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Sphingobacterium prati sp. nov., isolated from agricultural soil and involved in lignocellulose deconstruction

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Abstract

A bacterial strain, arapr2^T, was isolated from agricultural soil sampled in Reims, France. Based on its 16S rRNA gene sequence, the strain was affiliated to the family *Sphingobacteriaceae* and more specifically to the genus *Sphingobacterium*. The strain had 98.31% 16S rRNA gene sequence similarity to its closest relative *Sphingobacterium canadense* CR11^T and 98.25% to *Sphingobacterium pakistanensis* NCCP-246^T. Genome relatedness indexes revealed that the average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values between arapr2^T and its closest relative (*S. canadense* CR11^T) were 92.97% and 52.00%, respectively; for *S. pakistanensis* NCCP-246^T, the ANI and dDDH values were 82.46 and 27.6%, respectively. The genomic DNA of strain arapr2^T was 6.02 Mbp long, had a DNA G+C content of 40.4 mol% and had 5504 protein-coding genes. The results obtained in this study suggests that strain arapr2^T (CIP 111872^T=LMG 31848^T) represents a new species for which the name *Sphingobacterium prati* sp. nov. is proposed. Due to the fact that this strain has been isolated using wheat straw as carbon source, this novel bacterial strain represents a promising biotechnological tool for the fractionation of lignocellulosic biomass in the context of biorefinery development.

INTRODUCTION

The family *Sphingobacteriaceae*, which belongs to the phylum *Bacteroidetes*, consists of 16 genera: *Albibacterium*, *Anseongella*, *Arcticibacter*, *Daejeonella*, *Hevizibacter*, *Mucilaginibacter*, *Nubsella, Olivibacter*, *Parapedobacter* [1], *Pararcticibacter*, *Pedobacter* [2], *Pelobium* [3], *Pseudopedobacter*, *Pseudosphingobacterium*, *Solitalea* and *Sphingobacterium* (www.ncbi.nlm.nih.gov/Taxonomy/Browser). The genus *Sphingobacterium* was proposed by Yabuuchi *et al.* [4] and, at the time of writing, it contains 57 species with validly published names (www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=28453). These 57 species have been encountered in several environmental types such as Antarctic [5], compost [6], rhizosphere [7] and human respiratory tracts [8]. The strains belonging to the genus *Sphingobacterium* are characterized by the presence of high concentrations of sphingophospholipids in cellular lipid components [9]. The strains are Gram-stain-negative, aerobic, rod-shaped and have a low DNA G+C content [9].

ISOLATION AND ECOLOGY

Meadow soil was sampled near Reims, France (49.25° N, 4.03 °E). One gram was placed in 50 g l^{-1} M3 media [10] supplemented with wheat straw at 30 °C for 1 week at pH 7 in order to isolate new highly active lignocellulolytic microorganisms. Among all of the isolates, strain arapr2^T was further cultivated on Luria–Bertani (LB) medium and purified by repeated streaking on LB agar. The strain was subjected to polyphasic taxonomy (genomic, physiologic, phenotypic and chemotaxonomic) and, based on the obtained data, strain arapr2^T was found to represent a new species in the genus *Sphingobacterium*.

PHYLOGENY AND GENOMIC CHARACTERIZATION

Genomic DNA was extracted using the PureLink Genomic DNA Mini Kit (Invitrogen) according to the manufacturer's instructions. DNA was quantified using the ND-1000 spectrophotometer (NanoDrop Technologies). DNA quality

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Keywords: CAZymes; lignocellulose; *Sphingobacterium*.
Abbreviations: ANI, average nucleotide identity; CAZyme, carbohydrate active enzyme; dDDR, digital DNA–DNA relatedness; ISP2, International *Streptomyces* Project 2; MLSA, multi-locus sequence analysis; R2A, Reasoner's 2A; RAST, Rapid Annotation using Subsystem Technology; TSB, tryptic soy broth.
The GenBank accession numbers of the whole genome and 16S rRNA sequences are JABJWY000000000 and MT476935, respectively.
One supplementary table and five supplementary figures are available with the online version of this article.

was checked after electrophoresis on a 0.8% (w/v) agarose gel made with Tris–acetate–EDTA buffer. Genomic DNA was sequenced using a NovaSeq system (Illumina) and was performed by Novogene (Cambridge, UK).

