High-quality genome sequencing and description of *Dermabacter indicis* sp. nov.

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Abstract

Strain FF11^T was isolated from the wound on a researcher's finger who had been bitten by a fish (*Protopterus annectens*) in Senegal. Analysis by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry did not provide any identification, but the 16S rRNA sequence exhibited 97.9% identity with *Dermabacter hominis*. Phenotypic and genomic analyses demonstrated that strain FF11^T is Gram-positive, facultatively anaerobic, nonmotile and non–spore forming; it exhibited a genome of 2 222 902 bp encoding 2074 protein-coding and 50 RNA genes, with a 63.2% G+C content. We consequently proposed the creation of *Dermabacter indicis* strain FF11^T.

Keywords: Bacteria, culturomics, *Dermabacter indicis*, genome, taxonogenomics
Original Submission: 22 December 2015; **Revised Submission:** 8 February 2016; **Accepted:** 16 February 2016
Article published online: 23 February 2016

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Introduction

The *Dermabacter* genus is considered a common colonizer of human skin [1]. Currently this genus includes only one validly published species named *Dermabacter hominis* [2], which was formerly known as the coryneform bacteria of the Centers for Disease Control groups 3 and 5 [3,4]. Members of this genus are Gram-positive, non–spore forming, non–acid fast, nonmotile, short rods, facultatively anaerobic, catalase positive and oxidase negative [1]. *Dermabacter hominis* is involved in bacteraemia as a rare pathogen [5]. *D. hominis* has also been detected in clinical samples such as wound swabs, bronchial washings, abscesses and ear smears [3–6].

Recently, high-throughput genome sequencing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) analyses of bacteria have given unprecedented access to an abundance of genetic and proteomic information [7,8]. Thus, a polyphasic approach is currently proposed in our laboratory to describe new bacterial taxa, including their genome sequence, MALDI-TOF spectrum, and major phenotypic characteristics such as Gram staining, culture conditions, metabolic characteristics, habitat and, if applicable, pathogenicity [9].

Here we present a summary classification and a set of features for *Dermabacter indicis* sp. nov., together with a description of the complete genome sequencing and annotation. These characteristics support the circumscription of the *Dermabacter indicis* species.

Classification and features

Strain isolation and identification

In May 2014, while working at Dakar, the index finger of a researcher was bitten by a fish. Strain FF11^T (Table 1) was isolated from this wound by culture on 5% sheep's blood–enriched Columbia agar (bioMérieux, Marcy l'Etoile, France). In order to

http://dx.doi.org/10.1016/j.nmni.2016.02.007

TABLE 1. Classification and general features of *Dermabacter indicis* strain FF11^T

MIGS ID	Property	Term	Evidence code[a]
	Current classification	Domain: *Bacteria*	TAS [10]
		Phylum: *Actinobacteria*	TAS [11]
		Class: *Actinobacteria*	TAS [12,13]
		Order: *Micrococcales*	TAS [13,14]
		Family: *Dermabacteraceae*	TAS [13–15]
		Genus: *Dermabacter*	TAS [1]
		Species: *Dermabacter indicis*	IDA
		Type strain: FF11^T	IDA
	Gram stain	Positive	IDA
	Cell shape	Rods	IDA
	Motility	Nonmotile	IDA
	Sporulation	Non–spore forming	IDA
	Temperature range	30–37°C	IDA
	Optimum temperature	37°C	IDA
	pH range; optimum	7.4–7.2; 7.6	
	Carbon source	Unknown	
MIGS-6	Habitat	Human wound	IDA
MIGS-6.3	Salinity	Unknown	
MIGS-22	Oxygen requirement	Facultatively anaerobic	IDA
MIGS-15	Biotic relationship	Free-living	IDA
MIGS-14	Pathogenicity	Unknown	
MIGS-4	Geographic location	Senegal	IDA
MIGS-5	Sample collection	June 2014	IDA
MIGS-4.1	Latitude	14.6937000	IDA
MIGS-4.1	Longitude	−17.4440600	IDA
MIGS-4.4	Altitude	12 m above sea level	IDA

MIGS, minimum information about a genome sequence.
[a]Evidence 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) [16]. 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.

identify the strain FF11^T, MALDI-TOF protein analysis was performed using a Microflex LT (Bruker Daltonics, Leipzig, Germany), as previously reported [17,18]. The scores previously established by Bruker to identify or validate species compared to the instrument's database were applied. In short, a score of $\geq$2.000 with a species with a validly published name allows identification at the species level; scores of $\geq$1.700 and <2.000 allow identification at the genus level; and a score of <1.700 does not allow any identification to be made. We performed 12 distinct deposits from 12 isolated colonies of strain FF11^T. They were then imported into MALDI Biotyper software (version 2.0, Bruker) and analysed by standard pattern matching (with default parameter settings) against the main spectra. Scores ranging from 1.315 to 1.511 were obtained for FF11^T, suggesting that this strain was not a member of any known species. The reference mass spectrum from strain FF11^T was incremented in our database (Fig. 1).

