High-quality genome sequence and description of *Chryseobacterium senegalense* sp. nov.

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Abstract

Strain FF12^T was isolated from the mouth of a West African lungfish (*Protopterus annectens*) in Senegal. MALDI-TOF-MS did not provide any identification. This strain exhibited a 97.97% 16S rRNA sequence identity with *Kaistella flava*. Using a polyphasic study including phenotypic and genomic analyses, strain FF12^T is Gram-negative, aero-anaerobic, oxidase-positive, non-motile, non-spore-forming, and exhibited a genome of 4,397,629 bp with a G+C content of 35.1% that coded 4,001 protein-coding and 55 RNA genes. On the basis of these data, we propose the creation of *Chryseobacterium senegalense* strain FF12^T.

Keywords: *Chryseobacterium senegalense*, culturomics, genome, *Protopterus annectens*, taxono-genomics

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Introduction

The family *Flavobacteriaceae*, which formerly belonged to the *Cytophaga–Flexibacter–Bacteroides* group, represents the most important bacterial lineage in the phylum *Bacteroidetes* [1]. Likewise, *Chryseobacterium, Bergeyella, Ornithobacterium, Empedobacter, Weeksella, Wautersiella, Elizabethkingia, Sejongia* and *Kaistella* are the genera currently included in this family [1–3]. However, *Kaistella flava* and *Kaistella korensis* are reclassified in the genus *Chryseobacterium* [4,5]. The genus *Chryseobacterium* was proposed for the first time in 1994 [2]. Currently 90 species with validly published names are included in this genus [6]. Members of this genus have been isolated from a variety of environments, including soil [7,8], plant rhizosphere [9], wastewater [10], freshwater [11], compost [12], diseased fish [13] and clinical samples [14,15]. *Chryseobacterium* FF12^T strain (CSUR = P1490, DSM 100279) is the type strain of *Chryseobacterium senegalense* sp. nov. It was isolated from the mouth of a West African lungfish (*Protopterus annectens*). Cells are Gram negative, aeroanaerobic, nonmotile, non–spore forming and rods. The availability of genomic data for many bacterial species [16] inspired us to propose a new concept for the description of new bacterial species, integrating proteomic information obtained by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) [17] and genomic sequencing [18]. This concept changes the current methods of defining a new bacterial species, which are based on genetic, phenotypic and chemotaxonomic criteria that are poorly reproducible and cannot be applied to the entire bacterial genus [19–21].

Here we present a summary classification and a set of features for the type strain *Chryseobacterium senegalense* sp. nov., strain FF12^T (CSUR = P1490, DSM 100279), together with the description of the complete genomic sequence and its annotation. These characteristics support the circumscription of the species *Chryseobacterium senegalense*.

Organism Information

Classification and features

The strain FF12^T was isolated from the mouth of a West African lungfish (*Protopterus annectens*) in Senegal in June 2014 (Table 1). A sterile swab was introduced in the mouth of this fish. The sample was inoculated on a 5% sheep's blood–enriched Columbia agar (bioMérieux, Marcy L'Etoile, France) and incubated at 37°C during 48 hours. First identification of this strain by MALDI-TOF in Dakar was attempted [30]. Then in Marseille MALDI-TOF protein analysis was performed using

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TABLE 1. Classification and general features of *Chryseobacterium senegalense* strain FF12^T [22]

