

Hypericibacter terrae* gen. nov., sp. nov. and *Hypericibacter adhaerens* sp. nov., two new members of the family *Rhodospirillaceae* isolated from the rhizosphere of *Hypericum perforatum

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains R5913^T and R5959^T are MG271952 and MH450230, respectively. The GenBank accession numbers for the draft genome sequences of strain R5913^T and R5959^T are CP042906 and CP042582, respectively.

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Abstract

Two strains of the family *Rhodospirillaceae* were isolated from the rhizosphere of the medicinal plant *Hypericum perforatum*. Cells of both strains stain Gram-negative, are motile by means of a single polar flagellum, are non-spore-forming, non-capsulated, short rods that divide by binary fission. Colonies are small and white. Strains R5913^T and R5959^T are oxidase-positive, mesophilic, neutrophilic, and grow optimally without NaCl. Both grow under aerobic and microaerophilic conditions and on a limited range of substrates with best results on yeast extract. Major fatty acids are C_{19:0} cyclo ω 8c and C_{16:0}; in addition, C_{18:1} ω 7c is also found to be predominant in strain R5913^T. The major respiratory quinone is ubiquinone 10 (Q-10). The DNA G+C contents of strains R5913^T and R5959^T are 66.0 % and 67.4 %, respectively. 16S rRNA sequence comparison revealed that the closest relatives (<92% sequence similarity) of the strains are *Oceanibaculum pacificum* MCCC 1A02656^T, *Dongia mobilis* CGMCC 1.7660^T, *Dongia soli* D78^T, and *Dongia rigui* 04SU4-P^T. Both strains shared 98.6 % sequence similarity and represent different species on the basis of low average nucleotide identity of their genomes (ANI value, 83.8 %). Based on the combined phenotypic, genomic, and phylogenetic investigations the two strains represent two novel species of a novel genus in the family *Rhodospirillaceae*, for which the name *Hypericibacter* gen. nov. is proposed, comprising the type species *Hypericibacter terrae* sp. nov. (type strain R5913^T = DSM 109816^T = CECT 9472^T) and *Hypericibacter adhaerens* (type strain R5959^T = DSM 109817^T = CECT 9620^T).

The family *Rhodospirillaceae*, first proposed in 1971 by Pfennig and Trüper [1], consists of morphologically diverse taxa with a wide range of metabolic capabilities, including chemoheterotrophs, photoautotrophs, and photoheterotrophs. At the time of writing the family consisted of 48 different genera [2-4]. The members of this family have been found to inhabit different environments, like soils [5-7], desert sand [8], freshwater lakes [9], coastal water [10], rock biofilms [11], salt marshes [12, 13], antarctic white rocks [14], sludge of dye works [15], and even hydrothermal fields [16].

The newly described bacteria were obtained during a study of the rhizobiome of *Hypericum perforatum* (accession number: HyPR-01 [17]). This plant is a sexual diploid *Hypericum* line that was propagated by stem cuttings (sterile soil composition: 3 parts compost, 1 part Patzer substrate, 1.5 part sand; greenhouse conditions: 16 h of light at 26-28 °C, 8 h of darkness at 24-26°C, and 70% average humidity) and mostly known for the production of hypericin and hyperforin with antidepressant activity [17, 18]. The investigated bacterial communities originated from the rhizospheres of 1.5 years old plants. The plants were grown in the greenhouse of the Leibniz Institute of Plant Biochemistry in Halle, Germany (51° 29' 42.23" N, 11° 56' 36.56" E) and the soil had neutral pH (6.9 and 7.3 measured in distilled water and 2 mM CaCl₂, respectively). Two strains designated as R5913^T and R5959^T were isolated from the rhizosphere employing a high-throughput cultivation approach [19]. Briefly, total bacterial cell numbers were determined using SYBR Green I staining (Life Technologies, Ltd, Paisley, UK) as described previously [20]. The soil was first suspended in HEPES buffer (10 mM, pH 7) and 20 µL of this suspension, which comprised approximately either 25 or 50 cells, was transferred to 96-well microtiter plates containing 180 µL of SSE/HD 1:10 medium. SSE/HD is a medium consisting of soil solution equivalent [21] with the addition of 10-vitamin [22] and trace element SL-10 [23] solutions, buffered with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) at a pH of 7 and amended by peptone, yeast extract and glucose [24, 25]. The outer wells of the plates were left un-inoculated and the plates were incubated for 3 months at room temperature. Growth was detected by turbidity. Wells containing grown cultures were selected for the analysis of the 16S ribosomal RNA V1-V2 hypervariable regions, using a barcoded Illumina paired-end sequencing method [26] with modified PCR reactions (Supplementary Methods). Sequencing was performed on the Illumina MiSeq platform (San Diego, CA, USA).

