Spirosoma utsteinense sp. nov. isolated from Antarctic ice-free soils from the Utsteinen region, East Antarctica

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Depositories

Raw genome sequence data are available from the NCBI sequence read archive (https://www.ncbi.nlm.nih.gov/sra) under accession numbers SRR9265382 (LMG 31447^T) and SRR9265482 (LMG 31448).

The annotated Whole Genome Shotgun projects of strains LMG 31447^T (= R-68523^T = CECT 9925^T) and LMG 31448 (= R-68079) have been deposited at DDBJ/ENA/Genbank under the accession numbers VFIA00000000 and VFIC00000000, respectively. The complete 16S rRNA gene sequences of strains LMG 31447^T and LMG 31448 have been deposited at DDBJ/ENA/Genbank under the accession numbers MN031264 and MN031263, respectively.

Abbreviations

ANI, average nucleotide identity

CE, carboxyl esterase

GH, glycosyl hydrolase

dDDH, digital DNA-DNA hybridization

MA, marine agar

MALDI-TOF MS: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

NA, nutrient agar

R2A, Reasoner's 2A agar

TSA, trypticase soy agar

Abstract

Between 2014 and 2016, 16 Gram-negative, aerobic, rod-shaped and yellow-orange pigmented bacteria were isolated from exposed soils from the Utsteinen region, Sør Rondane Mountains, East Antarctica. Analysis of their 16S rRNA gene sequences revealed that the strains form a separate cluster in the genus *Spirosoma*, with *Spirosoma rigui* KCTC 12531^T as its closest neighbour (97.8% sequence similarity). A comparative genome analysis of two representative strains (i.e. R-68523^T and R-68079) of the new group with the types of *Spirosoma rigui* (its closest neighbour) and *Spirosoma linguale* (type species of the genus), yielded ANI values of 73.9 to 78.7%. Digital DNA-DNA reassociation values of the two strains and these type strains ranged from 20.3 to 22.0%. The predominant cellular fatty acids of the two novel strains were summed feature 3 (i.e. $C_{16:1}$ ω7c and/or iso-C_{15} 2-OH), $C_{16:1}$ ω5c, $C_{16:0}$ and iso-$C_{15:0}$. The new *Spirosoma* strains grew with 0-0.5% (w/v) NaCl, at pH 6.5-8.0 and displayed optimum growth between 15 and 25 °C. Based on phenotypic, genomic, phylogenetic and chemotaxonomic analyses, the new strains represent a novel species of the genus *Spirosoma* for which the name *Spirosoma utsteinense* sp. nov. is proposed. The type strain is R-68523^T (= LMG 31447^T = CECT 9925^T).

Introduction

The genus *Spirosoma* in the family *Cytophagaceae* (*Bacteroidetes*) was originally proposed by Migula [1]. Typically, *Spirosoma* species are yellow to orange, Gram-negative, catalase-positive bacteria with rod, filament and coil morphologies [2-5]. Strains motile by gliding as well as non-motile strains exist. On February 1 2021, the genus *Spirosoma* comprised 41 validly named species [6]. These have been isolated from a variety of environments including air conditioning systems, plants, pollen, soil, permafrost soil, air and glacier till. Furthermore, environmental studies have detected members of this taxon in biogas from a biogas plant, biofilm, an ammonium sulfate bioreactor, contaminated soil and many other environments [7-10], allowing the hypothesis that *Spirosoma* has a world-wide distribution [2-6, 11-44]. Additionally, several species have been found to exhibit some remarkable characteristics such a resistance against gamma and ultraviolet radiation [20, 30, 38, 40] and the ability to solubilize phosphate [21]. Its radiation resistance and the harsh nature of some of its habitats suggests that *Spirosoma* is a versatile taxon capable of surviving under extreme conditions.

As part of a study on aerobic anoxygenic phototrophic bacteria present in ice-free soils of the Utsteinen region, Sør Rondane Mountains, East Antarctica, a large number of bacterial isolates was obtained in 2014 [45]. Dereplication using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) indicated that 16 of them (i.e. strains R-68523^{T}, R-68522, R-68505, R-68502, R-68424, R-68376, R-68108, R-68079, R-6[6]8006, R-67957, R-67936, R-67915, R-67885, R-67884, R-67858 and R-67812) had highly similar MALDI-TOF MS spectra and formed a coherent cluster. Subsequent partial 16S rRNA gene sequencing (V1-V3 region, ~450 bp) confirmed that the strains have near-identical 16S rRNA gene sequences related to the genus *Spirosoma*. In this study, the novel *Spirosoma* isolates were further characterized using a polyphasic approach and comparative genomics. Based on the results, we propose that the 16 strains represent a novel species of the genus *Spirosoma*: *S. utsteinense* sp. nov.

Isolation and ecology

The 16 bacterial strains were isolated in 2014-2016 during an isolation campaign from exposed soils (top surface samples, 71° 56’ 45.8” to 71° 57’ 28.6” S, 23° 19’ 45.8” to 23° 20’ 44.6” E). The top surface soil samples were collected from the Utsteinen region, Sør Rondane Mountains, East Antarctica between January 31 and February 3 2009 [45]. Soil sample collection was performed as described by Peeters *et al.* [46]. All strains were isolated, sometimes as microcolonies, using PA or PH medium from the different setups used in the isolation campaign, as described by Tahon and Willems [45]. After primary isolation and purification, strains were stored in the corresponding liquid medium containing 15% (v/v) glycerol at -80 °C. The proposed type strain, R-68523^T, was deposited in the Belgian Co-ordinated Collections of Microorganisms (BCCM/LMG, Ghent, Belgium) and the Spanish Type Culture Collection under the accession numbers LMG 31447^T and CECT 9925^T, respectively. Additionally, strain R-68079 was deposited in the BCCM/LMG collection under the accession number LMG 31448. Strains R-68522, R-68505, R-68502, R-68424, R-68376, R-68108, R-68006, R-67957, R-67936, R-67915, R-67885, R-67884, R-67858 and R-67812 were stored in the research collection of the Laboratory of Microbiology at Ghent University and are available for further research.