MIGS ID	Property	Term	Evidence code[a]
	Classification	Domain: *Bacteria*	TAS [23]
		Phylum: *Bacteroidetes*	TAS [24,25]
		Class: *Flavobacteriia*	TAS [25,26]
		Order: *Flavobacteriales*	TAS [27,28]
		Family: *Flavobacteriaceae*	TAS [1]
		Genus: *Chryseobacterieum*	TAS [2]
		Species: *Chryseobacterieum senegalense*	IDA
		(Type) strain: FF12^T	IDA
	Gram stain	Negative	IDA
	Cell shape	Rod	IDA
	Motility	Nonmotile	IDA
	Sporulation	Non–spore forming	NAS
	Temperature range	Mesophile	IDA
	Optimum temperature	37°C	IDA
	pH range; optimum	6.0–6.4; 6.2	
	Carbon source	Unknown	
MIGS-6	Habitat	Fish	IDA
MIGS-6.3	Salinity	Unknown	
MIGS-22	Oxygen requirement	Aeroanaerobic	TAS
MIGS-15	Biotic relationship	Free-living	TAS
MIGS-14	Pathogenicity	Unknown	
MIGS-4	Geographic location	Senegal	TAS
MIGS-5	Sample collection	5 June 2014	TAS
MIGS-4.1	Latitude	14.6937000	TAS
MIGS-4.1	Longitude	−17.4440600	TAS
MIGS-4.4	Altitude	12 m above sea level	TAS

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) [29]. 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.

a Microflex LT (Bruker Daltonics, Leipzig, Germany) as previously reported [31]. An isolated colony was deposited in duplicate on a MALDI-TOF target for analysis. Scores ranging from 1.23 to 1.47 were obtained for FF12^T, suggesting that this strain was not a member of any known species in the MALDI-TOF database. The reference mass spectrum from strain FF12^T was incremented in our database (Fig. 1). Colonies that remained unidentified with MALDI-TOF after three tests are used for amplifying and sequencing the 16S rRNA sequence, as previously described elsewhere [32,33]. *Chryseobacterium senegalense* sp. nov. exhibited a 97.97% 16S rRNA sequence similarity with *Kaistella flava* [34], the phylogenetically closest bacterial species with standing in the nomenclature (Fig. 2). These values were lower than the 98.7% 16S rRNA gene sequence threshold recommended by Meier-Kolthoff *et al*. [37] in 2013 to delineate a new species within the phylum *Bacteroidetes* without carrying out DNA-DNA hybridization.

Different growth temperatures (25, 28, 37, 45 and 56°C) were tested. Growth was obtained between 25 and 37°C, with optimal growth at 28 and 37°C. Growth of the strain was also tested under anaerobic and microaerophilic conditions using GENbag anaer and GENbag microaer systems respectively (bioMérieux), and under aerobic conditions with or without 5% CO_2. Optimal growth was observed under aerobic and microaerophilic conditions. Weak growth was observed under anaerobic conditions at 25°C only. The colonies were opaque and light yellow in color, with a smooth surface on 5% sheep's blood–enriched Columbia agar and approximately 1 mm in diameter. A motility test was negative. Cells were Gram-negative, non-spore-forming rods (Fig. 3) with a mean diameter of 0.75 µm (range 0.5–1 µm) and a mean length of 2.25 µm (range 1.5–3 µm) (Fig. 4). Strain FF12^T was oxidase and catalase positive. Using an API ZYM strip (bioMérieux), positive reactions were observed for alkaline phosphatase, phosphatase acid, esterase, lipase, leucine arylamidase, α-glucosidase, β-glucosidase, naphthol-AS-BI-phosphohydrolase, α-fucosidase, β-galactosidase and α-galactosidase. Negative reactions were noted for β-glucuronidase, α-mannosidase, N-acetyl-β-glucosaminidase, α-chymotrypsin and cystine arylamidase. Using an API 50CH strip (bioMérieux), positive reactions were observed for D-glucose, D-maltose and starch. Negative reactions were observed for D-melibiose, D-trehalose, D-saccharose, D-raffinose, inositol, D-fructose, potassium 5-ketogluconate, D-mannitol, D-sorbitol, L-xylose, D-adonitol, methyl β-D-xylopyranoside, glycerol, ribose, D-xylose, D-mannose, D-melezitose and inulin. Four species with validly published names in the *Flavobacteriaceae* family were selected to make a phenotypic comparison with *C. senegalense* (Table 2). By comparison with other closer related *Chryseobacterium* species, *C. senegalense* differed in β-galactosidase production and 5-keto-gluconate utilization. The strain FF12^T is susceptible to amoxicillin, amoxicillin/clavulanic acid, ceftriaxone, trimethoprim/sulfamethoxazole, erythromycin, ciprofloxacin, nitrofurantoin, doxycycline, rifampicin and imipenem but resistant to gentamicin and metronidazole.