The phylogenetic identification of strain arapr2^T was performed by analysing its 16S rRNA gene. The 16S rRNA gene sequence was extracted from the whole genome sequence. The sequence was then compared to the closest type species of that genus using the EzBioCloud server [11] and then aligned with the closest relative sequences of representatives of the family *Sphingobacteriaceae* by using the ClustalW program [12].

The analysis of the 16S rRNA gene from strain arapr2^T showed that it is recognized as a member of the family *Sphingobacteriaceae* and the *Bacteroidetes*/*Chlorobi* group phylum. The phylogenetic tree showed that strain arapr2^T was closely related to the genus *Sphingobacterium*. Strain arapr2^T represented a new separate branch in the phylogenetic tree compared to the other *Sphingobacterium* species. Strain arapr2^T showed a 16S rRNA gene sequence identity value lower than 98.7% to the closest type strains (based on the results obtained on the EzBioCloud server): 98.31% for *Sphingobacterium canadense* CR11^T (completeness of 98.1%), 98.25% for *Sphingobacterium pakistanensis* NCCP-246^T (completeness of 98.6%), 97.78% for *Sphingobacterium ginsenosidimutans* THG 07^T (completeness of 96.2%), 97.52% for *Sphingobacterium caeni* DC-8^T (completeness of 100%) and only 92.12% for *Sphingobacterium spiritivorum* NCTC 11386^T, which is the type species of the genus (completeness of 99%) [13].

A maximum-likelihood phylogenetic tree was built using MEGA 7 software [14] using 1000 bootstraps. The almost-complete sequence of the 16S rRNA gene represented a sequence of 1455 nucleotides and was aligned on a total of 1268 unambiguous positions based on the *Escherichia coli* numbering system. The GenBank accession number for the 16S rRNA gene sequence of strain arapr2^T is MT476935. Neighbour-joining (Fig. 1) and maximum-likelihood (Fig. S1) phylogenetic trees were built based on the closest relative sequences of strain arapr2^T and using the sequence of *Flavobacterium terrae* R2A1-13^T as an outgroup taxon.

Multi-locus sequence analysis (MLSA) was performed with the 16S rRNA gene and additional partial sequences from the *rpoB* and *cpn60* genes as in Cheng *et al.* [15]. Alignments for each gene were performed separately using ClustalW supported on MEGAX [16] and the longest common fragments identified were selected for analysis. Next, the three alignments were concatenated. Phylogenetic analysis was performed based on finding the best DNA models in MEGA X. Maximum-likelihood and neighbour-joining trees were then reconstructed by using the GTR+G+I (general time reversible+gamma distribution+evolutionarily invariable] model, as in Cheng *et al.* [15]. Bootstrap analysis of 1000 replicates was performed to evaluate the phylogenetic tree topology. This realignment resulted in a sequence about 3603 bp long. MLSA based on those three concatenated genes (*rpoB*–*cpn60*–16S rRNA) confirmed that strain arapr2^T was most closely related to *S. canadense* CR11^T (=LMG 23727^T) with bootstrap support values of 97% (Fig. 2). Moreover, based on the genomes available, an automated multi-locus species tree was realized using the web server (https://automlst.ziemertlab.com/). Based on 83 housekeeping core genes (listed in Table S1), the phylogenomic tree obtained showed that strain arapr2^T is closely related to *Sphingobacterium athyrii* M46^T (Fig. S2).

The obtained genome sequence was annotated by using the RAST server (Rapid Annotation using Subsystem Technology; https://rast.nmpdr.org/) and deposited at DDBJ/ENA/GenBank under the accession number JABJWY000000000. Genomic comparisons against closely related strains for which the genomes are available were performed, such as *Sphingobacterium multivorum* JCM 21156^T [4], *Sphingobacterium detergens* 6.2S^T [17], *Sphingobacterium puteale* M05W1-28^T [9], *Sphingobacterium siyangense* SY1^T [18], *Sphingobacterium thalpophilum* NCTC 11429^T, *Sphingobacterium athyrii* M46^T [15], *Sphingobacterium pakistanensis* NCCP-246^T [19] and *Sphingobacterium spiritivorum* NCTC 11386^T [13], and presented in Table 1. The genome of *S. canadense* CR11^T was not available in public databases (GenBank of IMG); this strain was thus ordered from the BCCM/LMG Bacteria Collection (https://bccm.belspo.be/about-us/bccm-lmg) and subsequent sequencing of this strain was performed.