16S rRNA gene phylogeny

To identify the isolates, DNA was prepared using the alkaline lysis protocol [47]. Amplification and sequencing of the 16S rRNA gene was performed as previously described by Tahon and Willems [45]. First, only the V1-V3 region of all strains was sequenced. Comparison of these ~450 bp sequences using BioNumerics 7.5 (Applied Maths) indicated they were at least 99.7% similar to each other. Therefore, the near-complete 16S rRNA gene sequence was determined of only two strains, i.e. R-68079 (= LMG 31448) and R-68523^T (= LMG 31447^T). These strains were selected because they were located at different sides of the MALDI-TOF MS cluster obtained during the analysis of spectra obtained during the isolation campaign [45]. Comparison indicated these 1459 bp sequences were identical. Closest cultivated relatives to the strains were identified using the EzBioCloud database [48]. The highest similarities were all with type strains of the genus *Spirosoma*, however, with the exception of *Spirosoma rigui* KCTC 12531^T (97.8%) the similarities were below 94.6%.

To support the aforementioned result, phylogenetic placement of the near-complete 16S rRNA gene sequences of R-68523^T and R-68079 was performed together with those of the 41 validly named *Spirosoma* species. 16S rRNA gene sequences of closely related type strains from other genera were used as an outgroup. Sequences were aligned using MEGA7, after which a phylogenetic maximum-likelihood tree (1000 bootstraps) was constructed [49, 50]. Tree visualisation was done using the iTOL software [51].

The phylogenetic analysis revealed that strains R-68523^T and R-68079 represented a distinct subline within the genus *Spirosoma* and formed a robust cluster with *S. rigui* KCTC 12531^T and *S. arcticum* R2-35^T (Fig. 1) [4]. Thus, results using 16S rRNA gene sequence information indicate that the new strains represent a new species within the genus *Spirosoma*.

For further non-genome-based analyses, the types of *Spirosoma rigui* (its closest neighbour) and *Spirosoma linguale* (type species of the genus were selected as reference strains.

MALDI-TOF MS

In the MALDI-TOF MS dendrogram obtained during the isolation campaign [45], no spectra from reference type strains was included. Therefore, to corroborate the results obtained using 16S rRNA gene sequencing, new MALDI-TOF MS profiles were acquired in 2019 for strains R-68079, R-68523^T, and reference type strains *Spirosoma rigui* LMG 31158^T (= KCTC 12531^T) and *Spirosoma linguale* DSM 74^T according to Dumolin *et al.* [52]. In the dendrogram (Supplementary Figure 1), strains R-68079 and R-68523^T clearly cluster distantly from *Spirosoma rigui* LMG 31158^T, the closest neighbour based on 16S rRNA gene sequencing and *Spirosoma linguale* DSM 74^T, the type species of the genus *Spirosoma*.

Genome features

To allow genome-based analyses, the genomes of strains R-68523^T and R-68079 were sequenced. Genomic sequences for *S. linguale* DSM 74^T and *S. rigui* KCTC 12531^T were already publicly available. For sequencing, genomic DNA was extracted using an automated Maxwell® DNA preparation instrument (Promega) as detailed by Tahon *et al.* [53]. Subsequently, the genomic sequences were determined using the Illumina HiSeq 2500 platform with 2x150 bp cycles at the Oxford Genomics Centre at the Wellcome Centre for Human Genetics. The genomes were assembled using Shovill 1.0.4

(https://github.com/tseemann/shovill), with the adaptor trimming command (--trim) enabled. Contigs smaller than 500 bp were discarded. The QUAST program was used to generate the summary statistics of the assembly (e.g. N50, G+C%) [54]. Genome completeness and contamination was determined using CheckM 1.1.3 [55]. Final contigs were submitted for genome annotation using the Integrated Microbial Genomes-Expert Review (IMG-ER) platform [56]. Pairwise average nucleotide identity (ANI) was determined using the OrthoANIu tool [57]. Digital DNA-DNA hybridization (dDDH) was carried out using the Genome-to-Genome Distance Calculator 2.1 of DSMZ [58]. Identification and annotation of prophage sequences within the genome was performed using PHASTER [59]. Presence of clustered regularly interspaced short palindromic repeats (CRISPRs) was analysed using CRISPRCasFinder [60]. Identification of glycosyl hydrolases (GHs) and carbohydrate esterases (CEs) was performed using the dbCAN meta server [61]. Presence and identification of peptidases was performed using the MEROPS database [62]. Transporters were identified using TransAAP [63].

The annotated Whole Genome Shotgun projects have been deposited at DDBJ/ENA/GenBank (Table 1). Raw sequence data are available from the NCBI sequence read archive (https://www.ncbi.nlm.nih.gov/sra) under accession numbers SRR9265382 (R-68523^T = LMG 31447^T) and SRR9265482 (R-68079 = LMG 31448).

The final assemblies and their quality were in agreement with the minimal standards for the use of genome data as proposed by Chun *et al.* [64]. Genome characteristics of R-68523^T, R-68079 and reference type strains *S. linguale* DSM 74^T and *S. rigui* KCTC 12531^T are listed in Table 1. The draft genomes of R-68523^T and R-68079 were 6.86 and 7.04 Mb in size, respectively. Based on the genome analysis, the G+C content of strains R-68523^T and R-68079 was 52.12 and 52.11% respectively, which is within the range known for the genus *Spirosoma* (Table 1). Annotation using the IMG-ER platform resulted in 5975 and 6142 coding sequences for strains R-68523^T and R-68079, respectively. The ANI value of R-68523^T to R-68079, obtained using the OrthoANIu tool [57], was 99.96%. ANI values of these two strains to reference strains *S. linguale* LMG 10896^T and *S. rigui* LMG 31158^T were 73.9 to 78.7%, respectively (Table 2). Using the the Genome-to-Genome Distance Calculator 2.1 [58], the *is*DDH value of R-68523^T to R-68079 was 99.7%. *is*DDH values to reference strains *S. linguale* LMG 10896^T and *S. rigui* LMG 31158^T ranged from 20.3 to 22.0% (Table 2). These values were well below the recommended species thresholds (ANI: 95%; DDH 70%) [65, 66] indicating that the two strains indeed represent a novel species in the genus *Spirosoma*. Given the near-identical 16S rRNA

gene sequences and highly similar MALDI-TOF MS profiles, the 16 *Spirosoma* isolates are very likely strains of the same species.