Moreover, strain FF11^T exhibited 97.9% 16S rRNA sequence similarity with *Dermabacter hominis* [1] (GenBank accession no. X91034), the phylogenetically closest bacterial species with standing in the nomenclature (Fig. 2). This value was lower than the 98.7% 16S rRNA sequence identity threshold recommended by Meier-Kolthoff *et al.* [19] in 2013 to delineate a new species within the *Firmicutes* phylum without carrying out DNA-DNA hybridization.

Phenotypic and biochemical features

Different growth temperatures (25, 28, 37, 45 and 56°C) were tested. Growth was obtained at 37°C only. Growth of the strain was also tested under anaerobic and microaerophilic conditions using GENbag anaer and GENbag microaer systems (bioMérieux), respectively, and under aerobic conditions, with or without 5% CO_2. Optimal growth was observed under aerobic and microaerophilic conditions, but weak growth was observed under anaerobic conditions at 37°C. Strain FF11^T shows white convex colonies measuring approximately 1 mm in diameter on 5% sheep's blood–enriched Columbia agar (bioMérieux). Cells are Gram-positive, nonmotile, non–spore forming short rods (Fig. 3). The negative staining of the cells and observation under transmission electron microscopy (FEI Company, Hillsboro, Oregon, USA) displays cells lacking flagella (Fig. 4).

Dermabacter indicis is catalase positive and oxidase negative. Using an API 50CH strip (bioMérieux), fermentation was observed for D-galactose, D-glucose, N-acetyl-D-glucosamine, D-lactose, D-saccharose, D-trehalose, D-melezitose, D-raffinose, starch and D-turanose. Using the API Coryne strip (bioMérieux), positive reactions were also observed for pyrazinecarboxamide, pyroglutamic acid-β-naphthylamide, esculin ferric citrate, urea and D-maltose. Negative reactions were noted for potassium nitrate (reduction of nitrates), β-glucuronidase, gelatin, D-ribose, D-xylose, D-mannitol and glycogen. Using the API ZYM strip (bioMérieux), enzymatic reactions were observed for esterase, esterase–lipase, lipase, acid phosphatase, alkaline phosphatase, naphthol-AS-BI-phosphohydrolase, cystine arylamidase, trypsin, α-glucosidase, β-galactosidase, α-mannosidase, α-fucosidase and N-acetyl-β-glucosaminidase. Negative reactions were observed for