The genome size of strain arapr2^T (6.02 Mbp) fell in between those of the closest *Sphingobacterium* strains (5.84 Mbp for *S. pakistanensis* NCCP-246^T and 6.85 Mbp for *S. athyrii* M46^T). The same observation was made for the DNA G+C content of strain arapr2^T (40.4 mol%), which was 39.8% for *S. siyangense* SY1^T and 43.6% for *S. thalpophilum* NCTC 11429^T. The ANI values were determined by using an ANI calculator (http://jspecies.ribohost.com/jspeciesws/#home [20]). Digital DNA–DNA relatedness (dDDR) values were calculated using the genome-to-genome distance calculation [21] method between strain arapr2^T and the closely related strains. The results showed that the dDDR of strain arapr2^T was the highest with *S. canadense* CR11^T at 52.00%, which is lower than the threshold value of 70% (Table 1). The ANI values of strain arapr2^T compared to the other *Sphingobacterium* strains were lower than 92.67% for *S. canadense* CR11^T and consequently lower than the proposed threshold of 95–96%, thereby indicating a novel species [22]. Compared to the type species of the genus, *S. spiritivorum* NCTC 11386^T, the ANI and dDDR values were 20.20 and 69.31%, respectively.

A genomic comparison was made between *S. pakistanensis* NCCP-246^T and arapr2^T using the function-based approach on the RAST platform: 1030 functions were found in common, 47 were only present for strain arapr2^T and 28 only for *S. pakistanensis* NCCP-246^T. Unlike *S. pakistanensis* NCCP-246^T, strain arapr2^T harboured genes encoding for β-lactamase, resistance to chromium compounds (chromate transport protein ChrA) and genes involved in lactate and glycogen metabolism. In contrast, *S. pakistanensis* NCCP-246^T harboured genes involved in cAMP signalling in bacteria and chitin and *N*-acetylglucosamine utilization whereas strain