Genome-based phylogeny

A 16S rRNA gene analysis is often insufficient to discriminate between closely related species [67]. Therefore, a more robust genome-based phylogeny was also performed. For this analysis, the genomes of strains R-68523^T and R-68079, and the 20 publicly available *Spirosoma* genomes from validly named species (February 1 2021) were screened for the presence of 107 single-copy core genes, found in a majority of bacteria, using the automated bcgTree pipeline [68] with default parameters except that 1000 bootstraps were used. The genome of *Fibrella aestuarina* BUZ 2^T was used as an outgroup. The tree was visualized using the iTOL software [51]. The analysis showed that, with the exception of *Spirosoma telluris* HMF3257^T (96 of 107 single-copy core genes), the genomes contained at least 104 of the 107 core genes. Furthermore, the resulting maximum likelihood tree (Fig. 2) confirmed the placement of strains R-68523^T and R-68079 in a distinct subline within the genus *Spirosoma*, forming a robust cluster with *S. rigui* KCTC 12531^T. This analysis corroborates the results of the 16S rRNA gene analyses and confirms that the novel strains form a new species in the genus *Spirosoma*. However, it should be noted that, to obtain a complete genome-based phylogeny of the genus *Spirosoma*, the 21 remaining validly described species should also have their genome sequenced.

Spirosoma utsteinense sp. nov. metabolism

The draft genomes of the two strains also allowed first insights in their metabolism. Analysis of the genome annotations revealed that the central metabolism of the investigated *Spirosoma* strains very likely involves the glycolysis pathway as well as the tricarboxylic acid cycle and the pentose phosphate pathway. This was also corroborated by Biolog and API assays. Key enzymes for oxygenic and anoxygenic photosynthesis, and autotrophic pathways were absent in the four investigated genomes (i.e. R-68523^T, 68079, *Spirosoma linguale* DSM 74^T and *Spirosoma rigui* KCTC 12531^T).

As suggested by the annotated genomes, the sulfur metabolism of the two novel strains consist of the complete assimilatory sulfate reduction pathway, in which sulfate is converted into sulfide via several intermediates obtain sulfate.

To obtain phosphate, a component which can be scarce in the environment, the *Spirosoma* strains may rely on a phosphate regulon [69, 70]. Both of the annotated genomes contained

PhoR, an inner-membrane histidine kinase sensor and PhoP, a cytoplasmatic transcriptional response regulator. Together, these form a two-component regulatory system which controls the phosphate regulon. Upon phosphate scarcity, the PhoR-PhoP two-component system may be used to transcribe several important genes of the phosphate regulon, for example *phoA* and *phoD* [69, 71], both of which were present in the genomes of strains R-68523^T and R-68079.

Based on the genome annotations, nitrogen, another essential component for survival, may be obtained from the environment via nitrate and nitrite transporters. Additionally, the annotated genomes contained NarB and NirB/D, suggesting that these *Spirosoma* strains may also be capable of converting nitrate to nitrite and nitrite to ammonia, respectively.

One important genomic feature of members of the *Bacteroidetes* phylum is the presence of polysaccharide utilisation loci or PULs, which encode a number of cell surface glycan-binding proteins, TonB-dependent receptors, carbohydrate-active enzymes and carbohydrate sensors or transcriptional regulators [72, 73]. PULs constitute the major nutrient acquisition strategy in *Bacteroidetes* and typically contain at least one sequential pair of *susC/D* genes involved in polysaccharide uptake [73, 74]. The genomes of strains R-68079 and R-68523^T both contained ~40 of these *susC/D* pairs. The carbohydrate-active enzymes found in PULs are often carbohydrate esterases (CEs) and glycosyl hydrolases (GHs). Additionally, PULs also often contain sulfatases and peptidases. Using the dbCAN meta server, the genomes were found to contain ~4 CEs and ~17 GHs per Mb, many of which were located in PULs. The majority of CEs belonged to CE family 1, although both genomes contained CEs classified in 11 different CE families. GHs present in the genomes of strains R-68079 and R-68523^T were distributed among 48 different families. Although most of these were represented only by one or a couple of GHs, GH families 13 and 43 each contained more than 10 GHs.

The annotated genomes only contained one sulfatase gene, annotated as arylsulfatase. These are known to cleave sulfate esters to supply an organism with sulfur [75]. The low number of sulfatases present in the annotated genomes may be explained by the nature of terrestrial polysaccharides which are, contrary to marine polysaccharides, not highly sulfated [76].

The genomes of strains R-68079 and R-68523^T contained approximately 25 peptidases per Mb. This number is comparable to that of other *Bacteroidetes* [77, 78]. Peptidases were distributed among a large number of different families, with peptidase families S8, S9, S41, M16, M20 and M28 being represented by large numbers of peptidases in the genomes.

The novel strains all originate from Antarctic ice-free soils. In soil, which is an excellent matrix for phage-bacteria interaction, phages are an important integral part of the community. They are a vast reservoir of genetic elements, hence contributing to biological diversity. As a result,

phages influence soil bacterial communities and thus also the biogeochemical cycles [79]. To determine whether there might be contact with phages, the genomes of strains R-68079 and R-68523^T were screened for the presence of CRISPR regions. These CRISPRs encode functions for the prevention of infections with alien DNA [80]. The two genomes contained 13 and 12 CRISPR regions, respectively. These were all marked as questionable, except for one region in each genome which were marked with high evidence as CRISPRs. This was also corroborated by the presence of multiple *cas* genes in the annotated genomes. Additionally, the genomes were also screened for the presence of prophage sequences. Although strains R-68079 and R-68523^T contained 3 and 1 of these regions, they were all marked as putative and incomplete.

