previous works to define ORFans. Artemis [62] and DNA Plotter [63] were used for data management and visualization of genomic features, respectively. The Mauve alignment tool (version 2.3.1) was used for multiple genomic sequence alignment [64]. To estimate the mean level of nucleotide sequence similarity at the genome level between *E. timonensis* and five other members of the family *Coriobacteriaceae* (Table 6), we used the Average Genomic Identity Of gene Sequences (AGIOS) home-made software. Briefly, this software combines the Proteinortho software [65] for detecting orthologous proteins between genomes compared two by two, then retrieves the corresponding genes and determines the mean percentage of nucleotide sequence identity among orthologous ORFs using the Needleman-Wunsch global alignment algorithm. *Enorma timonensis* strain GD5^T was compared to *E. massiliensis* strain phI^T (GenBank accession number CAGZ000000000), *C. aerofaciens* strain ATCC 25986 (AAVN000000000), *C. tanakei* strain YIT 12063 (ADLS000000000) and *C. glomerans* strain PW2 (NC_015389).

Genome properties

The genome is 2,365,123 bp long (1 chromosome, no plasmid) with a 65.8% G+C content (Figure 6

and Table 4). Of the 2,060 predicted chromosomal genes, 2,006 were protein-coding genes and 52 were RNAs, including a complete rRNA operon, an additional 5S rRNA and 48 tRNAs. A total of 1,384 genes (67.18%) were assigned a putative function. Fifty-five genes were identified as ORFans (2.74%) and the remaining genes were annotated as hypothetical proteins. The properties and statistics of the genome are summarized in Tables 3 and 4. The distribution of genes into COGs functional categories is presented in Table 5.

Genome comparison of *E. timonensis* with other members of the *Coriobacteriaceae* family

We compared the genome of *E. timonensis* strain GD5^T with those of *E. massiliensis* phI, *Collinsella aerofaciens* strain ATCC 25986, *Collinsella tanakaei* strain YIT 12063 and *Coriobacterium glomerans* strain PW2 (Table 6).

The draft genome sequence of *E. timonensis* strain GD5^T is smaller than those of *C. aerofaciens* and *C. tanakaei* (2.36, 2.43 and 2.48 Mb, respectively), but larger than those of *E. massiliensis* and *C. glomerans* (2.26 and 2.11 Mb, respectively). The G+C content of *E. timonensis* is larger than those of *E. massiliensis*, *C. aerofaciens*, *C. tanakaei* and *C. glomerans* (65.80, 62.0, 60.54, 60.23 and 60.40%, respectively). The gene content of *E. timonensis* is smaller to those of *E. massiliensis*, *C. glomerans* and *C. tanakaei* (2,006, 2,159 and 2,195, respectively) but larger than those of *C. aerofaciens* and *C. tanakaei* (1,901 and 1,768, respectively). The distribution of genes into COG categories was not entirely similar in all compared genomes (Figure 7).

In addition, *E. timonensis* shared 1,109, 1,026, 880 and 1,077 orthologous genes with *E. massiliensis*, *C. aerofaciens*, *C. glomerans* and *C. tanakaei* respectively. The average genomic nucleotide sequence identity ranged from 66.37 to 79.44% among *Coriobacteriaceae* family members, and from 66.01 to 79.44% between *E. timonensis* and other species (Table 6 and Table 7).