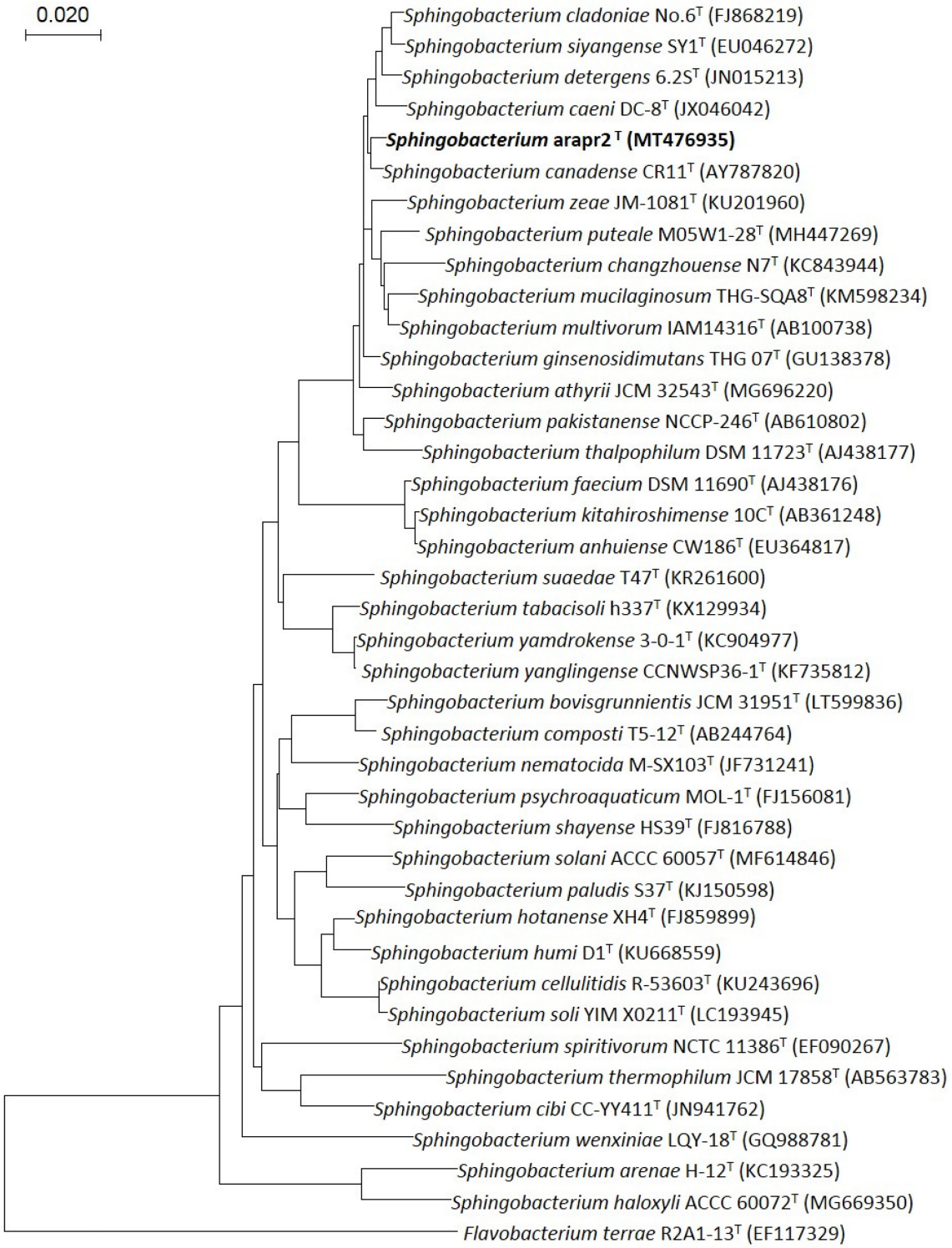

Fig. 1. Phylogenetic tree of type strains closely related to strain arapr2^T based on 16S rRNA gene sequences. The evolutionary history was inferred by MEGA 7.0 [32] using the the neighbour-joining method [33]. There were a total of 1315 positions in the final dataset. Bar, 0.02 substitutions per nucleotide position. The outgroup of the tree was *Flavobacterium terrae* R2A1-13^T.

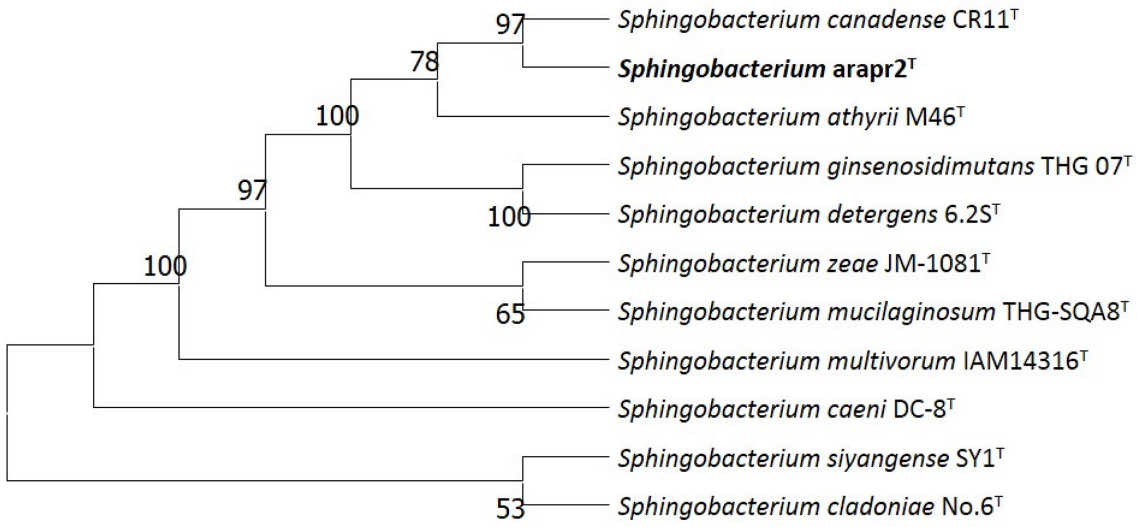

Fig. 2. Maximim-likelihood phylogenetic tree based on the sequences of the concatenated *rpoB–cpn60–16S rRNA* genes showing the relationship of strain arapr2^T with closely related *Sphingobacterium* species. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were removed.