Genome Sequencing Information

Genome sequencing and assembly

Genomic DNA (gDNA) of *Chryseobacterium senegalense* 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 biomass of one petri dish was scraped and resuspended in 500 µL phosphate-buffered saline. A total of 100 µL of this bacterial suspension was spun, and the pellet was resuspended in 160 µL of G2 buffer from the EZ1 DNA Tissue kit (Qiagen, Venlo, Netherlands). A first mechanical lysis was performed by glass powder on the FastPrep-24 device (MP Biomedicals, Santa Ana, CA, USA) during 2 × 20 seconds. DNA was then incubated with 40 µL of lysozyme at 40 mg/mL for 30 minutes at 37°C and

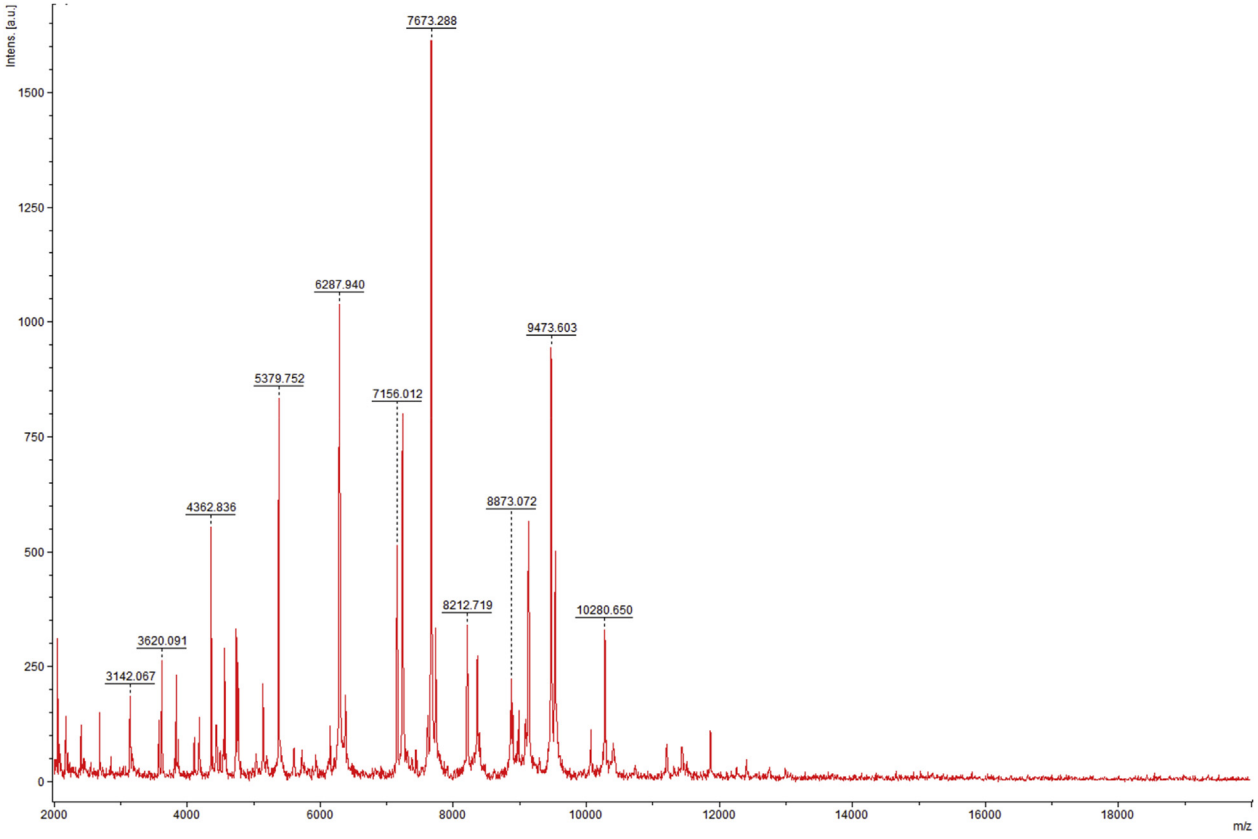

FIG. 1. Reference mass spectrum from *Chryseobacterium senegalense* strain FF12^T spectra.