The taxonomy of the reads was assigned to the SILVA database (v.123) [27] with UCLUST [28]. Cultures with sequences with less than 97% similarity to validly described strains were streaked on SSE/HD 1:10 medium buffered at pH 7 and solidified with 0.8% (w/v) gellan gum. Two strains were obtained, purified, and maintained in R2A medium (=DSMZ medium 830). For metabolic and physiological tests, both strains were grown without NaCl and at their optimum pH and temperatures (pH 7 and 30°C for the growth of R5913^T, pH 6 and 36°C for R5959^T). The strains were kept in 20%

glycerol at -80° C for long-term preservation. Strains R5913^T and R5959^T belong to the family *Rhodospirillaceae* and share 98.6 % 16S rRNA gene sequence similarity with one another. According to the EzBioCloud database [2], the closest relatives of strain R5913^T are *Oceanibaculum pacificum* MCCC 1A02656^T (91.9 %), *Dongia mobilis* CGMCC 1.7660^T (91.8 %) and *Dongia soli* D78^T (91.7 %), while the closest relatives of strain R5959^T were *Dongia mobilis* CGMCC 1.7660^T (91.6 %), *Oceanibaculum pacificum* MCCC 1A02656^T (91.4 %) and *Dongia rigui* 04SU4-P^T (91.3 %).

Phenotypic characterization was conducted by methods described previously [19, 29, 30]. Both strains stained Gram negative and appeared as small, round, smooth, shiny, convex, white colonies (1 mm in diameter) on R2A agar. Some cells were motile by means of a single polar flagellum. Capsules and endospores were not present. Within their closest relatives in the family *Rhodospirillaceae*, capsules are only known to be present in *Lacibacterium aquatile* LTC-2^T (Table 1). Cells of R5913^T were single short rods, sometimes slightly curved (0.7-1.5 μ m long and 0.5-0.8 μ m wide), as observed under the light microscope (Zeiss Axio Lab. A1; Carl Zeiss). Cells of strain R5959^T were slightly curved, short, and rod-shaped (0.5-2.5 μ m long and 0.3-1.0 μ m wide) and occurred as single cells or in short chains. Rod-shaped cells are a common feature of R5913^T and R5959^T closest relatives (Table 1), with the exception of the star-shaped cells of the genus *Stella* (currently classified as a member of the family *Acetobacteraceae*) [31]. For scanning electron microscopy, cells in liquid R2A media were fixed for 30 minutes at room temperature in 2 % glutaraldehyde, then mixed with 5 % paraformaldehyde and stored at 4°C. Cells were subsequently washed with TE buffer (10 mM Tris, 2 mM EDTA, pH 6.9) and dehydrated on ice in a graded series of acetone (10, 30, 50, 70, 90, and 100 %; each for 10 minutes). Samples were then subjected to critical-point drying by applying CO₂ (CPD 030, Bal-Tec, Liechtenstein). Dried samples were further coated with a gold/palladium (80/20) film by sputter coating (SCD 500, Bal-Tec, Liechtenstein) before examination in a field emission scanning electron microscope Zeiss Merlin (Oberkochen) using the Everhart Thornley HESE2-detector and the inlens SE-detector in a 25:75 ratio at an acceleration voltage of 5 kV. Images were recorded with Zeiss SEMSmart V 5.05 and contrast and brightness were adjusted with Adobe Photoshop CS5. Electron microscope pictures confirmed the presence of a single polar flagellum (Fig. 1).

Catalase activity was assessed by observing gas formation after exposing cells to 3 % (v/v) H₂O₂ [32] and cytochrome-*c* oxidase activity was determined by Bactident Oxidase (Merck), following the instructions of the manufacturer. Catalase activity was a feature distinguishing strain R5913^T from R5959^T since the first tested positive and the latter negative. Both strains were positive for cytochrome-*c* oxidase which is in agreement with the majority of their closest genera in the *Rhodospirillaceae* family (Table 1).