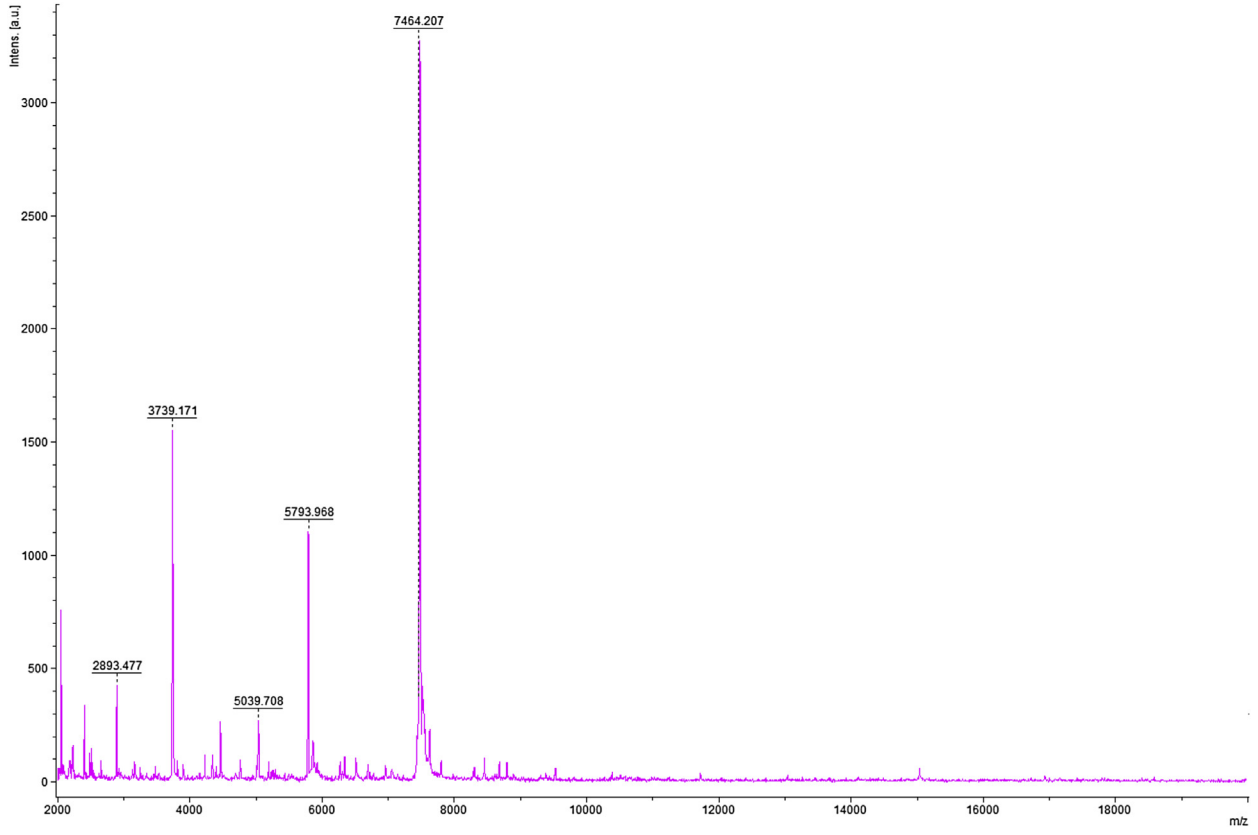

FIG. 1. Reference mass spectrum from *Dermabacter indicis* sp. nov. strain FF11^T. Spectra from 12 individual colonies were compared and reference spectrum generated.

leucine arylamidase, valine arylamidase, β-glucosidase, α-galactosidase and β-glucuronidase.

Strain FF11^T is susceptible to ciprofloxacin, amoxicillin/clavulanic acid, ticarcillin, ceftriaxone, imipenem, doxycycline, gentamicin and cefalotin, but it is resistant to colistin, trimethoprim/sulfamethoxazole, erythromycin and nitrofurantoin. A comparison of phenotypic characteristics with *Dermabacter hominis* [1], *Brachybacterium faecium* [20], *Brachybacterium muris* [21], and *Helcobacillus massiliensis* [22] is summarized in Table 2.

Genome sequencing information

Genome project history

The organism was selected for sequencing on the basis of its phylogenetic position, 16S rRNA similarity and phenotypic differences with other members of the *Dermabacteraceae* family. Here we present the first *Dermabacter indicis* sp. nov. genome. The EMBL/EBI accession number is CYUG00000000. Table 3 shows the project information and its association with MIGS (minimum information about a genome sequence) version 2.0 compliance [23].

Growth conditions and DNA isolation

Dermabacter indicis strain FF11^T (= CSUR P1488 = DSM 100283) was grown on 5% sheep's blood–enriched Columbia agar (bioMérieux) at 37°C. Bacteria grown on four petri dishes were resuspended in 5 × 100 µL of Tris-EDTA buffer and 150 µL of this suspension was diluted in: 350 µL Tris-EDTA buffer 10×, 25 µL proteinase K and 50 µL sodium dodecyl sulfate for lysis treatment. This preparation was incubated overnight at 56°C. Extracted DNA was then purified using three successive phenol–chloroform extractions and ethanol precipitations at −20°C overnight. After centrifugation, DNA was suspended in 65 µL of EB buffer. The genomic DNA concentration was measured at 69.3 ng/µL using the Qubit assay with the high sensitivity kit (Life Technologies, Carlsbad, CA, USA).

Genome sequencing and assembly

Genomic DNA (gDNA) of *Dermabacter indicis* FF11^T 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 mate pair library was prepared with 1.5 µg of gDNA using the Nextera Mate Pair