extracted through the BioRobot EZ1 Advanced XL (Qiagen) in an elution volume of 50 µL.

DNA was quantified by a Qubit assay with the high sensitivity kit (Life Technologies, Carlsbad, CA, USA) to 28.5 ng/µL. The mate pair library was prepared with 1 µg of genomic DNA using the Nextera mate pair Illumina guide. The genomic DNA sample was simultaneously fragmented and tagged with a mate pair junction adapter. The pattern of the fragmentation was validated on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) with a DNA 7500 LabChip. The DNA fragments ranged in size from 1 to 10 kb, with an optimal size at 3.5 kb. No size selection was performed, and only 479 ng of tagmented fragments were circularized. The circularized DNA was mechanically sheared to small fragments with 641 bp on a Covaris S2 device in microtubes (Covaris, Woburn, MA, USA). The library profile was visualized on a High Sensitivity Bioanalyzer LabChip (Agilent), and the final concentration library was measured at 57.9 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 27-hour run at 2 × 251 bp. A total of 8.6 Gb of information was obtained from a 950K/mm^2 cluster density, with a cluster passing quality control filters of 93.2% (18 182 000 clusters). Within this run, the index representation for *Chryseobacterium senegalense* was determined to be 8.35%. The 1 414 815 paired reads were filtered according to the read qualities. These reads were trimmed and then assembled using the CLC genomics WB4 software.

Genome annotation and comparison

Open reading frames (ORFs) were predicted using Prodigal [42] 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 [43] and the Clusters of Orthologous Groups (COGs) databases using BLASTP. The tRNAscan-SE tool [44] was used to find tRNA genes, whereas ribosomal RNAs were found using RNAmmer [45] and BLASTn against the GenBank database. Lipoprotein signal peptides and the number of transmembrane helices were predicted using SignalP [46] and TMHMM [47] respectively. ORFans were identified if their BLASTP *E* value was lower than 1e-03 for an alignment length greater than 80 amino acids. If the alignment lengths

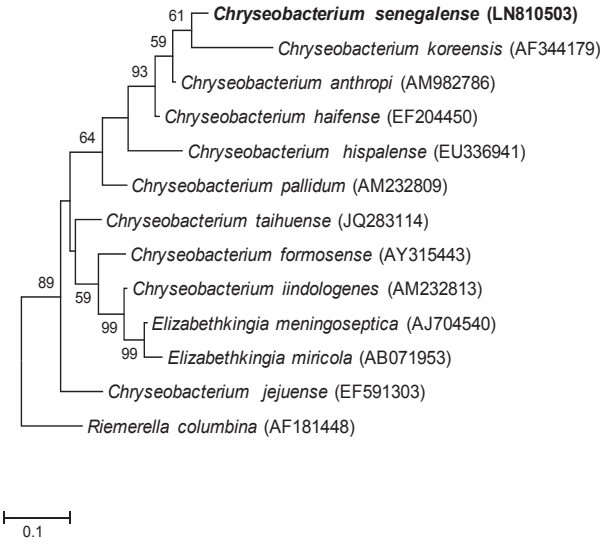

FIG. 2. Phylogenetic tree highlighting position of *Chryseobacterium senegalense* strain FF12^T (LN810503) relative to other type strains within *Flavobacteriaceae* family. GenBank accession numbers are indicated in parentheses. Sequences were aligned using MUSCLE [35], and phylogenetic tree was inferred by Maximum Likelihood method with Kimura two-parameter model from MEGA6 software [36]. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 1000 times to generate majority consensus tree. *Riemerella columbina* was used as outgroup. Scale bar = 0.1% nucleotide sequence divergence.