The growth ranges and optima for temperature, pH, and salinity were determined aerobically in liquid R2A medium. Growth was tested across the temperature range of 10-45 °C and pH optima were determined between pH values of 1.0 to 11.0 as described previously [19, 24, 25, 33]. Depending on the pH, MES, HEPES, HEPPS, or CHES (Sigma-Aldrich or AppliChem; 10mM) were used as buffers. Salt tolerance was determined in liquid R2A medium supplemented with 0, 0.25, 0.5, 1, 3, 5, 7.5, and 10 % NaCl (w/v). The experiments were conducted in three parallels for each pH, temperature, and salinity conditions and the growth was monitored by measuring the OD₆₆₀. Optimal growth was defined as ≥ 75 % of highest growth rate observed. Strain R5913^T grew between 10-36°C (optimum at 27-33°C), while strain R5959^T preferred slightly higher temperature in the range of 24-40°C (optimum at 33-36°C). The results are in accordance with the reported temperature ranges of some closest relatives that grew optimally at approximately 30°C (Table 1), such as *Oceanibaculum pacificum* MCCC 1A02656^T [16] and *Dongia mobilis* CGMCC 1.7660^T [34]. Strain R5913^T grew between pH 6.0 to 8.4 (optimum at pH 6.5-7.3) while strain R5959^T grew in the range of pH 5.1-8.2 (optimum at pH 5.6-7.4). The majority of the closest genera in the family *Rhodospirillaceae* have a higher alkalinity tolerance (Table 1) especially *Dongia rigui* 04SU4-P^T [35] and *Oceanibaculum indicum* P24^T [36] which can tolerate pH values of up to 11. Both strains grew optimally without addition of NaCl. Strain R5959^T could tolerate a concentration of NaCl up to 0.5 % while strain R5913^T grew slowly in 1 % NaCl but no growth was observed in 3 % NaCl. This trait is also shared within some of their closest genera that were isolated mostly from soil and freshwater lakes, such as *Reyranella* [37-41], *Dongia* [7, 34, 35], *Aliudongia* [5], *Stella* [31], *Lacibacterium* [9] and *Elstera* [11, 42]. Nevertheless, some genera like *Oceanibaculum* and *Nisaea*, which were isolated from marine environments, tolerate up to 6 and 9 % (w/v) NaCl, respectively [16, 36, 43, 44]. The doubling times of strains R5913^T and R5959^T under optimal conditions were 25.1 h and 31.7 h, respectively. The doubling times of most of the closest relatives have not been reported, except for *Elstera litoralis* Dia-1^T which grew slower (40 h) in VM-ethanol medium when compared to R5913^T and R5959^T [11].

The growth of strains R5913^T and R5959^T in microaerophilic and anaerobic conditions was assessed in a candle jar [45] or using Anaerocult® P (Merck), respectively. After 2 weeks of incubation, both strains grew under microaerophilic but not in anaerobic conditions. The results are in accordance with what has been observed for the genus *Stella* [31] and some species of *Reyranella* [37, 38] and *Elstera* [11] (Table 1). Most members of *Rhodospirillaceae* grow aerobically, with exception of phototrophic bacteria such as *Rhodocista pekingensis* 3-p^T [46], *Phaeovibrio sulfidiphilus* JA480^T [47] and species of *Phaeospirillum* [48-51] which display growth under anaerobic conditions.

Exoenzyme activities, along with nitrate reduction, indole production, fermentation of glucose, arginine dihydrolase, urease, and β -galactosidase (PNPG) activities, and the hydrolysis of aesculin and gelatin were tested using commercial API 20NE and API ZYM galleries (BioMérieux, Marcy

l'Etoile; France), according to the instructions of the manufacturer. The assimilation of different carbon substrates in API 20 NE was not conducted since the medium supplied was not suitable for the growth of R5913^T and R5959^T. Both strains were positive for esterase, leucine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase, and weakly positive for alkaline phosphatase. The results are similar to those for *Tagaea marina* TT1^T, *Aliidongia dinghuensis* 7M-Z19^T, *Lacibacterium aquatile* LTC-2^T, *Elstera litoralis* Dia-1^T, and *Elstera cyanobacterium* TH019^T (Supplementary Table 2). In addition, the majority of the closest relatives were positive for alkaline phosphatase but this was only weakly detected in both strains. Strain R5959^T also tested positive for esterase lipase and valine arylamidase but these were only weakly detected in strain R5913^T. Cystine arylamidase activity was weakly detected only in R5959^T. No activities were detected for lipase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase, and α -fucosidase for both strains, similar to *Aliidongia dinghuensis* 7M-Z19^T (Supplementary Table 2). In addition, strains R5913^T and R5959^T were negative for nitrate reduction and glucose fermentation. The capability of nitrate reduction varies across the members of *Rhodospirillaceae* and only strains of the genera *Lacibacterium* and *Elstera* are able to ferment glucose (Table 1). Indole production, arginine dihydrolase, urease and β -galactosidase activities, as well as hydrolysis of esculin and gelatin were negative for both strains. The closest relatives of strains R5913^T and R5959^T were not able to produce indole (Supplementary Table 2) while arginine dihydrolase activities were mostly negative and only present in some species of *Reyranella* and *Oceanibaculum*.