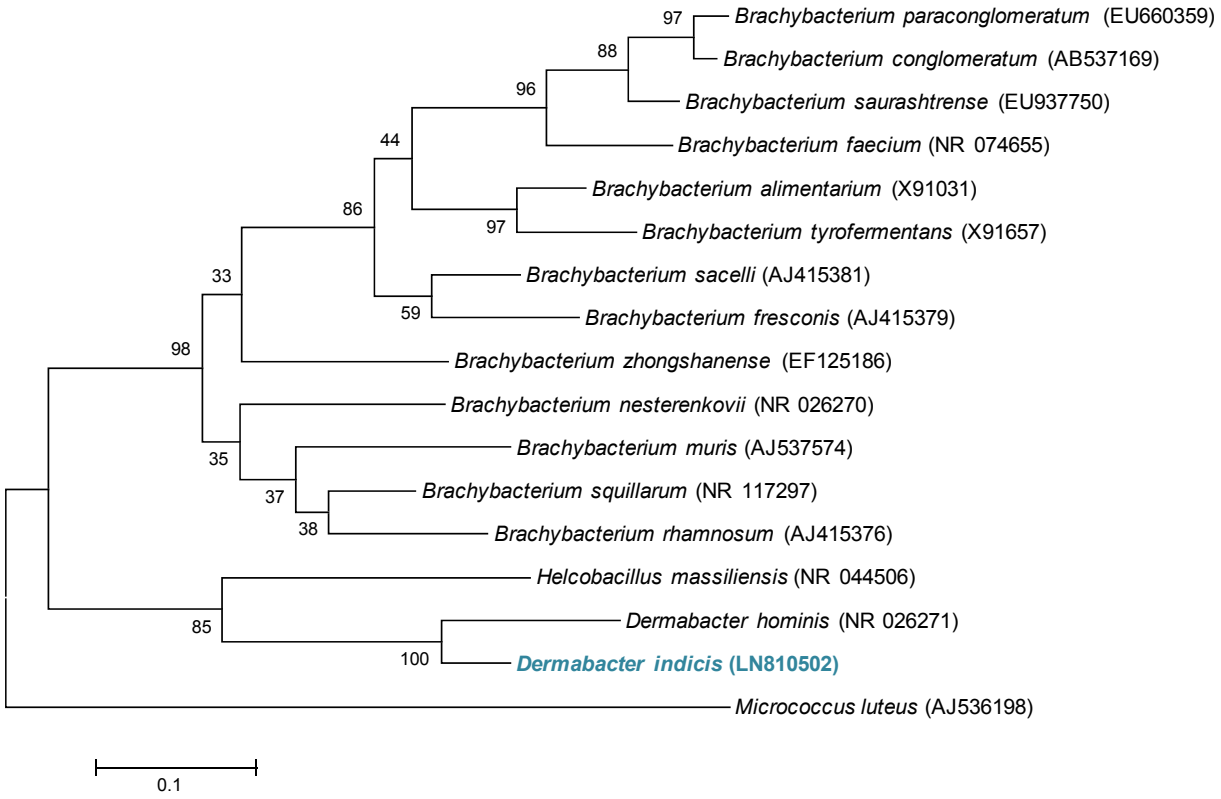

FIG. 2. Phylogenetic tree highlighting position of *Dermabacter indicis* sp. nov. strain FF11^T relative to other type strains within *Dermabacteraceae* family. Sequences were aligned using Clustal W, and phylogenetic inferences were obtained using maximum-likelihood method within MEGA6. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 1000 times to generate majority consensus tree. *Micrococcus luteus* strain was used as outgroup. Scale bar = 10% nucleotide sequence divergence.

Illumina guide. The gDNA sample was simultaneously fragmented and tagged with a mate pair junction adapter. The pattern of the fragmentation was validated on an Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) with

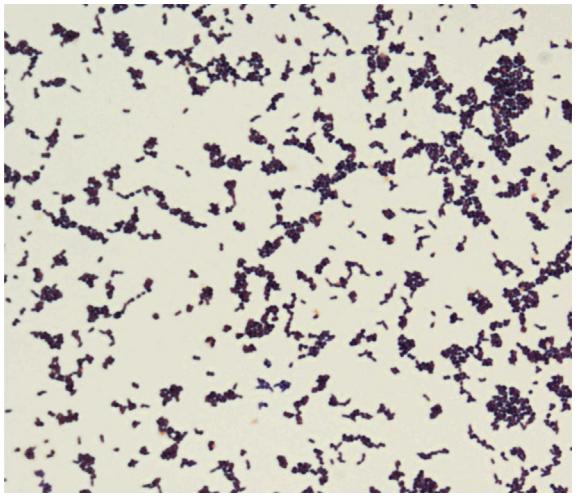

FIG. 3. Gram staining of *Dermabacter indicis* sp. nov. strain FF11^T.

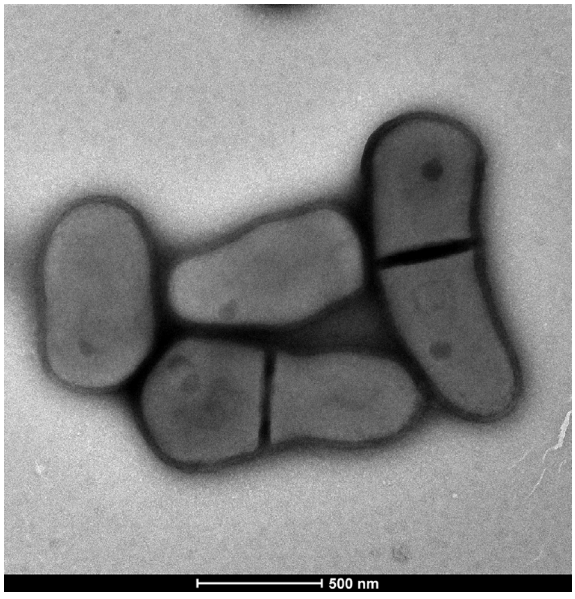

FIG. 4. Transmission electron microscopy of *Dermabacter indicis* strain FF11^T. Cells were observed on Tecnai G2 transmission electron microscope operated at 200 keV. Scale bar = 500 nm.

a DNA 7500 LabChip. The DNA fragments are ranged in size from 1.5 to 11 kb with an optimal size at 6.730 kb. No size selection was performed, and 636 ng of tagmented fragments were circularized. The circularized DNA was mechanically sheared into small fragments with an optimum at 653 bp on the Covaris device S2 in T6 tubes (Covaris, Woburn, MA, USA). The library profile was visualized on a High Sensitivity Bioanalyzer LabChip (Agilent), and the final concentration library was measured at 59.1 nmol/L.