Physiology, chemotaxonomy and environmental distribution

For cellular fatty acid analysis, strains R-68079, R-68523^T and the two reference type strains *S. rigui* LMG 31158^T and *S. linguale* LMG 10896^T were incubated on R2A at 20 °C for 7 days. After fatty acid methyl-ester extraction, separation by gas-liquid chromatography was performed using the MIDI system (MICROBIAL ID Inc.) as previously described [81]. Fatty acid methyl esters were identified by comparison to the MIDI Peak Library version 5.0.

In general, the whole-cell fatty acid composition of the new strains was similar to that of the reference type strains. The major cellular fatty acid were co-eluted fatty acids $C_{16:1}$ ω7c and/or iso-C_{15} 2-OH (38.9-50.7%; summed feature 3), $C_{16:1}$ ω5c (16.0-25.7%), $C_{16:0}$ (2.8-12.2%) and iso-$C_{15:0}$ (3.5-10.7%) (Table 3).

Single colonies of strains R-68079, R-68523^T and reference type strains *S. linguale* LMG 10896^T and *S. rigui* LMG 31158^T were grown on R2A at 20 °C for 7 days. Pigment extraction in methanol was performed as previously described by Tahon *et al.* [53]. Pigment extracts were characterized by spectrophotometry using a SPECTRAmax PLUS 384 spectrophotometer (Molecular Devices). The absorbance spectrum was measured between 190 and 1,000 nm. Subsequently, the samples were alkalinized with 0.1 M NaOH after which the spectra were examined for a bathychromatic shift characteristic of flexirubin-type pigments [82]. Additionally, pigment extracts were flooded with 20% KOH to examine presence of a color shift indicating presence of a flexirubin-type pigment [83].

Pigment extracts of strains R-68079 and R-68523^T showed a clear absorption maximum at 455 nm and shoulders at 433 and 479 nm (Supplementary Figure 2). The pigments are carotenoid-

like, because these values are in the range reported for carotenoid pigments [84]. Although the exact carotenoid type could not be determined, pigment colour, absorption maxima and data from annotated genomes suggest high similarities with the carotenoid lutein [85, 86]. Overall, these profiles were highly similar to those of the two reference type strains. However, for the latter, the absorption maximum was located at 447 nm, whereas the shoulders were located at 422 and 472 nm. For *S. rigui* LMG 31158^T these results corroborated previous results [3]. A small shift could be observed, although this was most likely due to the use of a different solvent in the previous study (i.e. ethanol). Flexirubin-type pigments were absent in all strains.

Cell morphology was observed using a phase-contrast microscope (Olympus BX40) after incubation on R2A at 20 °C for 7 days. Gram staining was performed as previously described by MacFaddin [87]. Catalase activity was determined by bubble production in 3% (v/v) H_2O_2 and oxidase activity was determined using 1% (w/v) tetramethyl *p*-phenylenediamine. Gliding-motility was evaluated using the hanging drop technique as described previously by Bernardet *et al.* [88]. Unless stated otherwise, metabolic profiling of the strains was performed at 20 °C in three replicates. Growth on other standard bacteriological media was evaluated using trypticase soy agar (TSA), nutrient agar (NA), marine agar (MA), R2A/10 and R2A/100. Salt tolerance was tested by growing the strains in R2A broth. Growth with 0, 0.5, 1, 2, 3, 4, 5, 6, 7 and 8% (w/v) NaCl was tested. Growth at different pH values (4.0-9.5 at intervals of 0.5 pH units) was assessed after growth in R2A broth. Because of very poor growth in R2A broth, growth of strains R-68523^T and R-68079 at different pH values was also assessed on R2A. Depending on the final pH needed, media were buffered with MES (pH 4.0-6.0), MOPS (pH 6.5-7.0), ACES (pH 7.5), TAPS (pH 8.0-8.5) or CHES (pH 9.0-9.5) in a final concentration of 10 mM. To determine pH and salt tolerance range and optimum, growth was monitored by measuring the optical density three times at 600 nm after 10 days. The value of the negative control was subtracted from all other wells. Resulting values >0.05 were considered as positive. Growth at 4, 10, 15, 20, 25, 28, 30, 35, 37, 41, 44 and 52 °C was determined on R2A. Growth in anaerobic atmosphere (80% N_2, 10% CO_2 and 10% H_2) and growth in microaerobic atmosphere (80% N_2, 15% CO_2 and 5% O_2) was tested at 15 and 20 °C on R2A. Antibiotic sensitivity was determined by growing the strains on R2A and using antimicrobial susceptibility paper discs. The following antibiotics (Oxoid) were tested: Gentamicin (10 µg), Ampicillin (10 µg), Tetracycline (10 µg), Chloramphenicol (30 µg), Bacitracin (4 µg) and Vancomycin (30 µg). Carbon source utilization, acid formation from carbohydrates and enzyme activity were determined using API ZYM, API 50 CH, API 20 NE (bioMérieux) and the GEN III

MicroPlate™ (Biolog). For the API 50 CH and API 20 NE, the manufacturer's protocols were changed as these did not result in growth or positive reactions. Briefly, inoculation fluids were modified to contain a final concentration 10% R2A broth as suggested by the manufacturer (personal communication). Additionally, a strip with all wells filled with this modified inoculation fluid was taken along as a negative control. After inoculation, these galleries were incubated for 7 (reference strains) or 10 (new strains) days at 20 °C before reactions were read. For the GEN III Microplate™, positive reactions were determined daily over the course of one week using a spectrophotometer (590 nm). The value of the negative control was subtracted from all other wells. Resulting values >0.075 were considered as positive.