arapr2^T did not. Compared to *S. canadense* CR11^T, the closest strain based on the ANI and dDDR indexes: 1080 functions were found in common, 22 were only present for strain arapr2^T and 30 only for *S. canadense* CR11^T. Strain arapr2^T harboured genes coding for aspartate racemase, mannose-6-phosphate isomerase, isoaspartyl aminopeptidase and sialidase. In contrast, *S. canadense* CR11^T harboured genes coding for hydroxymethylglutaryl-CoA reductase, proline dehydrogenase, chitinase, mannose-1-phosphate guanylyltransferase and choloylglycine hydrolase and was able to produce biotin whereas strain arapr2^T could not.

The genome of strain arapr2^T harboured six secondary metabolites regions predicted by the antiSMASH software (https://antismash.secondarymetabolites.org/) [23]. The six secondary metabolite regions encoded for terpene, RRE-containing, arylpolyene-T3PKS, betalactone, RRE-containing and T1PKS-hglE-KS. The terpene and RRE-containing

Table 1. Genome size, DNA G+C content, digital DNA–DNA relatedness and average nucleotide identity values between strain arapr2^T and closely related type strains of the genus *Sphingobacterium*

Strain	Size (bp)	DNA G+C content (mol%)	DNA–DNA relatedness (%)	Average nucleotide identity (%)
arapr2^T	6.020.522	40.4	–	–
Sphingobacterium canadense CR11^T	6.340.447	40.5	52.00	92.67
Sphingobacterium multivorum JCM 21156^T	5.984.896	40.0	22.30	77.49
Sphingobacterium athyrii M46^T	6.853.865	40.6	33.50	85.80
Sphingobacterium detergens 6.2S^T	6.732.474	39.8	29.50	83.70
Sphingobacterium pakistanensis NCCP-246^T	5.842.415	40.8	27.60	82.46
Sphingobacterium puteale M05W1-28^T	6.719.458	40.7	32.10	85.08
Sphingobacterium siyangense SY1^T	6.294.929	39.8	22.00	77.44
Sphingobacterium thalpophilum NCTC 11429^T	5.962.893	43.6	20.90	76.43
Sphingobacterium spiritivorum NCTC 11386^T	5138967	39.8	20.20	69.31
Sphingobacterium ginsenosidimutans THG 07^T*	NA	40.6	NA	NA

*Genomic sequence data not available.

Table 2. The major cellular fatty acid composition (%) derived from fatty acid methyl ester analysis of strain arapr2^T and closely related reference strains of *Sphingobacterium* species

Strains:1, arapr2^T; 2, *S. canadense* LMG 23727^T; 3, *S. pakistanensis* NCCP-246^T ; 4, *S. spiritivorum* NCTC 11386^T. Data for strain arapr2^T were obtained after 48 h of growth in tryptic soy broth; the data for the closest strains (2 and 3) were obtained from the reference publication [15]. The data for strain 4 were obtained from its reference publication [13].

Fatty acid	1	2	3	4
$C_{14:0}$	1.5	0.9	2.4	2.4
anteiso-$C_{15:0}$	2.2	3.1	_	1.4
iso-$C_{15:0}$	28.1	26.5	28	24
iso $C_{15:0}$ 3-OH	3.6	3.3	2.7	4.9
$C_{16:0}$	3.1	2.9	11.9	4.4
$C_{16:0}$ 3-OH	1.3	1.5	4.81	5.0
$C_{16:1}$ ω5*c*	0.3	–	_	_
iso-$C_{17:0}$	9.2	8.1	_	_
Summed feature 1*	0.9	0.7	_	_
Summed feature 3*	38.3	41.5	37.1	32.31
Summed feature 4*	0.5	0.4	_	_
Summed feature 9*	1.6	1.4	_	_

*Summed features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total. Summed feature 1 contained iso-$C_{15:1}$ h and/or $C_{13:0}$ 3-OH; summed feature 3 contained $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*; summed feature 4 contained iso-$C_{17:1}$I and/or anteiso-$C_{17:1}$B; summed feature 9 contained iso-$C_{17:1}$ *ω9c* and/or $C_{16:0}$10-methyl

regions produced carotenoid and pseudaminic acid, respectively; the other regions had such lower similarity compared to known clusters, that the secondary metabolites could not be identified.