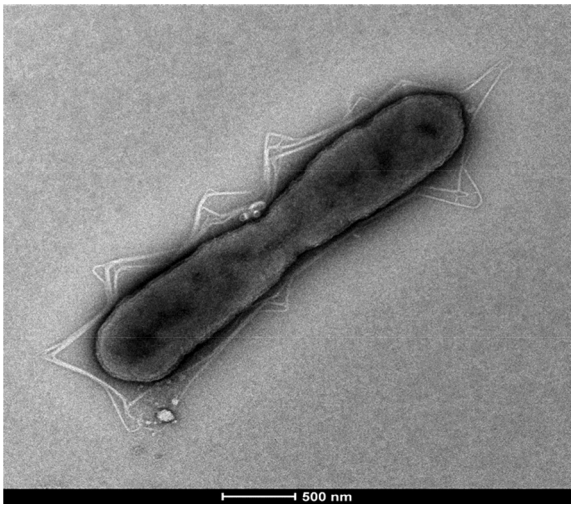

FIG. 4. Transmission electron microscopy of *Chryseobacterium senegalense* strain FF12^T. Cells are observed on Tecnai G2 transmission electron microscope operated at 200 keV. Scale bar = 500 nm.

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 [48] was used for data management and DNA Plotter [49] to visualize genomic features. The Mauve alignment tool (version 2.3.1) was used for multiple genomic sequence alignment [50]. To estimate the mean level of nucleotide sequence similarity at the genome level, we used the MAGI homemade software to calculate the average genomic identity of gene sequences (AGIOS) among compared genomes [18]. Briefly, this software combines the Proteinortho software [51] 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. Genomes from the genus *Chryseobacterium* and closely related genera were used to calculate AGIOS values.

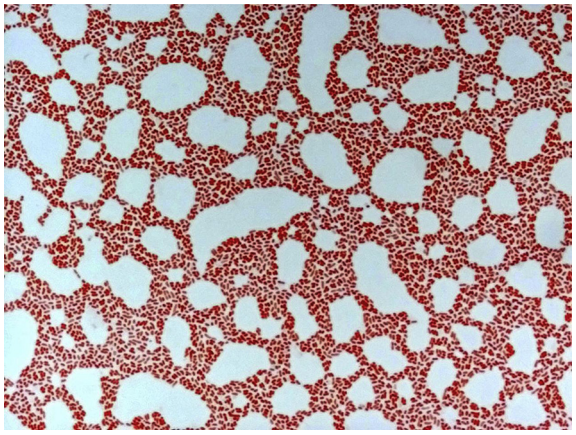

FIG. 3. Gram staining of *Chryseobacterium senegalense* strain FF12^T.

The genome of *Chryseobacterium senegalense* strain FF12^T (GenBank accession no. CYUH01000001–CYUH01000015CYUH01000001CYUH01000002CYUH01000003-CYUH01000004CYUH01000005CYUH01000006CYUH01000007CYUH01000008CYUH01000009CYUH01000010CYUH01000011CYUH01000012CYUH01000013CYUH01000014-CYUH01000015) was compared to *Chryseobacterium haifense* strain DSM 19056^T (GenBank accession no. JASZ00000000), *Chryseobacterium indologenes* strain NBRC 14944^T (GenBank accession no. BAVL01000000), *Chryseobacterium formosense* strain LMG 24722 (GenBank accession no. JPRP00000000) and *Elizabethkingia miricola* strain ATCC 33958 (GenBank accession no. JRFN00000000).