Strains R-68523^T and R-68079 grew well on R2A, R2A/10 and R2A/100, but not on MA, NA and TSA, and very poorly in R2A broth. Both strains formed rod-shaped cells of approximately 2-4 µm long and 1 µm wide. When grown on R2A, cells sometimes appeared filamentous, forming filaments up to 35 and 12 µm long for strains R-68079 and R-68523^T, respectively. Cells were Gram-negative, aerobic, non-motile and non-spore forming. Strains were aerobic, but growth also occurred under microaerobic conditions. No growth occurred under anaerobic conditions. Colonies were orange on full strength R2A, but more transparent and less colourful on diluted R2A. Colonies formed on R2A after 7 days of incubation at 20 °C were circular, convex and had smooth edges. The strains grew between 4 and 28 °C with optimal growth between 15 and 25 °C. The NaCl range tolerated for growth was 0-0.5% (w/v). Optimal growth occurred without NaCl. Growth occurred on R2A over the range of pH 6.5-8.0 (optimum pH 6.5-7.0). Neither strain R-68523^T nor R-68079 produced flexirubin-like pigments. Other physiological characteristics of strains R-68523^T and R-68079 are found in the species description. The two strains of the newly proposed species shared many characteristics with reference type strains of the genus *Spirosoma*, but also showed differences. For example, strains R-68523^T and R-68079 did not produce acid from erythritol, L-sorbose and xylitol, whereas the reference type strains did. Additionally, differences could be observed for the assimilation of L-galactonic acid lactone, D-sorbitol and D-fructose-6-PO_4, and resistance against troleandomycin and 1% sodium lactate. Characteristics that differentiate strains R-68523^T and R-68079 from the closest neighbour *Spirosoma rigui* LMG 31158^T and the type strain of the genus *Spirosoma* (i.e. *Spirosoma linguale* LMG 10896^T) are shown in Table 4.

To determine the environmental distribution of the new taxon, the 16S rRNA gene sequences of strains R-68523^T and R-68079 were subjected to a blast search [89]. Interestingly, with the

exception of the other novel *Spirosoma* strains included here, only two publicly available sequences (accession numbers JF192916.1 and JF156465.1) displayed a higher similarity (i.e. 98.2-98.3%) than *Spirosoma rigui* KCTC 12531^T, the closest type strain. Both sequences originate from human skin and were obtained from clone libraries [7]. The two sequences were added to the alignment used to determine the 16S rRNA gene phylogeny of the novel strains. After realigning the sequences, a maximum likelihood phylogenetic analysis using MEGA7 [49] (Fig. 3) showed that the clone sequences formed a coherent cluster with *Spirosoma rigui* KCTC 12531^T and not with the sequences of strains R-68523^T and R-68079. Therefore, based on currently available data, the environmental distribution of *Spirosoma utsteinense* sp. nov. is restricted to Antarctic top surface soil.

As shown using physiological, morphological and chemotaxonomic characteristics, and 16S rRNA gene sequence analysis, strains R-68523^T, R-68079, and the 14 other strains (i.e. R-68522, R-68505, R-68502, R-68424, R-68376, R-68108, R-68006, R-67957, R-67936, R-67915, R-67885, R-67884, R-67858 and R-67812) represent a novel species of the genus *Spirosoma*, for which the name *Spirosoma utsteinense* sp. nov. is proposed.

Description of *Spirosoma utsteinense* sp. nov.

Spirosoma utsteinense (ut.stein.en'se. N.L. neut. adj. referring to Utsteinen, the location in Antarctica where the first strain was isolated)

Cells are Gram-negative, oxidase- and catalase-positive, rod-shaped, non-motile and do not form endospores. Cells are visualized as rods of ~1 µm wide and 2-4 µm long, sometimes forming filaments of up to 12 µm. When grown on R2A for 2 weeks, colonies are orange, circular and 1-2 mm in diameter. Growth is observed at 4-30 °C with an optimum between 15 and 25 °C, and at pH 6.5-8.0 with an optimum of 6.5-7.0. Grows with 0-0.5% (w/v) NaCl (optimum of 0%). Growth occurs under microaerobic (80% N_2, 15% CO_2 and 5% O_2), but not under anaerobic atmosphere (80% N_2, 10% CO_2 and 10% H_2). Does not reduce nitrate. Does not produce flexirubin.

In the API ZYM gallery, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase activities are present. Lipase (C14), trypsin, β-glucuronidase, α-mannosidase and α-fucosidase activities are absent.

According to the API 20 NE gallery, strain R-68523^T is positive for positive for arginine dehydrolase, urease, esculine, the PNPG test (β-galactosidase), hydrolysis of gelatin and assimilation of glucose, mannose, N-acetyl-glucosamine, mannitol, adipic acid, malate, citric acid, gluconate, maltose and phenylacetic acid.

In the API 50 CH, strain R-68523^T is positive for acid formation from D-arabinose, L-arabinose, D-xylose, L-xylose, methyl-D-xylopyraniside, D-galactose, D-glucose, D-fructose, D-mannose, methyl-D-mannopyranoside, methyl-D-glucopyranoside, N-acetylglucosamine, amygdaline, arbutine, esculine, salicine, D-celiobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, inuline, D-melezitose, D-raffinose, starch, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-ribose and L-rhamnose.

Using Biolog GEN III, strain R-68523^T gave positive reactions for the following assays: dextrin, D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, stachyose, D-raffinose, α-D-lactose, D-melibiose, β-methyl-D-glucoside, D-salicin, N-acetyl-D-glucosamine, α-D-glucose, D-mannose, D-fructose, D-galactose, L-fucose, D-rhamnose, D-glucose-6-PO_4, D-gluconic acid, tetrazolium blue, tween 40, aztreonam, N-acetyl-β-D-mannosamine, N-acetyl-

D-galactosamine, D-arabitol, myo-inositol, D-serine, troleandomycin, L-glutamic acid, L-galactonic acid lactone, glucuronamide, acetic acid.

The type strain was susceptible to bacitracin, vancomycin, ampicillin, tetracycline and chloramphenicol, but resistant to gentamicin.

The major cellular fatty acids are co-eluted fatty acids $C_{16:1}\,\omega 7c$ and/or iso-C_{15} 2-OH, $C_{16:1}\,\omega 5c$, $C_{16:0}$ and iso-$C_{15:0}$.