The ability of strains R5913^T and R5959^T to utilize different carbon substrates was tested in triplicate using liquid R2A. The 108 carbon sources tested included sugars, various organic acids, keto acids, alcohols, amino acids, casamino acid, casein hydrolysate, laminarin, peptone, yeast extract and Tween 80. The final concentrations were as described previously [19, 24]. A positive result was defined when the mean OD₆₆₀ of the three parallels was equal or surpassed the mean of the control (culture without substrate) by 1.5 times. Weak positive growth was defined as reaching values between 1.2-1.5 times the controls. Strain R5913^T was able to metabolize yeast extract and grew weakly with casein hydrolysate, pepton, β -hydroxybutyrate, trimethoxybenzoate, acetate, ethylene glycol, butyrate, glycerol, α -hydroxybutyrate, sodium pyruvate, and isovaleric acid. Similarly, strain R5959^T was only able to utilize yeast extract and grew weakly on casein hydrolysate. A narrow range of growth substrates was also observed for *Tagaea marina* TT1^T [10] and *Thalassobaculum litoreum* DSM 18839^T [52]. The first tests negative for all the substrates in Biolog GN2 and the latter is only able to utilize D-ribose, L-arabinose, sucrose, and yeast extract. Nevertheless, most of the closest relatives of R5913^T and R5959^T, such as members of *Elstera*, *Lacibacterium*, *Inquilius*, *Reyranella* (except for *Reyranella terrae* 11G32^T [40]), and *Stella* can utilize a wider range of substrates (Supplementary Table 3). Additionally, the abilities of both strains to utilize complex substrates such as starch,

cellulose, carboxymethyl cellulose, chitin, lignin, polygalacturonic acid, pectin, xylan and 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonate) (ABTS) were tested in solidified R2A medium. Specific staining solutions were employed, as described previously [29], to detect degradation of the polymeric substrates after 1 month incubation. Both strains tested negative for the utilization of all substrates while some of the species of *Reyranella* are able to utilize cellulose (Supplementary Table 3).

Strains R5913^T and R5959^T were harvested during late logarithmic phase for detection of fatty acids, polar lipids, and respiratory quinones. Fatty acids were extracted, saponified, and methylated using standard protocols and identified by TSBA40 library (MIDI Microbial Identification System.; version 6.1; [53]). Polar lipids were analysed by two-dimensional thin layer chromatography [54, 55]. Isoprenoid quinones were extracted from dried biomass using chloroform/methanol (2:1, v/v) [56] and analysed further with HPLC [57]. The major fatty acids of strains R5913^T and R5959^T were C_{19:0} cyclo ω 8c (43.0 and 53.1 %, respectively) and C_{16:0} (15.0 and 25.1 %, respectively) (Supplementary Table 1). In contrast, C_{18:1} ω 7c (27.2 %) was found to be one of the major fatty acids of strain R5913^T but was much less abundant in strain R5959^T (1.6 %). These three fatty acids are commonly found as the major fatty acids of other members of the *Rhodospirillaceae* family such as *Reyranella*, *Oceanibaculum*, *Thalassobaculum* and *Dongia* (Table 1) but C_{19:0} cyclo ω 8c and C_{18:1} ω 7c are absent in several genera, such as *Constrictibacter* and *Magnetospirillum* [58, 59]. Strains R5913^T and R5959^T also contained C_{18:0} (1.9 and 5.2 %, respectively) and C_{16:0} 2-OH (1.7 and 5.1 %, respectively). Fatty acids C_{18:1} 2-OH (5.1 %) and C_{14:0} 2-OH (4.6 %) were found in R5913^T while traces were found in R5959^T (0.5 and 0.7 %, respectively). C_{18:0} 3-OH (5.2 %) was only detected in R5959^T (Table 1). The major polar lipids of strain R5913^T were phosphatidylethanolamine, an unknown aminoglycolipid and two unidentified aminoglycophospholipids (Supplementary Figure 1). Strain R5959^T contained phosphatidylethanolamine, an unidentified aminolipid, an unknown phosphoglycolipid, an unknown aminoglycophospholipids, and three unidentified aminoglycolipids (Supplementary Figure 2). Phosphatidylethanolamine and phosphatidylglycerol are commonly reported for members of the *Rhodospirillaceae* family (Table 1), but the latter was not identified in strains R5913^T and R5959^T. Ubiquinone-10 was the sole respiratory quinone found in both strains, and it is also the major quinone of their phylogenetically closest genera (Table 1).