Due to its potential role in wheat straw degradation (a lignocellulosic substrate considered as recalcitrant), the genomic content in CAZyme (carbohydrate active enzyme) [24] was determined by annotating its genome against the CAZy database using the web server dbCAN2 (http://bcb.unl.edu/dbCAN2/) [25]. The genome harboured 297 CAZymes which were divided into: 185 glycosyl hydrolases, 47 glycosyl transferases, 35 carbohydrate esterases, 13 carbohydrate bonding modules, 10 polysaccharide lyases and seven auxiliary activities based on the CAZyme annotation. In total, the CAZyme gene content represented 5.81% of the proteins expressed, which is high compared to other genera previously characterized as lignocellulose degraders, such as *Clostridium phytofermans*, which had only 161 CAZymes [26].

PHENOTYPIC, PHYSIOLOGIC AND CHEMOTAXONOMIC AFFILIATION

Regarding the physiological parameters, pH range for growth was assessed in LB medium from pH 4.0 to 10.0 (intervals of 1.0). Tolerance to NaCl was determined using LB medium supplemented with final NaCl concentrations of 0–10.0 g l^{-1}. Those physiological parameters were monitored by measuring the optical density at λ=600 nm. Growth range temperature was determined at 4, 10, 15, 20, 25, 30, 37, 40 and 45 °C using LB medium agar plates during 90 h. The growth on different media [LB, Reasoner's 2A (R2A), tryptic soy broth (TSB), International *Streptomyces* Project 2 (ISP2), Plate Count Agar (PCA), MacConkey] was tested by plating arapr2^T onto them for 90 h at 30 °C. Pigmentation was determined by observing colonies on different medium agar plates after incubation at 30 °C for 90 h. Catalase activity was determined by dropping 3% (v/v) H_2O_2 onto a fresh culture grown on R2A agar and oxidase activity was tested using the oxidase reagent kit (bioMérieux) according to the manufacturer's instructions. Biochemical tests (oxidation of carbon compounds and presence of enzymatic activities) were carried out using the GEN III MicroPlate (Biolog) and the API 10S system (bioMérieux), respectively, according to the manufacturers' instructions under the optimal growth conditions. All physiological tests were performed in duplicate for each strain. Susceptibility to antibiotics was investigated on LB agar medium using the disc-diffusion method as described previously [27]. Antibiotic resistance was correlated with the results obtained from the GEN III MicroPlate. Cell morphology was examined under light microscope (BH-2, Olympus) as well as a scanning electron microscope (5400 LV, JEOL) after coating with gold–palladium (Ion Sputter JFC-1100, JEOL).

Regarding the chemotaxonomic affiliation, the fatty acid methyl esters were obtained from 40 mg of fresh scraped colonies from Petri dishes by saponification, methylation and extraction using methods from [28] and [29]. The respiratory quinones were extracted from 100 mg of freeze-dried cell material using methanol–hexane extraction first and separation into hexane secondly as described previously [30, 31]. The polar lipids were extracted from 200 mg freeze-dried cell material using a choroform–methanol–0.3% aqueous NaCl mixture; polar lipids were separated by two-dimensional silica gel thin-layer chromatography. The first direction was developed in chloroform–methanol–water and the second in chloroform–methanol–acetic acid–water. The fatty acid, respiratory quinone and polar lipid analyses were carried out by DSMZ (Braunschweig, Germany).