The DNA G+C content of the type strain R-68523^T, as derived from the genome sequence is 52.1%, its approximate size 6.86 Mb, its Genbank deposit SAMN11964661. The complete 16S rRNA gene sequences of strain LMG 31447^T has been deposited at DDBJ/ENA/Genbank under the accession number MN031264.

The description of strain R-68079 is identical to that of the type strain, with the following differences: filaments up to 35 µm can be formed. Resistant to bacitracin (4 µg). Negative for acid hydrolysis of D-ribose and L-rhamnose in the API 50 CH gallery. Positive for pectin, but negative for myo-inositol, D-serine, acetic acid, glucuronamide and L-glutamic acid in the Biolog GEN III microplate. Negative for assimilation of maltose and phenylacetic acid in the API 20 NE gallery. The DNA G+C content of strain R-68079, as derived from the genome sequence is 52.1%, its approximate size 7.04 Mb, its Genbank deposit SAMN11964662. The complete 16S rRNA gene sequences of strain LMG 31448 has been deposited at DDBJ/ENA/Genbank under the accession numbers MN031263.

The type strain, R-68523^T (= LMG 31447^T = CECT 9925^T), and strains R-68079 (= LMG 31448), R-68522, R-68505, R-68502, R-68424, R-68376, R-68108, R-68006, R-67957, R-67936, R-67915, R-67885, R-67884, R-67858 and R-67812 were isolated from ice-free terrestrial samples taken in the proximity of the Belgian Princess Elisabeth Station, Utsteinen, Sør Rondane Mountains, East Antarctica.

Figure captions

Fig. 1. Maximum-likelihood phylogenetic tree (1000 bootstrap replicates) of near-complete 16S rRNA gene sequences showing the relationship between strains R-68523^{T} and R-68079 and the type strains of species of the genus *Spirosoma*. Several other members of the family *Cytophagaceae* were used as an outgroup. Only bootstrap values greater than 70% are shown. Scale bar indicates 0.01 substitutions per nucleotide position.

Fig. 2. Maximum-likelihood phylogenetic tree (1000 bootstraps) built using the concatenated sequence of 107 highly conserved single copy genes extracted from strains R-68523^{T} and R-68079, and reference *Spirosoma* genomes. Only bootstrap values higher than 50% are shown. *Fibrella aestuarina* BUZ 2^{T} was used as an outgroup. Scale bar indicates 0.01 substitutions per position.

Fig. 3. Detailed view of the maximum likelihood phylogenetic tree cluster (1000 bootstraps) generated using near-complete 16S rRNA gene sequences from strains R-68523^{T} and R-68079, *Spirosoma* type strains and closely-related environmental sequences. Only bootstrap values greater than 50% are shown. Scale bar indicates 0.01 substitutions per nucleotide position.

Table 1. Characteristics of genomes of *Spirosoma utsteinense* R-68523^{T} and R-68079, and reference type strains *S. linguale* DSM 74^{T} and *S. rigui* KCTC 12531^{T}

Features	***S. utsteinense*** **R68523^{T}**	***S. utsteinense*** **R-68079**	***S. linguale*** **DSM 74^{T}**	***S. rigui*** **KCTC 12531^{T}**
Size (Mb)	6.86	7.04	8.49	5.83
Contigs	180	195	9	1
N50 (kb)	136	149	8080	5830
G+C%	52.12	52.11	50.15	54.40
Genes (Total)	5971	6138	7130	4774
CDS (Coding)	5914	6078	7067	4647
Genes (RNA)	51	56	63	54
5S rRNA	2	2	3	3
16S rRNA	1	1	4	3
23S rRNA	1	1	4	3
tRNA	43	44	49	43
CRISPR (questionable)	12	13	2	1
CRISPR (high evidence)	1	1	1	0
Prophage region (incomplete)	1	3	1	1
Glycosyl hydrolases	113	113	157	94
Glycosyl transferases	95	95	113	99
Carboxyl esterases	26	27	37	20
Auxiliary activities	6	6	3	2
Carbohydrate-binding modules	18	20	18	17
Transporters	340	353	394	301
Accession number	VFIA00000000	VFIC00000000	GCA_000024525.1	CP020105.1

Table 2. Results of Average Nucleotide Identity (ANI) and digital DNA-DNA hybridization (dDDH) between *Spirosoma utsteinense* R-68523^{T} and R-68079, and representative genomes of *Spirosoma* species. Values in the lower (in bold) and upper triangle correspond to ANI and dDDH, respectively

	S. utsteinense **R-68523^{T}**	***S. utsteinense*** **R-68079**	***S. linguale*** **DSM 74^{T}**	***S. rigui*** **KCTC 12531^{T}**
S. utsteinense R-68523^{T}		99.7	20.3	21.9
S. utsteinense R-68079	**99.9**		20.3	22.0
S. linguale DSM 74^{T}	**74.0**	**73.9**		20.2
S. rigui KCTC 12531^{T}	**78.7**	**78.6**	**74.0**	

Table 3. Major fatty acid composition (i.e. ≥1%) of *Spirosoma utsteinense* R-68523^T (1), R-68079 (2), *Spirosoma linguale* LMG 10896^T (3) and *Spirosoma rigui* LMG 31158^T (4). Values shown are percentages of total fatty acids obtained in this study.

TR: Trace amount (i.e. < 1%). [a]: Summed features represent groups of multiple fatty acids that cannot be separated by the Microbial Identification System. [b]: Unknown fatty acid with chain length (ECL) 14.959. [c]: Unknown fatty acid with chain length (ECL) 16.582. -: Not detected

	1	2	3	4
$C_{12:0}$	2.4	2.1	4.0	5.9
iso-$C_{13:0}$	-	-	1.8	-
$C_{15:0}$	TR	TR	1.7	-
anteiso-$C_{15:0}$	4.6	4.6	2.0	3.0
iso-$C_{15:0}$	8.6	10.7	6.9	3.5
iso-$C_{15:0}$ 3-OH	2.1	2.1	2.8	4.3
$C_{16:0}$	11.1	12.1	2.8	4.0
$C_{16:0}$ 3-OH	2.2	1.8	1.9	3.8
$C_{16:1}$ ω5c	16.0	17.0	25.2	21.0
$C_{17:0}$ 2-OH	1.0	TR	-	-
iso-$C_{17:0}$ 3-OH	5.7	5.3	5.0	4.0
cyclo-$C_{19:0}$ ω8c	-	-	1.6	-
Summed Features[a]				
3 $C_{16:1}$ ω7c and/or iso-C_{15} 2-OH	42.4	38.6	42.1	50.7
Unknown ECL 14.959[b]	-	-	1.6	-
Unknown ECL 16.582[c]	1.0	TR	-	-

Table 4. Phenotypic characteristics of *Spirosoma utsteinense* R-68523^T and R-68079, and reference type strains *S. linguale* LMG 10896^T and *S. rigui* LMG 31158^T. +, positive; -, negative; w, weakly positive; R, resistant; S, sensitive.