The almost full-length 16S rRNA gene sequences of strains R5913^T and R5959^T (both 1441 bp) were amplified and sequenced with the method described previously [30]. The multiple sequence alignment was done using the SINA alignment tool in the ARB-SILVA website [60] and the alignment result was visually inspected to re-evaluate and improve some uncertain alignments. The phylogenetic analysis was performed using MEGA version 7.0 [61] and involved the construction of Neighbour-joining (NJ; with Kimura's two parameter evolutionary model) and maximum likelihood (ML; T92+I+G evolutionary model) trees. Tree topology was evaluated by bootstrap analysis with 1000

replications. The phylogenetic trees confirmed the affiliation of the strain R5913^T and R5959^T within the family *Rhodospirillaceae* (Fig. 2 and Supplementary Figure 3). Both strains formed a well-defined monophyletic group supported by a high bootstrap value. In both ML (Fig. 2) and NJ (Supplementary Fig. 3) trees, *Reyranella* was the phylogenetically closest genus of the new isolates with a high statistical support.

For genome sequencing, DNA was isolated using the Qiagen Genomic-tip 100/G (Qiagen, Hilden Germany) according to the instructions of the manufacturer. A SMRTbellTM template library was prepared according to the instructions from PacificBiosciences, Menlo Park, CA, USA, following the Procedure & Checklist – Greater Than 10 kb Template Preparation. Genomic DNA (8µg) was sheared briefly for preparation of 15kb libraries using g-tubesTM from Covaris, Woburn, MA, USA according to the instructions of the manufacturer. DNA was end-repaired and ligated overnight to hairpin adapters applying components from the DNA/Polymerase Binding Kit P6 from Pacific Biosciences, Menlo Park, CA, USA. Reactions were carried out according to the instructions of the manufacturer. BluePippinTM Size-Selection of fragments greater than 4 kb was performed according to the instructions of the manufacturer (Sage Science, Beverly, MA, USA). Conditions for annealing of sequencing primers and binding of polymerase to purified SMRTbellTM template were assessed with the Calculator in RS Remote, PacificBiosciences, Menlo Park, CA, USA. 1 SMRT cell was sequenced on the PacBio *RSII* (PacificBiosciences, Menlo Park, CA, USA) taking one 240-minutes movie for each strain. Libraries for sequencing on Illumina platform were prepared applying Nextera XT DNA Library Preparation Kit (Illumina, San Diego, USA) with modifications according to Baym et al. [62]. Samples were sequenced on NextSeqTM 500. Genome assembly was performed applying the RS_HGAP_Assembly.3 protocol included in SMRT Portal version 2.3.0. Error-correction was performed by a mapping of the Illumina short reads onto finished genomes using the Burrows-Wheeler Aligner bwa 0.6.2 in paired-end (sampe) mode using default setting [63] with subsequent variant and consensus calling using VarScan 2.3.6 [64]. Automated genome annotation was carried out using DFAST [65]. The genome sequences of strains R5913^T and R5959^T have been deposited at GenBank/EMBL/DDBJ under the accessions CP042906 and CP042582, respectively. Genomes of both strains R5913^T and R5959^T are organized in one circular chromosome with a size and G+C content of 5,894,118 bp and 66.0 %, and 5,865,246 bp and 67.4 %, respectively. The total number of predicted genes of strains R5913^T and R5959^T were 5459 and 5336, comprising 5361 and 5247 protein-coding sequences, and 56 and 53 tRNAs, respectively. Both strains have 6 rRNA genes organized in two operons.

The average nucleotide identity (ANI) value between the two strains, calculated with OrthoANIu algorithm using EzGenome web service (www.ezbiocloud.net/tools/ani) [66], was 83.8 %. The ANI values calculated between the genomes of both strains and the

genomes of their closest genera were lower. Strains R5913^T and R5959^T shared ANI values of 72.0 % and 72.2 % with *Dongia mobilis* CGMCC 1.7660^T and values of 70.8 % and 71.0 % with *Oceanibaculum pacificum* MCCC 1A02656^T, respectively. A phylogenomic tree based on nucleotide sequences was generated with the purposes of obtaining more accurate phylogenetic inference of both strains R5913^T and R5959^T. The UBCG v. 3.0 pipeline (up-to-date bacterial core gene set) [67] was used to construct a maximum likelihood tree based on a multiple alignment of a set of 92 universal and single copy gene sequences with the tool FastTree v2,10,1 (Fig. 3). Confirming the results of the 16S rRNA-based analysis, the phylogenomic tree showed that both strains form a monophyletic group, with *Dongia mobilis* CGMCC 1.7660^T being the closest neighbor. The branching pattern was supported by high bootstrap values and gene support indices. In addition, we observed that the genomes of *Thalassobaculum salexigens* DSM 19539^T and *Thalassobaculum litoreum* DSM 18839^T were very similar and therefore performed digital DNA-DNA hybridization (DDH), using the genome-to-genome distance calculator (GGDC) (<http://ggdc.dsmz.de>) [68], yielding a DDH value of 90.5%. This value is above the threshold for the differentiation of bacterial species (cut-off 70%) [69], clearly indicating that both *Thalassobaculum* strains are members of the same species. Therefore, *Thalassobaculum salexigens* DSM 19539^T likely has to be considered a later heterotrophic synonym of *Thalassobaculum litoreum* DSM 18839^T, but further studies are needed to confirm this finding. On the basis of phenotypic, genomic, and phylogenetic investigations, we confirmed the affiliation of both strains within the family *Rhodospirillaceae*. Both strains could be differentiated by cell morphology, enzymatic activities, optimal growth conditions (temperature and pH), substrate preferences, polar lipids and fatty acids compositions, and thus should be assigned to different species, as also supported by low ANI value (83.8 %) which is below the threshold for the delineation of bacterial species (95.0 - 96.0 %) [70].