The main fatty acid found in strain arapr2^T was the iso-$C_{15:0}$ (28.1%) branched fatty acid, which is similar to the other *Sphingobacterium* strains. The second most abundant fatty acid encountered in strain arapr2^T was iso $C_{17:0}$ (9.2%) and was among the values measured for the other *Sphingobacterium* strains. Among the saturated fatty acids, the most dominant in strain arapr2^T was $C_{16:0}$ (3.1%) (Table 2). The profile of strain arapr2^T was different from its phylogenetically closest neighbour *S. canadense* CR11^T; indeed, the relative

Table 3. Differential characteristics between strain arapr2^T and its closest related species in the genus *Sphingobacterium*

Strains: 1, arapr2^T; 2, *S. canadense* LMG 23727^T; 3, *S. pakistanensis* NCCP-246^T; 4, *S. spiritivorum* NCTC 11386^T. +, positive; –, negative ; ND, not determined; W, weakly positive. The data for the closest strains (2 and 3) were obtained from the reference publication [15]. The data for strain 4 were obtained from its reference publication [13].

Characteristics	1	2	3	4
Growth conditions:				
Temperature (°C)	15–37	20–37	16–37	15–30
pH	6–9	5–10	6–8	6–9
Tolerance to NaCl (%, w/v)	0–4	0–4	0–4	0–3.5
Reduction of nitrates to nitrogen	–	+	+	–
Enzyme activities:				
Lipase (C14)	+	–	–	ND
Trypsine	+	–	–	W
α-Chymotrypsin	–	–	–	W
β-Galactosidase	+	–	+	+
α-Mannosidase	+	–	+	–
α-Fucosidase	–	–	+	+
Assimilation of L-arabinose	+	+	–	+
Fermentation of:				
Glucose	+	–	+	–
Melibiose	+	+	+	W
Arabinose	+	+	–	–
Degradation of xylan	–	+	+	+

amounts of $C_{14:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$ and $C_{14:0}$ were higher in strain arapr2^T. Regarding *S. pakistanensis* NCCP-246^T, higher percentages of $C_{16:0}$ and $C_{16:0}$ 3-OH were detected in this strain compared to arapr2^T. Fatty acid iso-$C_{17:0}$ was detected in arapr2^T whereas it was not present in *S. pakistanensis* NCCP-246^T and *S. spiritivorum* NCTC 11386^T.

Only one quinone, MK7, was found in strain arapr2^T. MK-7 was detected as the predominant menaquinone (98%) and traces of menaquinones 6 and 8 were also detected in *S. canadense* CR11^T. MK-7 was found to be the major isoprenoid quinone in *S. pakistanensis* NCCP-246^T.

The major polar lipids in strain arapr2^T were phospholipids and phosphatidylethanolamine (Fig. S3). A majority of unknown lipids was detected for strain arapr2^T. Strain arapr2^T harboured aminolipid, phosphoaminolipid and phosphoaminoglycolipid. For *S. canadense* CR11^T, eight lipids were detected with five of them different from strain arapr2^T. The type species of the genus, *S. spiritivorum* NCTC 11386^T, had phosphatidylethanolamine, unidentified phosphoglycolipid, unidentified polar lipid and unidentified aminolipids [13] which is different from strain arapr2^T.

The overall cellular fatty acid, polar lipid and quinone profiles supported the affiliation of strain arapr2^T to the genus *Sphingobacterium* and differed enough from the closest representatives to define it as a new bacterial species.

Biolog tests (GEN III MicroPlate) were performed to determine which carbon sources were oxidized by strain arapr2^T and compared to the reference *Sphingobacterium* strains: *S. canadense* LMG 23727^T, *S. pakistanensis* NCCP-246^T and *S. spiritivorum* NCTC 11386^T. Strain arapr2^T was unable to degrade xylan in comparison to the other strains (Table 2). Melibiose was strongly oxidized by all the strains with the exception of *S. spiritivorum* NCTC 11386^T. Arabinose was oxidized by strain arapr2^T and *S. canadense* LMG 23727^T, whereas the two other reference strains did not. For the enzymatic activities, strain arapr2^T produced a lipase whereas the other reference strains did not. *S. spiritivorum* NCTC 11386^T had trypsine and chymotrypsine activities whereas the other strains did not. Compared to its closest neighbour, strain arapr2^T had β-galactosidase and α-mannosidase activities whereas *S. canadense* LMG 23727^T did not.