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

Total information of 5.9 GB was obtained from a 624K/mm^2 cluster density with cluster passing quality control filters of 96.33% (12 040 000 clusters). Within this run, the index representation for *Dermabacter indicis* FF11^T was determined at 16.54%. The 1 918 640 paired reads were filtered according to the read qualities. These reads were trimmed and then assembled using the CLC genomicsWB4 software.

Genome annotation

Open reading frames (ORFs) were predicted using Prodigal [24] with default parameters, but the predicted ORFs were excluded if they spanned a sequencing gap region. The predicted bacterial protein sequences were searched against the GenBank database [25] and the Clusters of Orthologous Groups (COGs) database using BLASTP. The tRNAScan-SE tool [26] was used to find tRNA genes, while ribosomal RNAs were found using RNAmmer [27] and BLASTn against the GenBank database. Transmembrane topology and signal peptide predictors were provided using the Phobius server [28]. ORFans were identified if their BLASTP *E* value was lower than 1e-03 for an alignment length greater than 80 aa. If alignment lengths were smaller than 80 aa, we used an *E* value of 1e-05. Such parameter thresholds have been used in previous works to define ORFans. Artemis [29] was used for data management and DNA Plotter [30] for the visualization of genomic features. The Mauve alignment tool (version 2.3.1) was used for multiple genomic sequence alignment [31]. Briefly, this software combines the Proteinortho software [32] for detecting orthologous proteins in pairwise genomic comparisons, then retrieves the corresponding genes and determines the mean percentage of nucleotide sequence identity among orthologous ORFs using the Needleman-Wunsch global alignment algorithm. Annotation and comparison processes were performed in the Multi-Agent Software System DAGOBAH [33], including Figenix [34] libraries that provide pipeline analyses. Genome-to-Genome Distance Calculator (GGDC) analysis was also performed using the GGDC web server as previously reported [35,36]. Here, the genome of

TABLE 3. Project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	High-quality draft
MIGS-28	Libraries used	Mate-pair library
MIGS-29	Sequencing platforms	Illumina MiSeq
MIGS-30	Assemblers	CLC GENOMICSWB4
MIGS-32	Gene calling method	Prodigal
	BioProject ID	PRJEB10922
	GenBank accession numbers	CYUG01000001/CYUG01000017
	GenBank Date of Release	25 September 2015
	Project relevance	MALDI-TOF implementation in Dakar

MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MIGS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

TABLE 2. Differential characteristics of *Dermabacter indicis* strain FF11^T with *Dermabacter hominis* [1], *Brachybacterium faecium* [20], *Brachybacterium muris* [21] and *Helcobacillus massiliensis* [22]

Character	*D. indicis*	*D. hominis*	*B. faecium*	*B. muris*	*H. massiliensis*
Gram stain	+	+	+	+	+
Motility	−	−	−	−	−
Endospore formation	−	−	−	−	−
Production of:					
Alkaline phosphatase	+	NA	−	NA	−
Acid phosphatase	+	NA	−	NA	−
Catalase	+	+	+	+	+
Oxidase	−	−	−	−	−
β-Hemolysis	−	−	−	−	−
Nitrate reductase	−	−	+	+	+
α-Galactosidase	−	−	NA	NA	−
β-Galactosidase	+	NA	NA	NA	−
α-Glucosidase (PNPG)	+	+	NA	NA	+
β-Glucosidase	−	NA	NA	NA	−
Esterase	+	NA	NA	NA	−
Esterase lipase	+	NA	NA	NA	−
N-acetyl-β-glucosaminidase	+	+	NA	NA	+
Utilization of:					
D-Fructose	−	NA	−	+	+
D-Mannose	−	+	−	+	−
D-Xylose	−	−	−	−	+
D-Glucose	+	+	+	+	+
Habitat	Human wound	Human skin	Faeces	Mouse	Human skin

+, positive result; −, negative result; NA, data not available.