Description of *Hypericibacter* gen. nov.

Hypericibacter (Hy.pe.ri.ci.bac'ter. L. neut. n. *Hypericum* a botanical genus; N. L. masc. n. *bacter* a rod; N.L. masc. n. *Hypericibacter* a rod-shaped bacterium isolated from *Hypericum*).

Gram negative, motile, non-spore-forming, non-capsulated, straight to slightly curved, short, rod-shaped bacteria that divide by binary fission. Oxidase positive, grow under aerobic and microaerophilic conditions, mesophilic chemo-organotroph, which are unable to reduce nitrate and ferment glucose. The predominant respiratory quinone is Q-10. The major fatty acids are C_{19:0} cyclo ω8c and C_{16:0}. The type strain is *Hypericibacter terrae*.

Description of *Hypericibacter terrae* sp. nov.

Hypericibacter terrae (ter'rae. L. gen. n. *terrae* of the soil, referred to the isolation source of the type strain.)

Exhibits the following characteristics in addition to those given in the genus description. Cells are 0.7-1.5 μm long and 0.5-0.8 μm wide and appear as single cells. Colonies in solidified R2A are small, round, smooth, convex, and shiny white with a diameter of 1 mm. Growth occurs between 20 and 36°C (optimum 27-33°C), between pH 6.0 and 8.4 (6.5-7.5), and at up to 1 % (w/v) NaCl (grows optimally without NaCl). Doubling time under optimal conditions is 25.1 h. Assimilates yeast extract but grows weakly on casein hydrolysate, pepton, β -hydroxybutyrate, trimethoxybenzoate, acetate, ethylene glycol, butyrate, glycerol, α -hydroxybutyrate, sodium pyruvate, and isovaleric acid. No growth is observed on glucose, lactose, fructose, cellobiose, galactose, mannose, melezitose, raffinose, fucose, sorbose, lyxose, maltose, rhamnose, sucrose, trehalose, xylose, adonitol, arabitol, mannitol, *myo*-inositol, sorbitol, xylitol, lysin, hydroxyproline, casamino acid, glycolate, malonate, propionate, oxaloacetate, lactate, butanol, ethanol, methanol, propanol, *N*-acetylglucosamine, caproate, caprylate, dulcitol, erythrose, erythrulose, isocitrate, laevulinate, arabinose, glucosamine, gluconate, glucuronate, lyxitol, 2-oxoglutarate, sodium pyruvate, acetoin, ascorbate, glyoxylate, 2-oxovalerate, 2-oxogluconate, *N*-acetylgalactosamine, maleic acid, 1,2-butandiol, 2,3-butandiol, 1,2-propandiol, alanine, arginine, asparagine, cysteine, glutamine, isoleucine, ornithine, proline, benzoate, tryptophan, formate, γ -hydroxybutyrate, isobutyrate, tyrosine, serine, phenylalanine, glycine, leucine, histidine, valine, methionine, threonine, succinate, nicotinic acid, Tween 80, adipate, shikimate, aspartate, glutamate, laminarin, malate, citrate, tartrate, heptanoic acid, and fumarate. Positive for esterase, leucine arylamidase, acid phosphatase, and Naphthol-AS-BI-phosphohydrolase activities. Alkaline phosphatase, esterase lipase, and valine arylamidase activities are weak. No activities of lipase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase, and α -fucosidase. Negative for nitrate reduction, indole production, and glucose fermentation. Activities of urease, arginine dihydrolase, and β -galactosidase (PNPG) are not detected. Aesculin and gelatin are not hydrolyzed.

The type strain is R5913^T (=DSM 109816^T = CECT 9472^T), isolated from the rhizosphere of *Hypericum perforatum* (accession number: HyPR-01) retrieved from a greenhouse of the Leibniz Institute of Plant Biochemistry (IPB), Halle, Germany (51° 29' 42.23" N, 11° 56' 36.56" E). The DNA G+C content of the type strain is 66.0 %. The 16S rRNA gene and whole-genome sequences have been deposited in GenBank/EMBL/DDBJ under accession numbers MG271952 and CP042906, respectively.