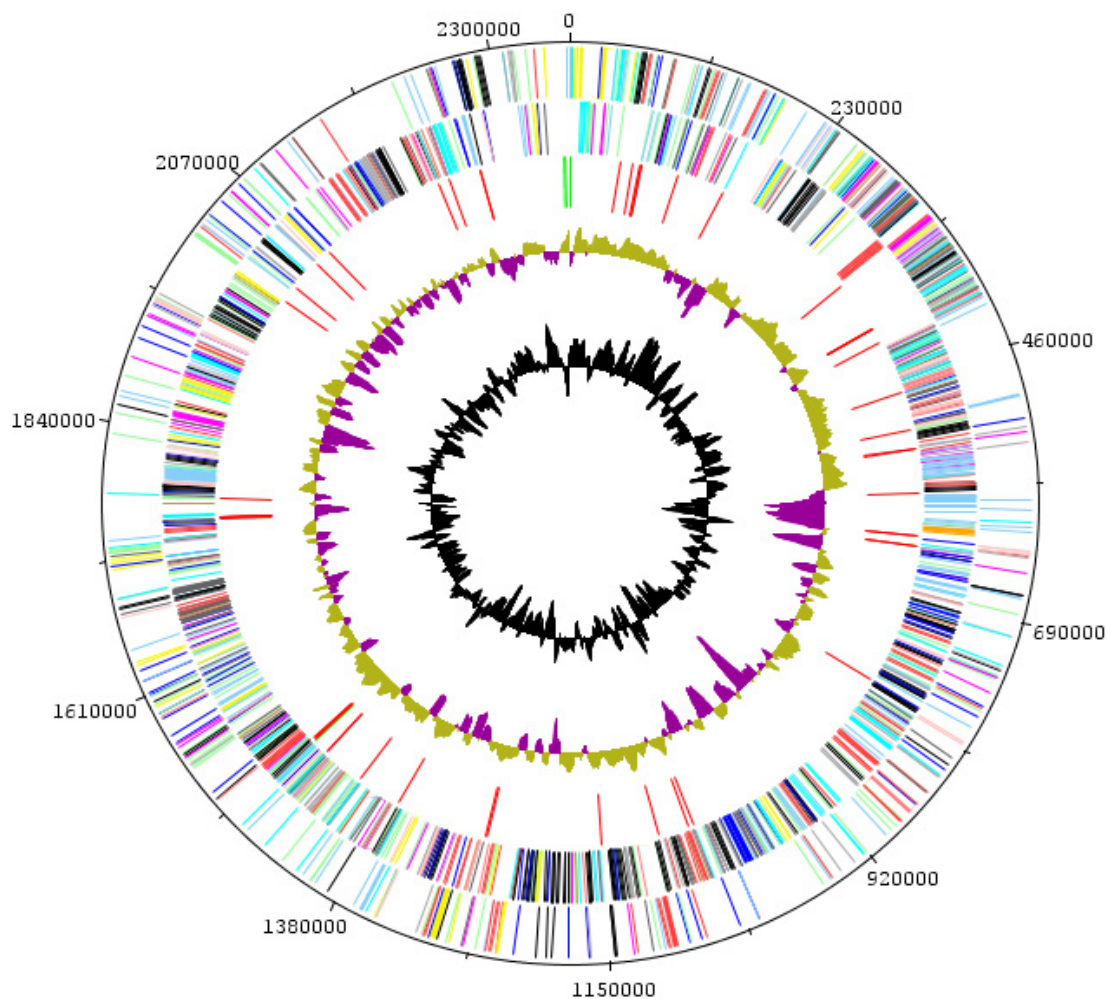


Figure 6. Graphical circular map of the chromosome. From the outside in: genes on the forward strand (colored by COG categories), genes on the reverse strand (colored by COG categories), RNA genes (rRNAs green, tRNAs red), GC skew (purple: negative values, olive: positive values), and G+C content plot.

Table 4. Nucleotide content and gene count levels of the genome

Attribute	Value	% of total ^a
Genome size (bp)	2,365,123	
DNA coding region (bp)	2,061,753	87.17
DNA G+C content (bp)	1,556,250	65.8
Total genes	2,060	100
RNA genes	52	2.62
Protein-coding genes	2,006	97.37
Genes with function prediction	1,384	67.18
Genes assigned to COGs	1,518	73.68
Genes with peptide signals	88	4.27
Genes with transmembrane helices	466	22.62

^aThe 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

Table 5. Number of genes associated with the 25 general COG functional categories

Code	Value	% of total ^a	Description
J	140	6.98	Translation
A	0	0	RNA processing and modification
K	152	7.58	Transcription
L	90	4.48	Replication, recombination and repair
B	1	0.05	Chromatin structure and dynamics
D	20	1.0	Cell cycle control, mitosis and meiosis
Y	0	0	Nuclear structure
V	52	2.59	Defense mechanisms
T	61	3.04	Signal transduction mechanisms
M	92	4.58	Cell wall/membrane biogenesis
N	3	0.15	Cell motility
Z	0	0	Cytoskeleton
W	0	0	Extracellular structures
U	16	0.80	Intracellular trafficking and secretion
O	45	2.24	Posttranslational modification, protein turnover, chaperones
C	78	3.88	Energy production and conversion
G	224	11.16	Carbohydrate transport and metabolism
E	172	8.57	Amino acid transport and metabolism
F	50	2.49	Nucleotide transport and metabolism
H	40	1.99	Coenzyme transport and metabolism
I	38	1.89	Lipid transport and metabolism
P	71	3.54	Inorganic ion transport and metabolism
Q	13	0.65	Secondary metabolites biosynthesis, transport and catabolism
R	205	10.22	General function prediction only
S	118	5.88	Function unknown
-	488	24.32	Not in COGs