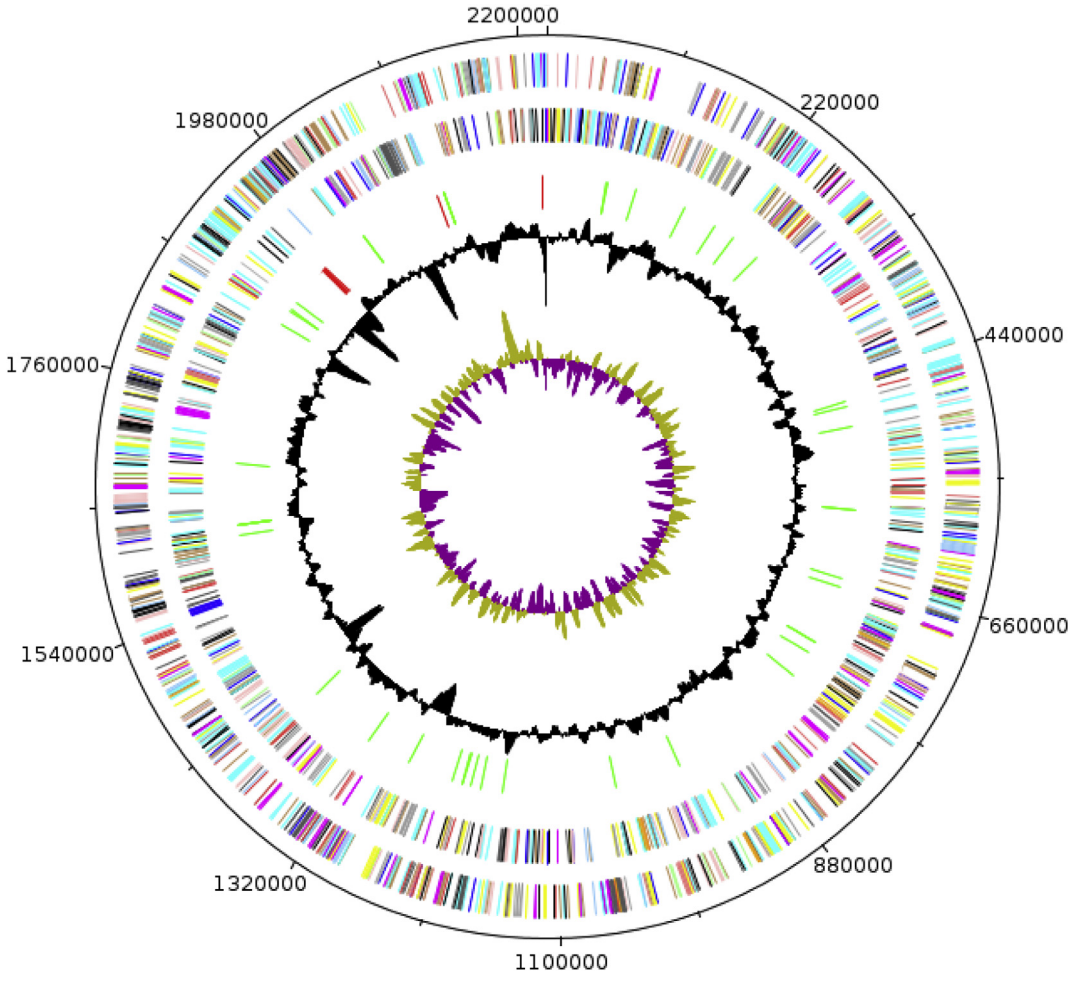

FIG. 5. Graphical circular map of *Dermabacter indicis* sp. nov. strain FF11^T chromosome. From outside in, outer two circles show open reading frames oriented in forward (coloured by COGs categories) and reverse (coloured by COGs categories) directions, respectively. Third circle marks tRNA genes (green). Fourth circle shows G+C% content plot. Innermost circle shows GC skew, with purple indicating negative values and olive positive values.

Dermabacter indicis strain FF11^T (EMBL/EBI accession no. CYUG00000000) is compared to those of *Dermabacter hominis* strain 1368 (JDRS00000000), *Brachybacterium faecium* strain DSM 4810^T (CP001643), *Brachybacterium paraconglomeratum* strain LC44 (AGSO00000000), *Brachybacterium squillarum* strain M-6-3^T (AGBX00000000) and *Brachybacterium muris* strain UCD-AY4 (AORC00000000).

