Herbaspirillum canariense sp. nov., *Herbaspirillum aurantiacum* sp. nov. and *Herbaspirillum soli* sp. nov., isolated from volcanic mountain soil, and emended description of the genus *Herbaspirillum*

Lorena Carro,¹ Raúl Rivas,¹ Milagros León-Barrios,²
María González-Tirante,³ Encarna Velázquez¹ and Angel Valverde³

¹Departamento de Microbiología y Genética, Universidad de Salamanca, Salamanca, Spain

²Departamento de Microbiología y Biología Celular, Universidad de la Laguna, Tenerife, Spain

³Instituto de Recursos Naturales y Agrobiología, CSIC, Salamanca, Spain

Correspondence
Encarna Velázquez
evp@usal.es

Three Gram-negative, motile and slightly curved rod-shaped bacteria, strains SUEMI03^T, SUEMI08^T and SUEMI10^T, were isolated from an old volcanic mountain soil on Tenerife (Canary Islands). The three strains were related phylogenetically to *Herbaspirillum seropedicae*. 16S rRNA gene sequence similarity was 99.2–99.6 % among strains SUEMI03^T, SUEMI08^T and SUEMI10^T, which presented 97.5, 97.8 and 97.7 % identity, respectively, with respect to *H. seropedicae* DSM 6445^T. The three strains grew optimally in TSB at 28 °C and contained summed features 3 (C_{16:1}ω6c and/or C_{16:1}ω7c) and 8 (C_{18:1}ω6c and/or C_{18:1}ω7c) and C_{16:0} as major cellular fatty acids. The DNA G + C contents of strains SUEMI03^T, SUEMI08^T and SUEMI10^T were 61.6, 60.4 and 61.9 mol%, respectively. Strains SUEMI03^T, SUEMI08^T and SUEMI10^T presented less than 60 % interstrain DNA relatedness and less than 30 % relatedness with respect to *H. seropedicae* DSM 6445^T. In spite of their common geographical origin, the three strains isolated in this study presented several phenotypic differences, presenting phenotypic profiles highly divergent from that of *H. seropedicae*. Therefore, we propose that the strains isolated in this study represent three novel species of the genus *Herbaspirillum*, named *Herbaspirillum canariense* sp. nov. (type strain SUEMI03^T = LMG 26151^T = CECT 7838^T), *Herbaspirillum aurantiacum* sp. nov. (type strain SUEMI08^T = LMG 26150^T = CECT 7839^T) and *Herbaspirillum soli* sp. nov. (type strain SUEMI10^T = LMG 26149^T = CECT 7840^T).

The genus *Herbaspirillum* belongs to the family *Oxalobacteraceae* within the class *Betaproteobacteria*, with *Herbaspirillum seropedicae* as the type species (Baldani *et al.*, 1986). Since the description of *H. seropedicae*, ten additional species have been described, one of which, *Herbaspirillum putei*, was recently reclassified as a subspecies of *Herbaspirillum huttiense* (Dobritsa *et al.*, 2010). The members of the genus *Herbaspirillum* have been found in waters (Ding & Yokota, 2004) and in association with plant tissues (Baldani *et al.*, 1986; Kirchhof *et al.*, 2001; Valverde *et al.*, 2003; Rothballer *et al.*, 2006), the plant rhizosphere (Jung *et al.*, 2007) and soil (Im *et al.*, 2004).

During screening to search for bacteria with plant growth promotion (PGPR) potential, we isolated three

siderophore-producing strains from an old volcanic mountain soil in the north-east of Tenerife (Canary Islands). The vegetation at this site is dominated by herbaceous nitrophilous communities with species such as *Galactites tomentosa*, *Calendula arvensis*, *Oxalis pes-caprae*, *Sinapis arvensis* and *Brachypodium distachyon*. Patches of diverse shrubs and plantations of *Eucalyptus globulus* are also common. The bacterial isolates were classified in the genus *Herbaspirillum* after 16S rRNA gene sequence analysis. The phenotypic and genetic characteristics of these strains showed that SUEMI03^T, SUEMI08^T and SUEMI10^T belong to three novel species of the genus *Herbaspirillum*.

The strains were isolated on TSA medium (Difco) containing 1 % soil extract prepared by suspending 10 g of the same soil from which the strains were isolated in 90 ml sterile water that was maintained under shaking conditions for 2 h. Then, 10 ml of this suspension was added to 90 ml water to which the corresponding amount of TSA powder was added. The medium was sterilized by

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains SUEMI03^T, SUEMI08^T and SUEMI10^T are HQ830496, HQ830497 and HQ830498, respectively.

Three supplementary figures and two supplementary tables are available with the online version of this paper.

autoclaving at 121 °C for 20 min and dispensed into Petri dishes. Decimal dilutions of the soil extract were obtained and 1 ml from each dilution was spread on these plates. The strains isolated were transferred to Petri dishes containing M9-CAS agar (Alexander & Zuberer, 1991) and three colonies surrounded by an orange halo indicative of siderophore production were transferred to TSA plates for identification.

The strains were grown in nutrient broth (Difco) for 48 h at 22 °C to check for motility by phase-contrast microscopy using the hanging drop method. Gram staining was carried out by the procedure described by Doetsch (1981) after 24 h of incubation at 28 °C. The flagellation type was determined by electron microscopy after 48 h of incubation of strains in TSB at 22 °C as described previously (Rivas *et al.*, 2007). The three strains were Gram-negative short rods, motile by a polar flagellum (Fig. S1, available in IJSEM Online).