Description of *Hypericibacter adhaerens* sp. nov.

Hypericibacter adhaerens (ad.hae'rens. L. part. adj. *adhaerens* sticky, referring to the attachment of the colonies to solid growth media).

Exhibits the following characteristics in addition to those given in the genus description. Cells are 0.5-2.5 μm long and 0.3-1.0 μm wide. Colonies in solidified R2A are small, round, smooth, convex, and shiny white with a diameter of 1 mm. Growth occurs between 24 and 40°C (optimum 33 to 36°C), between pH 5.0 and 8.2 (optimum 5.6 to 7.4), and up to 0.5 % (w/v) NaCl (grows optimally without NaCl). Doubling time under optimal condition is 31.7 h. Grows on yeast extract and weak growth is observed on casein hydrolysate. No growth detected with glucose, lactose, fructose, cellobiose, galactose, mannose, melezitose, raffinose, fucose, sorbose, lyxose, maltose, rhamnose, sucrose, trehalose, xylose, adonitol, arabitol, mannitol, *myo*-inositol, sorbitol, xylitol, lysin, hydroxyproline, casamino acid, glycolate, malonate, propionate, oxaloacetate, lactate, butanol, ethanol, glycerol, methanol, propanol, pepton, *N*-acetylglucosamine, caproate, caprylate, dulcitol, ethylenglycol, erythrose, erythrulose, α -hydroxybutyrate, isocitrate, laevulinate, arabinose, glucosamine, gluconate, glucuronate, lyxitol, 2-oxoglutarate, sodium pyruvate, acetoin, ascorbate, glyoxylate, 2-oxoalate, 2-oxogluconate, *N*-acetylgalactosamine, maleic acid, 1,2-butandiol, 2,3-butandiol, 1,2-propandiol, alanine, arginine, asparagine, cysteine, glutamine, isoleucine, ornithine, proline, benzoate, tryptophane, acetate, butyrate, formate, β -hydroxybutyrate, γ -hydroxybutyrate, isobutyrate, tyrosine, serine, phenylalanine, glycine, leucine, histidine, valine, methionine, threonine, succinate, nicotinic acid, Tween 80, adipate, shikimate, aspartate, glutamate, laminarine, malate, citrate, tartrate, isovaleric acid, heptanoic acid, fumarate, and trimethoxybenzoate. Tests positive for enzymatic activities of esterase, esterase lipase, leucine arylamidase, valine arylamidase, acid phosphatase, and Naphthol-AS-BI-phosphohydrolase, while alkaline phosphatase and cystine arylamidase activities are detected only weakly. No activities of lipase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase, and α -fucosidase are detected. Nitrate reduction, indole production, and glucose fermentation are negative. Aesculin and gelatin are not hydrolysed. Urease, arginine dihydrolase, and β -galactosidase (PNPG) activities are not detected.

The type strain is R5959^T (=DSM 109817^T = CECT 9620^T), isolated from the rhizosphere of *Hypericum perforatum* (accession number: HyPR-01) retrieved from a greenhouse of the Leibniz Institute of Plant Biochemistry (IPB), Halle, Germany (51° 29' 42.23" N, 11° 56' 36.56" E). The DNA G+C content of the type strain is 67.4 %. The 16S rRNA gene and whole-genome sequences have been deposited in GenBank/EMBL/DDBJ under accession numbers MH450230 and CP042582, respectively.

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Table 1. Phenotypic differences of strains R5913^T and R5959^T with other related genera in the family *Rhodospirillaceae*.

Strains: 1, R5913^T; 2, R5959^T; 3, *Reyranella* [37-41]; 4, *Tagea* [10]; 5, *Oceanibaculum* [16, 36, 43]; 6, *Nisaea* [44]; 7, *Thalassobaculum* [52, 71, 72]; 8, *Dongia* [7, 34, 35]; 9, *Inquilinus* [73, 74]; 10, *Aliidongia* [5]; 11, *Stella* [31]; 12, *Lacibacterium* [9]; 13, *Elstera* [11, 42]. +, Positive; -, negative; V, variable response; NA, no data available.