Cells of strain arapr2^T were Gram-stain-negative rods, with a length and width of 0.8–1.7 µm and 0.7–0.9 µm, respectively (Fig. S4) when grown on LB medium at pH 7, at 30 °C for 48 h. Growth occurred at 15–37 °C and pH 6.0–9.0. The NaCl tolerance range was between 0 and 4 %

(w/v). Compared to its closest strain by 16S rRNA affiliation (*S. canadense* CR11^T), strain arapr2^T was smaller in terms of length and width, had a greater range of growth temperature and salinity tolerance but a lower range in terms of pH (Table 3 and Fig. S5). Strain arapr2^T was able to grow on LB, R2A, TSB or PCA media but was not able to grow on ISP2 and MacConkey. The production of slightly yellowish pigments was observed when grown on LB, PCA or TSB for 90 h.

Based on the polyphasic characteristics determined in this study (the presence of new polar lipids, the ANI values and the degradation of different carbon compounds) for strain arapr2^T, it should be classified as representing a novel species of the genus *Sphingobacterium* for which the name *Sphingobacterium prati* sp. nov. is proposed.

DESCRIPTION OF *SPHINGOBACTERIUM PRATI* SP. NOV.

Sphingobacterium prati (pra'ti L. gen. n. *prati* of a meadow).

Cells are Gram-stain-negative, non-motile, non-spore-forming, strictly aerobic rods, 0.8–1.7 µm long and 0.7–0.9 µm wide. After 2 days incubation on LB agar, colonies are 1.0–2.5 mm in diameter, slightly yellowish, convex, circular and smooth. Growth occurs at 15–37 °C (optimum, 30 °C), pH 6.0–9.0 (optimum, pH 7.0) and with 0–4% NaCl (optimum, 0%). Growth occurs on nutrient agar and LB agar, but not on MacConkey agar. Positive for catalase and oxidase. Acid is produced from sodium lactate, acetoacetic acid, cellobiose, dextrin, D-fructose, D-galactose, D-galacturonic acid, D-gluconic acid, maltose, D-mannose, melibiose, D-salicin, turanose, gelatin, gentiobiose, glycyl-L-proline, guanidine HCl, L-fucose, L-rhamnose, *N*-acetyl-D-galactosamine, *N*-acetyl-β-D-mannosamine, nalidixic acid, pectin, sodium butyrate, sucrose, D-glucose and lactose. Acid is not produced from 3-methyl-glucose, bromo-succinic acid, citric acid, D-arabitol, D-aspartic acid, D-fructose-6-PO_4, D-fucose, D-glucose-6-PO_4, D-gluronic-acid, D-lactic acid, methyl ester, D-malic acid, D-mannitol, raffinose, D-saccharic acid, D-serine, D-sorbitol, trehalose, formic acid, fusidic acid, glucuronamide, glycerol, inosine, L-alanine, L-arginine, L-aspartic acid, L-galactonic acid, lactone, L-glutamic acid, L-histidine, L-lactic acid, L-malic acid, L-pyroglutamic acid, L-serine, methyl pyruvate, mucic acid, myo-anositol, *N*-acetyl neuraminic acid, *N*-acetyl-D-glucosamine, *p*-hydroxy-phenylacetic acid, propionic acid, quinic acid, stachyose, Tween 40, α-hydroxy-butyric acid, α-keto-butyric acid, α-keto-glutaric acid, β-hydroxy-D acid, L-butyric acid, methyl β-D-glucoside and γ-amino-butyric acid. Resistant to aztreonam, rifamycin and sensitive to lincomycin, minocycline, troleandomycin and vancomycin. Strain arapr2^T harbours β-galactosidase but not lysine decarboxylase, ornithine decarboxylase, urease and tryptophane desaminase (API 10S). Strain arapr2^T can oxidize glucose and produce NO_2 but cannot oxidize arabinose or produce H_2S (API 10S). The predominant fatty acids are iso-$C_{15:0}$, $C_{16:0}$ and $C_{17:0}$ iso 3-OH. Menaquinone-7 is the major respiratory quinone. The major polar lipids are glycolipid, aminolipid, phospholipid, phosphatidylethanolamine, phosphoaminolipid and phosphoaminoglycolipid. The type strain, arapr2^T (CIP 111872^T=LMG 31848^T), was isolated from meadow soil collected near Reims, France. The DNA G+C content of the type strain is 40.4 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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