All strains are positive for catalase, oxidase, growth under microaerobic conditions, growth on R2A, R2A/10 and R2A/100, and negative for Gram-stain, and growth on marine agar. All strains are sensitive to ampicillin (10 µg), vancomycin (30 µg), tetracycline (10 µg) and chloramphenicol (30 µg), but resistant to gentamicin (10 µg). In API ZYM, all strains are positive for the alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase, but negative for the Lipase (C14), β-glucuronidase and α-fucosidase enzyme activity tests. In API 50 CH, all strains are positive for acid formation from D-arabinose, L-arabinose, D-xylose, L-xylose, methyl-D-xylopyraniside, D-galactose, D-glucose, D-fructose, D-mannose, methyl-D-mannopyranoside, methyl-D-glucopyranoside, N-acetylglucosamine, amygdaline, arbutine, esculine, salicine, D-celiobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, inuline, D-melezitose, D-raffinose, amidon, D-turanose, D-lyxose, D-tagatose, D-fucose and L-fucose, but negative for acid formation from glycerol, D-adonitol, dulcitol, inositol, D-mannitol, D-sorbitol, glycogen, gentiobiose, L-arabitol, potassium gluconate, potassium 2-keto-gluconate and potassium 5-keto-gluconate. In Biolog GEN III, all strains are positive for the dextrin, D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, stachyose, D-raffinose, α-D-lactose, D-melibiose, β-methyl-D-glucoside, D-salicin, N-acetyl-D-glucosamine, α-D-glucose, D-mannose, D-fructose, D-galactose, L-fucose, D-rhamnose, D-glucose-6-PO_4, D-gluconic acid, tetrazolium blue, tween 40 and aztreonam assays, but negative for the pH 5, 4% NaCl, 8% NaCl, 3-methyl glucose, D-fucose, inosine, fusidic acid, D-serine, D-mannitol, glycerol, D-aspartic acid, minocycline, glycyl-L-proline, L-alanine, L-aspartic acid, L-histidine, L-pyroglutamic acid, L-serine, lincomycin, guanidine HCl, Niaproof 4, mucic acid, quinic acid, D-saccharic acid, vancomycin, D-lactic acid methyl ester, L-lactic acid, citric acid, α-keto-glutaric acid, D-malic acid, L-malic acid, bromo-succinic acid, nalidixic acid, lithium chloride, potassium tellurite, γ-amino-butyric acid, α-hydroxy-butyric acid, β-hydroxy-D,L-butyric acic, α-keto-butyric acid, acetoacetic acid, propionic acid, formic acid, sodium butyrate and sodium bromate assays. In API 20 NE, all strains are positive for arginine dehydrolase, urease, esculine, the PNPG test (β-galactosidase) and assimilation of glucose, mannose and N-acetyl-glucosamine, but negative for reduction of nitrates to nitrites, reduction of nitrates to nitrogen, indole production, fermentation (glucose) and assimilation of arabinose and capric acid.

Characterstic	**R-68523^T**	**R-68079**	**LMG 10896^T**	**LMG 31158^T**
Cell shape	Rod-shaped, filaments can be present	Rod-shaped, filaments can be present	Rod- and horseshoe-shaped	Rod-shaped, filaments can be present
Cell size (µm)				
Gliding motility	-	-	+	+
Anaerobic	-	-	+	+
Growth conditions				
Temperature range (°C)	4-30	4-30	4-37	4-37
Temperature optimum (°C)	15-25	15-25	20-30	28-30
Salinity range (% NaCl, w/v)	0-0.5	0-0.5	0-1	0-2
Salinity optimum (% NaCl, w/v)	0	0	0	0
pH range	6.5-8.0	6.5-8.0	6-9.5	5.5-9.5
pH optimum	6.5-7.0	6.5-7.0	6-6.5	6.5
TSA, NA	-	-	+	+
Antibiotic susceptibility:				
Bacitracin (4 µg)	S	R	R	R
Enzyme activity (API ZYM):				
Cystine arylamidase	w	w	-	w
Trypsin	-	-	+	+
α-chymotrypsin	w	+	-	-
β-galactosidase	+	+	-	w
α-mannosidase	-	-	-	w
Acid hydrolysis (API 50 CH):				
Erythritol, L-sorbose, xylitol	-	-	+	+
D-ribose, L-rhamnose	+	-	+	+
D-arabitol	-	-	+	-
Assimilation of/resistance to (Biolog GEN III):				

Characterstic	R-68523^T	R-68079	LMG 10896^T	LMG 31158^T
pH 6, 1% sodium lactate, D-sorbitol, D-fructose-6-PO_4	-	-	+	+
Methyl pyruvate, p-hydroxy-phenylacetic acid, tetrazolium violet, D-glucuronic acid, D-galacturonic acid, L-arginine, N-acetyl neuraminic acid	-	-	+	-
1% NaCl, rifamycin SV, gelatin	-	-	-	+
N-acetyl-β-D-mannosamine, N-acetyl-D-galactosamine	+	+	+	-
Myo-inositol, D-serine, acetic acid	+	-	-	-
Troleandomycin, L-galactonic acid lactone	+	+	-	-
Glucuronamide, L-glutamic acid	+	-	+	-
D-arabitol	+	+	-	+
Pectin	-	+	+	+
Hydrolysis/assimilation of (API 20 NE):				
Gelatin	+	+	-	-
Mannitol, adipic acid, malate, citric acid	w	w	-	-
Gluconate	w	w	-	+
Maltose	w	-	+	+
Phenylacetic acid	w	-	-	-