^a The total is based on the total number of protein coding genes in the annotated genome

Table 6. Genomic comparison of *E. timonensis* and four other members of the *Coriobacteriaceae* family

Species	Strain	Genome accession number	Genome size (Mb)	G+C content
<i>E. timonensis</i>	GD5 ^T	CAPF00000000	2,365,123	65.80
<i>E. massiliensis</i>	phI	CAGZ01000000	2,263,008	62.0
<i>C. aerofaciens</i>	ATCC 25986	AAVN00000000	2,439,869	60.54
<i>C. glomerans</i>	PW2	NC_015389	2,115,681	60.40
<i>C. tanakaei</i>	YIT 12063	ADLS00000000	2,482,197	60.23

Table 7. Genomic comparison of *E. timonensis* and four other members of the *Coriobacteriaceae* family^a

	<i>E. timonensis</i>	<i>E. massiliensis</i>	<i>C. aerofaciens</i>	<i>C. glomerans</i>	<i>C. tanakaei</i>
<i>E. timonensis</i>	2,006	1109	1026	880	1077
<i>E. massiliensis</i>	79.44	1,901	1046	899	1103
<i>C. aerofaciens</i>	66.37	66.01	2,159	880	1062
<i>C. glomerans</i>	73.39	72.38	66.15	1,768	913
<i>C. tanakaei</i>	74.02	73.43	64.96	71.27	2,195

^aUpper right triangle: numbers of orthologous protein shared between genomes; lower left triangle: average percentage similarity of nucleotides corresponding to orthologous proteins shared between genomes; bold face: numbers of proteins per genome.