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12	13
Colony pigmentation	White	White	Grey-white, milk colored	Greyish	Grey colored	Cream	Cream yellow, yellowish-brown	White	Creamy white	White	Milky white, greyish white	Creamy white	Milky white to creamed colored, light brown
Cell shape	Short to slightly curved rods	Slightly curved short rods	Rods	Rods	Rods	Pleomorphic rods	Slightly curved to straight rods	Slightly curved to straight rods	Rods	Rods	Star-shaped	Slightly curved rods	Slightly curved rods
Flagellar motility	+	+	-	+	+	+	V	+	-	+	-	+	V
Capsules	-	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA
Catalase	+	-	V	+	+	+	+	V	+	+	+	+	+
Oxidase	+	+	+	+	V	+	+	V	+	+	+	+	+
Nitrate reduction	-	-	V	+	V	+	V	V	-	+	NA	-	V
Salt is required for growth	-	-	-	+	-	-	V	-	V	-	-	-	-
Temperature range (°C) (optimum)	20-36 (27-33)	24-40 (33-36)	15-37 (20-35)	20-42 (30-35)	10-45 (25-37)	15-44 (30)	10-40 (30-35)	15-40 (25-37)	15-42	10-37 (28)	(28-30)	10-37 (20-30)	10-37 (20-28)
pH range (optimum)	6.0-8.4 (6.5-7.5)	5.0-8.2 (5.6-7.4)	4.0-10.0 (5.0-9.0)	6.0-9.0 (7.0-8.0)	6.0-11.0 (7.0-9.0)	5.0-9.0 (6.0)	5.0-10.0 (6.5-8.0)	5.0-11.0 (7.0-7.5)	NA	4.5-7.5 (6.0-6.5)	(near neutral/ slightly alkaline)	6.0-9.0 (7.0-8.0)	5.0-9.0 (6.5-7.0)
Glucose fermentation / acidification	-	-	-	-	-	NA	-	-	-	-	NA	+	+
Relation to O ₂ [†]	AE, MAE	AE, MAE	AE, MAE	AE	AE	FA	FA, AE	AE	AE	AE	AE, MAE	FA	AE, MAE
DNA G+C content (% or mol%)	66.02	67.35	59.8-66.5	56.4	64.8-67.7	60.1-60.2	65-69	54.7-71.5	69-9-70.3	65.8	69.3-73.5	58.5	61.2-62.4
Major quinone (s)	Q-10	Q-10	Q-10, Q-9	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	NA	Q-10	Q-10
Major polar lipids:													
Phosphatidylethanolamine	+	+	+	NA	+	-	-	+	NA	+	NA	+	+
Hydroxyphosphatidylethanolamine	-	-	-	NA	-	-	-	+	NA	-	NA	-	-
Phosphatidylmonomethylethanolamine	-	-	+	NA	+	-	-	-	NA	-	NA	+	+

Phosphatidyl dimethylethanolamine	-	-	-	NA	-	-	-	-	NA	+	NA	+	-
Phosphatidylglycerol	-	-	+	NA	+	+	+	+	NA	+	NA	+	+
Diphosphatidylglycerol	-	-	+	NA	+	-	-	-	NA	+	NA	-	-
Phosphatidylcholine	-	-	-	NA	-	-	-	-	NA	-	NA	-	-
Major fatty acids													
C19:0 cyclo ω 8c	+	+	+	+	+	-	+	+	+	+	NA	-	-
C18:1 ω 7c	+	-	+	+	+	+	+	+	+	+	NA	+	+
C16:0	+	+	+	-	+	+	+	+	-	-	NA	+	+
Isolation source	Rhizosphere soil	Rhizosphere soil	Forest and agricultural soil, bamboo litter, river, lake	Coastal seawater	Surface and deep seawater, hydrothermal vent	Surface seawater	Coastal and deep seawater	Freshwater, activated sludge of a sequencing batch reactor, soil	Respiratory secretions of a CF patient, soil	Forest soil	Soils; freshwater sediments, sewage, sludge, horse manure	Freshwater lake	Stones biofilms, lake

† AE, aerobic; AN, anaerobic; FA, facultative anaerobic; MAE, microaerophilic.

Figure Legends

Fig. 1. Electron micrographs of strains R5913^T (a) and R5959^T (b). Bars, 1 μm .

Fig. 2. Maximum-likelihood (ML) phylogenetic tree based on the almost full-length 16 ribosomal RNA gene sequences illustrating the position of strains R5913^T and R5959^T within the closest genera in the *Rhodospirillaceae* family. The best evolutionary model for the nucleotide substitution, calculated by Mega 7.0, and applied for the tree was T92+G+I. Bar, 0.1 fixed nucleotide substitutions per site. *Burkholderia cepacia* ATCC 25416^T was used as outgroup. Bootstrap values above 50% (of 1000 replicates) are indicated at the tree branching points.

Fig. 3. Phylogenomic tree of strains R5913^T and R5959^T and their closest relatives. Unrooted maximum likelihood phylogenetic tree based on a multiple alignment of a set of 92 gene (nucleotidic) sequences from using the UBCG v. 3.0 pipeline. Bootstrap analysis was carried out using 100 replications. Gene support indices (max. value; 92 genes) and percentage bootstrap values (max. value; 100%) are given at branching points. Bar, 0.10 substitution per position.