Genome properties

The EMBL/EBI BioProject number is PRJEB10922 and consists of 248 large contigs. Finally, the draft genome of *D. indicis* FF11^T generated a 2 222 902 bp long genome with a 63.2% G+C content (Fig. 5). Of the 2124 predicted genes, 2074 were protein-coding genes and 50 were RNAs (three 5S rRNA

TABLE 4. **Nucleotide content and gene count levels of genome**

Attribute	Genome (total)	
	Value	% of total[a]
Size (bp)	2 222 902	100
G+C content (bp)	1 400 428	63.2
Coding region (bp)	2 019 684	90.85
Total genes	2124	100
RNA genes	50	2.35
Protein-coding genes	2074	97.64
Genes with function prediction	1557	73.30
Genes assigned to COGs	1422	66.94
Genes with peptide signals	110	5.17
Genes with transmembrane helices	435	20.48

COGs, Clusters of Orthologous Groups database.
[a]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 25 general COGs functional categories[a]

Code	Value	% value	Description
J	151	7.28	Translation
A	1	0.04	RNA processing and modification
K	124	5.97	Transcription
L	135	6.50	Replication, recombination and repair
B	0	0	Chromatin structure and dynamics
D	21	1.01	Cell cycle control, mitosis and meiosis
Y	0	0	Nuclear structure
V	43	2.07	Defense mechanisms
T	63	3.03	Signal transduction mechanisms
M	82	3.95	Cell wall/membrane biogenesis
N	1	0.04	Cell motility
Z	0	0	Cytoskeleton
W	0	0	Extracellular structures
U	22	1.06	Intracellular trafficking and secretion
O	69	3.32	Posttranslational modification, protein turnover, chaperones
C	93	4.48	Energy production and conversion
G	179	8.63	Carbohydrate transport and metabolism
E	144	6.94	Amino acid transport and metabolism
F	64	3.08	Nucleotide transport and metabolism
H	72	3.47	Coenzyme transport and metabolism
I	42	2.02	Lipid transport and metabolism
P	106	5.11	Inorganic ion transport and metabolism
Q	22	1.06	Secondary metabolites biosynthesis, transport and catabolism
R	198	9.54	General function prediction only
S	105	5.16	Function unknown
—	1183	35.23	Not in COGs

COGs, Clusters of Orthologous Groups database.
[a]Total is based on total number of protein-coding genes in annotated genome.

genes, one 16S rRNA gene, one 23S rRNA gene and 45 tRNA genes). A total of 59 genes (2.77%) were identified as ORFans. The remaining genes were annotated as hypothetical proteins. The properties and statistics of the genome are summarized in Table 4. The distribution of genes into COGs functional categories is presented in Table 5.

Genomic comparison with other *Dermabacteraceae* species

The draft genome of *D. indicis* is smaller than *D. hominis, B. faecium, B. paraconglomeratum, B. squillarum* and *B. muris* (2.22, 2.51, 3.61, 3.78, 3.19 and 3.26 Mb respectively). The G+C content of *D. indicis* is higher than that of *D. hominis* (63.2 and 62.7%, respectively) but lower than that of *B. faecium, B. paraconglomeratum, B. squillarum* and *B. muris* (72.0, 72.4, 72.8 and 70.0% respectively). The gene content of *D. indicis* is smaller than that of *D. hominis, B. faecium, B. paraconglomeratum, B. squillarum* and *B. muris* (2124, 2302, 3191, 3432, 2869 and 2914 respectively). However, the distribution of genes into COGs categories was similar in all the genomes compared (Fig. 6). In addition, *D. indicis* shared 2074, 2226, 3068, 3341, 2765 and 2806 orthologous genes with *D. hominis, B. faecium, B. paraconglomeratum, B. squillarum* and *B. muris* (Fig. 6). The genomic similarity between strain FF11^T and the closely related *Brachybacterium* species was also estimated using GGDC (Table 6).

Conclusion

The results of phenotypic, phylogenetic and genomic analyses allow us to propose the creation of *Dermabacter indicis* sp. nov., which contains strain FF11^T. The strain was isolated from a human wound in Dakar, Senegal.