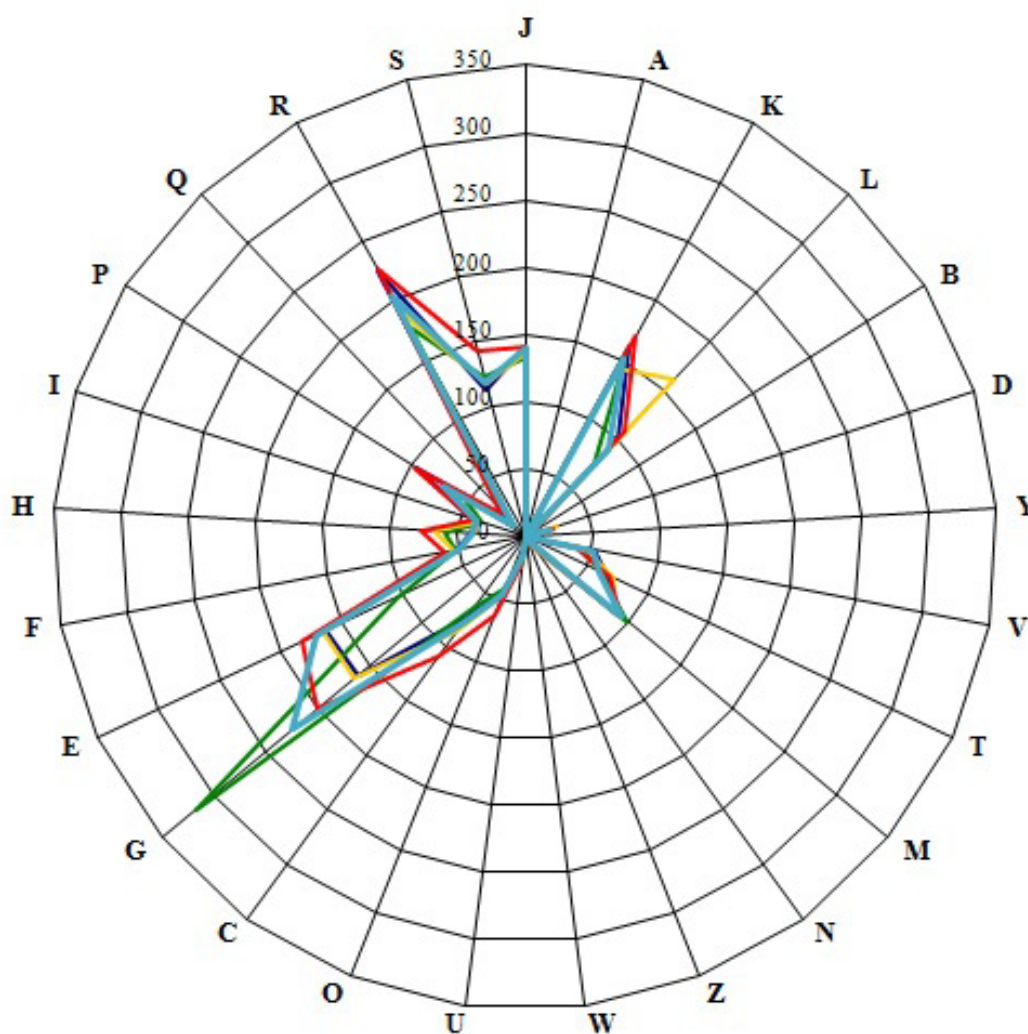


Figure 7. Distribution of functional classes of predicted genes in the *E. timonensis* (colored in light blue), *E. massiliensis* (dark blue), *Coriobacterium glomerans* (green), *Colinsella aerofaciens* (yellow) and *Colinsella tanakaei* (red) chromosomes, according to the clusters of orthologous groups of proteins.

Conclusion

On the basis of phenotypic, phylogenetic and genomic analyses (taxono-genomics), we formally propose the creation of *Enorma timonensis* sp. nov. that contains strain GD5^T. This bacterium has been found in France.

Description of *Enorma timonensis* sp. nov.

Enorma timonensis (ti.mo.nen'sis. L. gen. fem. *timonensis*, of Timone, the name of the hospital where strain GD5^T was cultivated). Colonies are translucent grey and 0.4 mm in diameter on blood-enriched Columbia agar. Cells are rod-shaped with a mean diameter of 0.58 µm and a mean length of 1.32 µm. Optimal growth is achieved in anaerobic conditions. No growth is observed in aerobic or microaerophilic conditions. Growth occurs between 37-45°C, with optimal growth being observed at 37°C on blood-enriched Columbia agar. Cells are Gram-positive, non-endospore forming, and non-motile. Cells are negative for catalase and oxidase. Using an API ZYM strip, positive reactions are observed for leucine arylamidase, valine arylamidase, cystin arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucuronidase, α-glucosidase and β-glucosidase. Negative reactions are observed for acid phosphatase, nitrate reduction, urease alka

line phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), trypsin, α-chemotrypsin, acid phosphatase, α-galactosidase, N-actetyl-β-glucosaminidase, α-mannosidase, α-fucosidase. Using an API Rapid ID 32A strip, positive reactions are observed for proline arylamidase, phenylalanine arylamidase, histidin arylamidase, serine arylamidase. Negative reactions are observed for urease, arginine dihydrolase, tyrosin arylamidase, leucyl-glycyl arylamidase, alanine arylamidase, glycine arylamidase and arginine arylamidase. Using an API 50 CH strip, fermentation or assimilation was not observed.

Cells are susceptible to amoxicillin-clavulanic acid, metronidazole, imipenem, vancomycin, rifampicin, gentamicin and resistant to penicillin G, amoxicillin, ceftriaxon, erythromycin, and trimethoprim/sulfamethoxazole. The 16S rDNA and genome sequences are deposited in GenBank under accession numbers JX424767 and [CAPF00000000](https://doi.org/10.1093/nar/gkt000), respectively. The G+C content of the genome is 65.8%. The habitat of the organism is the human digestive tract. The type strain GD5^T (= CSUR P900 = DSM 26111) was isolated from the fecal flora of a 53-year old French patient hospitalized in an intensive care unit. This strain has been found in Marseille, France.

Acknowledgments

The authors thank the Xegen Company for automating the genomic annotation process. This

study was funded by the Mediterranee Infection Foundation.

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