Genome properties

The GenBank BioProject number is PRJEB10923. The draft genome of *C. senegalense* FF12^T consists of 68 contigs and generated a 4 397 629 bp long genome with a 35.1% G+C content (Fig. 5). Of the 4056 predicted genes, 4001 were protein-coding genes, three were RNAs (one 5S rRNA gene,

TABLE 2. **Differential characteristics of *Chryseobacterium senegalense* strain FF12^T (data from this study), *Chryseobacterium haifense* [38], *Chryseobacterium hispalense* [39], *Chryseobacterium formosense* [40] and *Elizabethkingia meningoseptica* [41]**

Character	*C. senegalense*	*C. haifense*	*C. hispalense*	*C. formosense*	*E. meningoseptica*
Cell diameter (μm)	0.5–1	0.6–0.9	0.2–0.6	0.5–1	0.5–1.0
Oxygen requirement	Aeroanaerobic	Aerobic	Aerobic	Aerobic	Aerobic
Gram stain	–	–	–	–	–
Motility	–	–	–	–	–
Endospore forming	–	–	–	–	–
Catalase	+	+	+	NA	+
Oxidase	+	+	+	+	+
Alkaline phosphatase	+	+	+	+	+
Nitrate reductase	–	–	+	–	–
Acid production from:					
Trehalose	+	NA	–	+	+
D-Glucose	+	+	+	+	+
Mannose	+	+	+	+	NA
Rhamnose	+	NA	+	+	NA
Mannitol	–	–	–	+	+
Naphthol-AS-BI-phosphohydrolase	+	NA	+	+	+
β-Galactosidase	+	NA	–	–	+
N-acetyl-β-glucosaminidase	–	NA	–	–	NA
Utilization of:					
5-keto-gluconate	–	NA	+	+	NA
D-Xylose	–	–	NA	+	+
D-Fructose	+	+	+	–	+
L-Fucose	–	NA	+	–	NA
D-Arabitol	–	NA	NA	+	NA
Habitat	Fish	Raw milk	Clinical samples	Rhizosphere	Human

NA, not available.