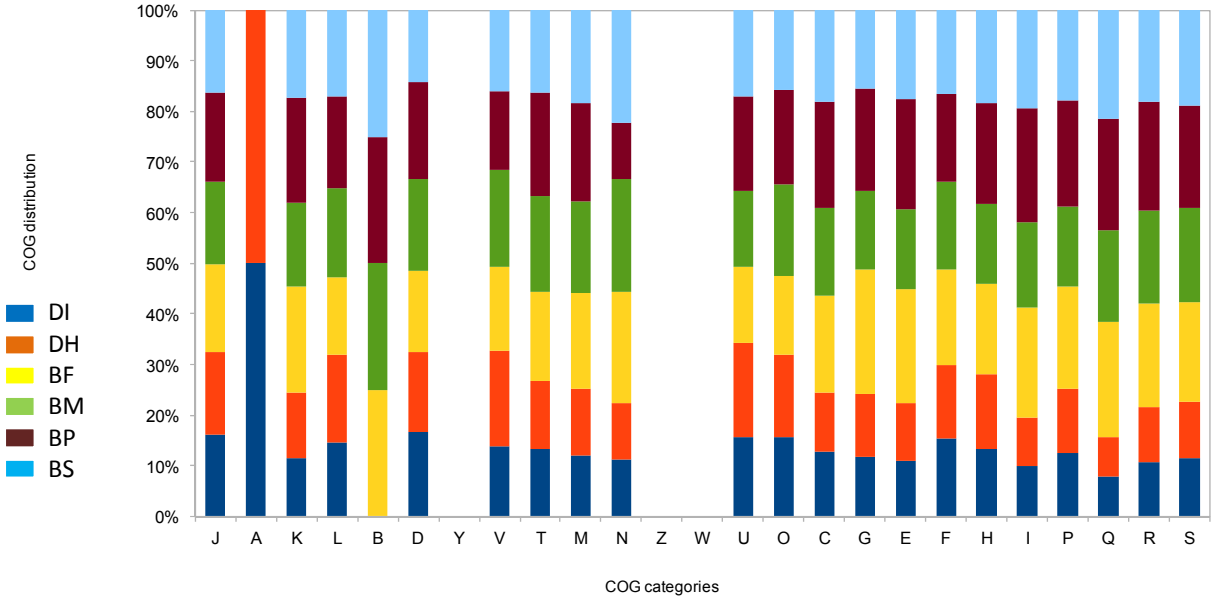

FIG. 6. Distribution of functional classes of predicted genes in genomes of indicated chromosomes according to clusters of orthologous groups of proteins. BF, *Brachybacterium faecium;* BM, *Brachybacterium muris;* BP, *Brachybacterium paraconglomeratum;* BS, *Brachybacterium squillarum;* DH, *Dermabacter hominis;* DI, *Dermabacter indicis.*

TABLE 6. Pairwise comparisons of *Dermabacter* species and *Brachybacterium* species using GGDC formula 2 (DDH estimates based on identities/HSP length)[a]

	DI	DH	BF	BM	BP	BS
DI	100.00%	26.9% ± 3.05	20.7% ± 2.57	20.7% ± 2.57	20.7% ± 2.58	20.3% ± 2.58
DH		100.00%	20.1% ± 2.57	21.1% ± 2.56	20.1% ± 2.58	20.7% ± 2.57
BF			100.00%	21.7% ± 2.94	25.0% ± 3.01	21.9% ± 2.97
BM				100.00%	22.2% ± 2.96	22.0% ± 2.97
BP					100.00%	22.7% ± 2.99
BS						100.00%

BF, *Brachybacterium faecium*; BM, *Brachybacterium muris*; BP, *Brachybacterium paraconglomeratum*; BS, *Brachybacterium squillarum*.; DDH, DNA-DNA hybridization; DH, *Dermabacter hominis*; DI, *Dermabacter indicis;* GGDC, Genome-to-Genome Distance Calculator; HSP, high-scoring segment pair.
[a]The confidence intervals indicate the inherent uncertainty in estimating DDH values from intergenomic distances based on models derived from empirical test data sets (which are always limited in size); details are provided elsewhere [19]. The distance formulas are explained elsewhere [35]; formula 2 is recommended, particularly for draft genomes.

Taxonomic and nomenclatural proposals

Description of *Dermabacter indicis* strain FF11^T sp. nov.

Dermabacter indicis (in.di.cis, L. gen. neutr. n. *indicis,* pertaining to the Latin name of the index finger, from which the type strain was isolated).

Strain FF11^T is a Gram-positive bacterium, facultatively anaerobic, with small (1 mm) and white colonies on 5% sheep's blood–enriched Columbia agar. Strain FF11^T is nonmotile, non–spore forming, oxidase negative and catalase positive. Strain FF11^T grows at 37°C. Strain FF11^T presents positive reactions for D-galactose, D-glucose, D-trehalose, D-melezitose, D-raffinose, starch, D-turanose, esterase, esterase-lipase, pyrazinecarboxamide, pyroglutamic acid-β-naphthylamide, esculin ferric citrate, urea, D-lactose, D-maltose, D-saccharose, acid phosphatase, naphthol-AS-BI-phosphohydrolase, cystine arylamidase, trypsin, α-glucosidase, β-galactosidase, α-mannosidase, α-fucosidase and N-acetyl-β-glucosaminidase. *Dermabacter indicis* strain FF11^T is susceptible to ciprofloxacin, amoxicillin/clavulanic acid, ticarcillin, ceftriaxone, imipenem, doxycycline, gentamicin and cefalotin but resistant to colistin, trimethoprim/sulfamethoxazole, erythromycin and nitrofurantoin.

The G+C content of the genome is 63.2%. The 16S rRNA and genome sequences are deposited in GenBank under accession numbers LN810502 and CYUG00000000, respectively. The type strain FF11^T (= CSUR P1488 = DSM 100283) was isolated from a human finger wound in Dakar, Senegal.

Acknowledgements

The authors thank the Xegen Company (http://www.xegen.fr/) for automating the genomic annotation process. This study was funded by the Fondation Méditerranée Infection. We also thank C. Andrieu for administrative assistance.

Conflict of interest

None declared.

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