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Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Committee approval for human or animal research

NA

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Supplementary Material

Spirosoma utsteinense sp. nov. isolated from Antarctic ice-free soils from the Utsteinen region, East Antarctica

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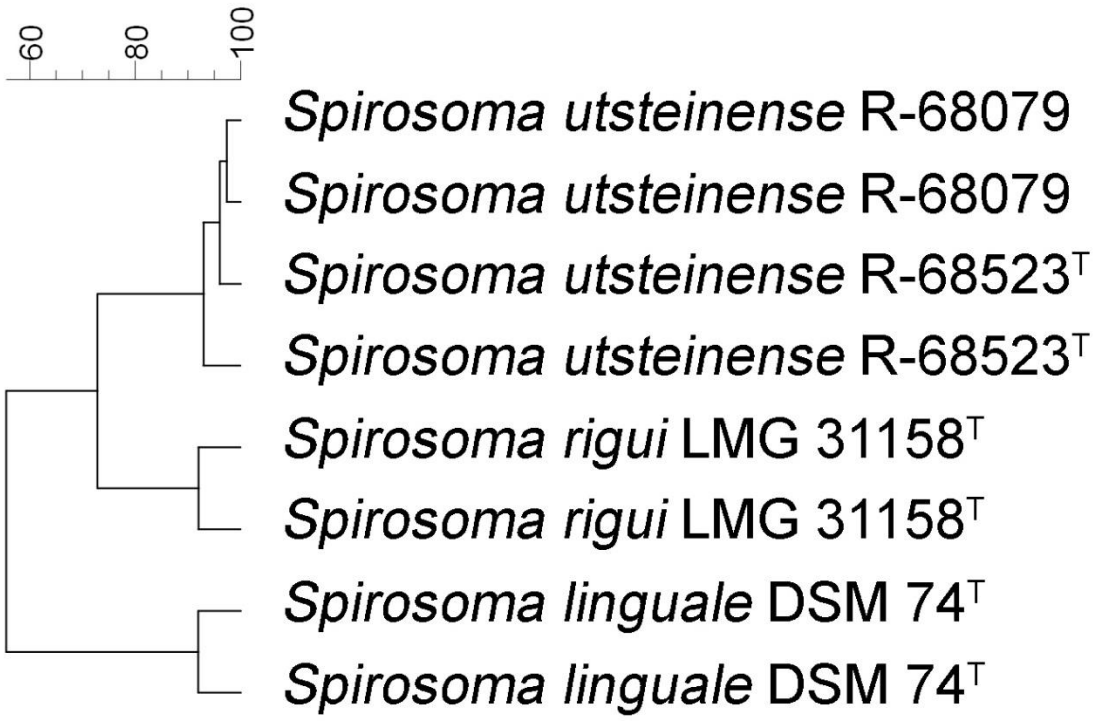
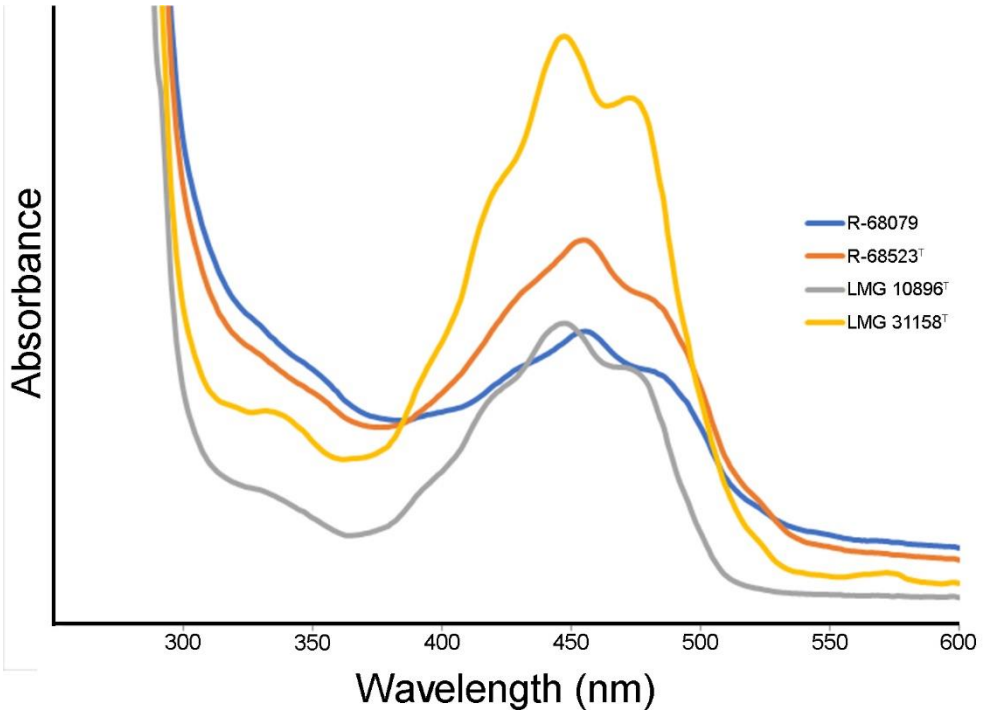
1. Supplementary Figures



Supplementary Figure 1. Curve-based cluster analysis of mass spectra obtained from protein extracts using the Pearson product moment correlation coefficient and UPGMA cluster algorithm of strain R-68523^{T}, strain R-68079, and type strains *Spirosoma rigui* LMG 31158^{T} and *Spirosoma linguale* DSM 74^{T}.



Supplementary Figure 2. Absorption spectrum of extracted pigment (in methanol) of strain R-68523^{T}, strain R-68079, and type strains *Spirosoma rigui* LMG 31158^{T} and *Spirosoma linguale* DSM 10896^{T}.