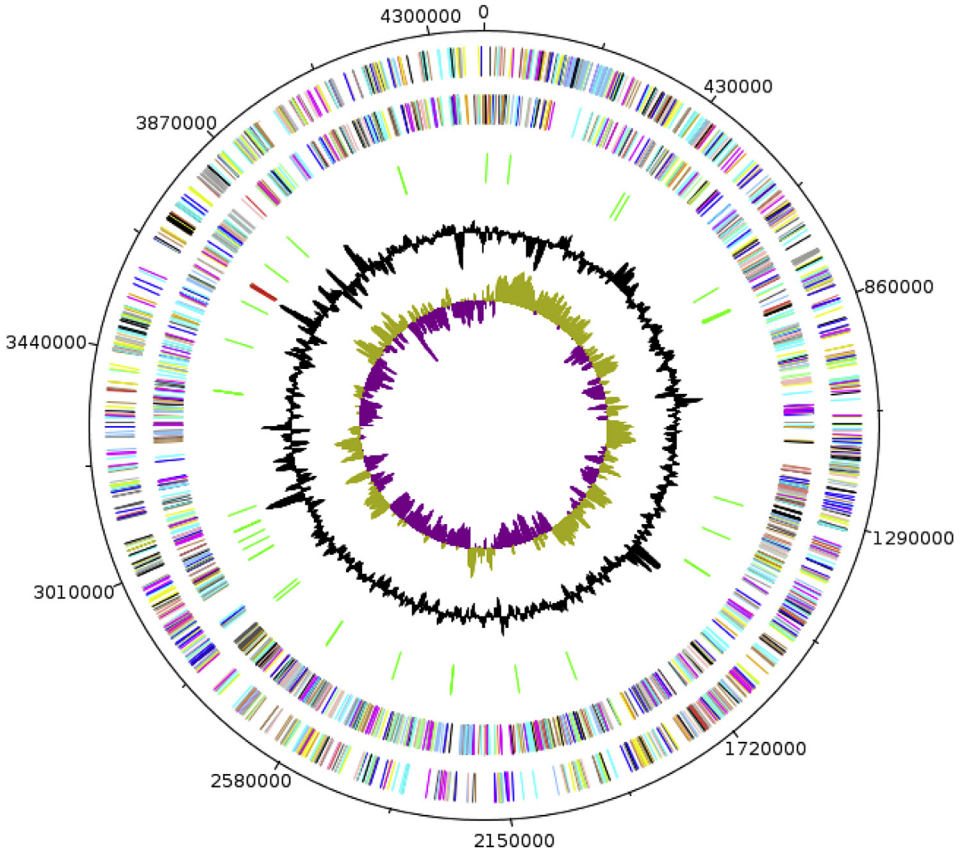

FIG. 5. Graphical circular map of genome. From outside to center, contigs (red/grey), COGs category of genes on forward S strand (three circles), genes on forward strand (blue circle), genes on reverse strand (red circle), COGs category on reverse strand (three circles), GC content.

TABLE 3. Nucleotide content and gene count levels of genome

Attribute	Value	% of total[a]
Size (bp)	4 397 629 bp	100
G+C content (bp)	1 543 567 bp	35.1
Coding region (bp)	3 945 189 bp	89.71
Total genes	4056	100
RNA genes	55	1.35
Protein-coding genes	4001	98.64
Genes with function prediction	2385	58.80
Genes assigned to COGs	2118	52.21
Genes with peptide signals	478	11.78
Genes with transmembrane helices	819	20.19

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

TABLE 4. Number of genes associated with 25 general COGs functional categories[a]

Code	Value	% value	Description
J	142	3.54	Translation
A	0	0	RNA processing and modification
K	147	3.67	Transcription
L	133	3.32	Replication, recombination and repair
B	0	0	Chromatin structure and dynamics
D	23	0.57	Cell cycle control, mitosis and meiosis
Y	0	0	Nuclear structure
V	59	1.47	Defense mechanisms
T	79	1.97	Signal transduction mechanisms
M	186	4.64	Cell wall/membrane biogenesis
N	2	0.04	Cell motility
Z	0	0	Cytoskeleton
W	0	0	Extracellular structures
U	24	0.59	Intracellular trafficking and secretion
O	93	2.32	Posttranslational modification, protein turnover, chaperones
C	116	2.89	Energy production and conversion
G	90	2.24	Carbohydrate transport and metabolism
E	157	3.92	Amino acid transport and metabolism
F	55	1.37	Nucleotide transport and metabolism
H	91	2.27	Coenzyme transport and metabolism
I	72	1.79	Lipid transport and metabolism
P	187	4.67	Inorganic ion transport and metabolism
Q	84	2.09	Secondary metabolites biosynthesis, transport and catabolism
R	254	6.34	General function prediction only
S	333	8.32	Function unknown
—	267	6.67	Not in COGs

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

one 16S rRNA gene, one 23S rRNA gene) and 52 were tRNA genes assigned a putative function. A total of 56 genes were identified as ORFans (1.38%). The remaining genes were annotated as hypothetical proteins. The genome properties and statistics are summarized in Table 3. The distribution of genes into COGs functional categories is presented in Table 4.

Genome comparison

The draft genome of *C. senegalense* is larger than that of *C. haifense* and *C. formosense* (4.39, 2.85 and 4.36 Mb respectively) but smaller than that of *C. indologenes* and *E. miricola* (4.75 and 4.58 Mb respectively). The G+C content of *C. senegalense* is higher than that of *C. formosense* (35.1 and 34.8% respectively) but lower than that of *C. haifense, C. indologenes* and *E. miricola* (36.7, 37.2, and 35.9% respectively). The gene content of *C. senegalense* is higher than that of *C. haifense* and *C. formosense* (4001, 2085 and 3695 respectively) but lower than that of *C. indologenes* and *E. miricola* (4258 and 4159 respectively). However, the distribution of genes into COGs categories was similar in all compared genomes. In addition, *C. senegalense* shared 4056, 2905, 4258, 3789 and 4159 orthologous genes with *C. haifense, C. indologenes, C. formosense* and *E. miricola* (Table 5). Among the species with standing in nomenclature, AGIOS values ranged from 61.15% between *C. haifense* and *E. miricola* to 74.70% between *C. formosense* and *C. indologenes.* The genomic similarity level between strain FF12^T and closely related *Chryseobacterium* species was also estimated using the genome-to-genome distance calculator (GGDC) (Table 6).

TABLE 5. Numbers of orthologous proteins shared between genomes (upper right) and AGIOS values obtained (lower left)[a]

	CS	CF	CH	CI	EM
CS	**4001**	2259	1517	2356	1964
CF	77.41	**3695**	1477	2274	1881
CH	64.29	64.78	**2085**	1538	1381
CI	76.28	74.70	63.91	**4192**	1980
EM	66.59	66.41	61.15	66.95	**4052**

AGIOS, average genomic identity of orthologous gene sequences; CF, *Chryseobacterium formosense;* CH, *Chryseobacterium haifense;* CI, *Chryseobacterium indologenes;* CS, *Chryseobacterium senegalense;* EM, *Elizabethkingia miricola.*
[a]Shown is average percentage similarity of nucleotides corresponding to orthologous protein shared between genomes (lower left) and numbers of proteins per genome (bold).

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

	CS	CF	CH	CI	EM
CS	100.00%	22.9% ± 2.91	21.4% ± 2.58	22.7% ± 2.83	22.0% ± 2.59
CF		100.00	19.8% ± 2.58	21.8% ± 2.73	19.7% ± 2.57
CH			100.00%	27.2% ± 2.59	24.9% ± 2.58
CI				100.00%	22.3% ± 2.60
EM					100.00%

CF, *C. formosense;* CH, *C. haifense;* CI, *C. indologenes;* CS, *C. senegalense;* DDH, DNA-DNA hybridization; EM, *Elizabethkingia miricola;* GGDC, Genome-to-Genome Distance Calculator; HSP, high-scoring segment pair.
[a]Confidence intervals indicate inherent uncertainty in estimating DDH values from intergenomic distances based on models derived from empirical test data sets (which are always limited in size) [52]. Formula 2 is recommended, particularly for draft genomes [53].

Conclusion

On the basis of phenotypic, phylogenetic and genomic analyses, we formally propose the creation of *C. senegalense* sp. nov., which

contains strain FF12^T. The strain was isolated from the mouth of a West African lungfish (*Protopterus annectens*) in Senegal.

Description of *Chryseobacterium senegalense* strain FF12^T sp. nov.

Chryseobacterium senegalense (se.ne.gal.e′n.sis, L. gen. masc. n. senegalense, pertaining to Senegal, the country where the type strain was isolated). Isolated from the mouth of a West African lungfish (*Protopterus annectens*), *C. senegalense* is Gram negative, aeroanaerobic, non–spore forming, a rod and catalase and oxidase positive. The strain grows easily on 5% sheep's blood–enriched Columbia agar with colonies 1 mm in diameter and comprise aerobic and nonmotile cells with a mean diameter of 0.75 µm (range 0.5–1 µm) and a mean length of 2.25 µm (range 1.5–3 µm). Positive reactions were observed for alkaline phosphatase, phosphatase acid, esterase, lipase, leucine arylamidase, α-glucosidase, β-glucosidase, naphthol-AS-BI-phosphohydrolase, α-fucosidase, β-galactosidase, α-galactosidase, D-glucose, D-maltose and starch. *Chryseobacterium senegalense* strain FF12^T is susceptible to amoxicillin, amoxicillin/clavulanic acid, ceftriaxone, trimethoprim/sulfamethoxazole, erythromycin, ciprofloxacin, nitrofurantoin, doxycycline, rifampicin and imipenem but resistant to gentamicin and metronidazole.

The G+C content of the genome is 35.1%. The 16S rRNA and genome sequences of *C. senegalense* strain FF12^T (CSUR = P1490, DSM 100279) are deposited in GenBank under accession numbers LN810503 and CYUH01000001–CYUH01000015 respectively.

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Conflict of Interest

None declared.

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