The 16S rRNA genes of strains SUEMI03^T, SUEMI08^T and SUEMI10^T were analysed as described by Rivas *et al.* (2007). The sequences obtained were compared with those from GenBank using the BLASTN (Altschul *et al.*, 1990) and EzTaxon (Chun *et al.*, 2007) programs. Sequences were aligned using the CLUSTAL_X software (Thompson *et al.*, 1997). Distances were calculated according to Kimura's two-parameter model (Kimura, 1980). The phylogenetic tree was inferred using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Felsenstein, 1983) and maximum-likelihood (Rogers & Swofford, 1998) methods. Bootstrap analysis was based on 1000 resamplings. MEGA4 (Tamura *et al.*, 2007) was used for neighbour-joining and

maximum-parsimony analyses and ARB (Ludwig *et al.*, 2004) was used for maximum-likelihood analysis. The resulting neighbour-joining tree is shown in Fig. 1 and those obtained using maximum-parsimony and maximum-likelihood in Figs S2 and S3, respectively.

The results of the phylogenetic analyses showed that the strains isolated in this study belong to the genus *Herbaspirillum*, forming a cluster clearly divergent from the remaining species of this genus (Figs 1, S2 and S3). All phylogenetic analysis showed that the genus *Herbaspirillum* does not constitute a compact group, since the species *Herbaspirillum hiltneri*, *H. lusitanum*, *H. rhizosphaerae* and *H. autotrophicum* formed a separated cluster. However, the three strains isolated in this study clearly belong to the genus *Herbaspirillum*, because all analysis showed that the closest related species in all cases was *H. seropedicae*, the type species of the genus (Figs 1, S2 and S3). The type strain of this species, DSM 6445^T, presented 97.5, 97.8 and 97.7 % 16S rRNA gene sequence similarity, respectively, with respect to strains SUEMI03^T, SUEMI08^T and SUEMI10^T according to the EzTaxon program. The 16S rRNA gene sequence similarity between the three isolates from this study was 99.2 % between SUEMI03^T and SUEMI08^T, 99.6 % between SUEMI03^T and SUEMI10^T and 99.5 % between SUEMI08^T and SUEMI10^T. Similarities higher than those found between SUEMI10^T and the two other strains have been reported between other *Herbaspirillum* species: for example, 99.9 % identity between the type strains of *H. hiltneri* and *H. lusitanum* (Rothballer *et al.*, 2006) or 99.8 % identity between those of *H. aquaticum* and *H. huttiense* (Dobritsa *et al.*, 2010).

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences of *Herbaspirillum canariense* sp. nov. SUEMI03^T, *Herbaspirillum aurantiacum* sp. nov. SUEMI08^T and *Herbaspirillum soli* sp. nov. SUEMI10^T and currently accepted species and subspecies within the genus *Herbaspirillum*. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets. Bar, 1 substitution per 100 nucleotide positions.

DNA–DNA hybridization experiments were carried out by using the method of Ezaki *et al.* (1989) following the recommendations of Willems *et al.* (2001). DNA samples from strains SUEMI03^T, SUEMI08^T and SUEMI10^T were hybridized with each other and with DNA from *H. seropedicae* DSM 6445^T. Strains SUEMI03^T, SUEMI08^T and SUEMI10^T showed 25, 12 and 11 % DNA relatedness, respectively, with respect to *H. seropedicae* DSM 6445^T. Strain SUEMI03^T showed 58 and 59 % relatedness with respect to SUEMI08^T and SUEMI10^T, respectively, and strains SUEMI08^T and SUEMI10^T showed 59 % interstrain relatedness. In the case of the species mentioned above, 25 % DNA–DNA relatedness was found between the type strains of *H. hiltneri* and *H. lusitanum* (Rothballer *et al.*, 2006) and 40–59 % between the type strains of *H. aquaticum* and *H. huttiense* (Dobritsa *et al.*, 2010). All these values are lower than the currently accepted threshold value of 70 % DNA–DNA relatedness for definition of bacterial species (Wayne *et al.*, 1987), and we therefore consider that the strains isolated in this study represent three novel species of the genus *Herbaspirillum*.

DNA for analysis of DNA base composition was prepared according to Chun & Goodfellow (1995) and the G+C content was determined using the thermal denaturation method (Mandel & Marmur, 1968). The DNA G+C contents of strains SUEMI03^T, SUEMI08^T and SUEMI10^T were 61.6, 60.4 and 61.9 mol%, respectively.

The presence of the *nifD* and *nifH* genes was analysed by using primers FdB261 and FdB260 (for *nifD*) and PolF and PolR (for *nifH*) under conditions described previously by Stoltzfus *et al.* (1997) and Poly *et al.* (2001), respectively. These genes were not amplified from any of the strains isolated in this study. Although these genes are carried by *H. seropedicae* (Baldani *et al.*, 1986), negative results have also been obtained for other *Herbaspirillum* species, such as *H. rhizosphaerae* (Jung *et al.*, 2007), *H. chlorophenolicum* (Im *et al.*, 2004), *H. autotrophicum* and *H. huttiense* (Ding & Yokota, 2004) and *H. hiltneri* (Rothballer *et al.*, 2006).

Cellular fatty acids were analysed by using the Microbial Identification System (MIDI; Microbial ID) Sherlock 6.1 and the library RTSBA6 according to the technical instructions provided with this system (Sasser, 1990). The strains were grown on TSA plates (Difco) for 48 h at 28 °C and the biomass was then recovered and lyophilized. *H. seropedicae* DSM 6445^T was included in this analysis together with the strains isolated in this study. The major fatty acids of the strains isolated in this study were C_{16:0} and those summed in features 3 (C_{16:1}ω6c and/or C_{16:1}ω7c) and 8 (C_{18:1}ω6c and/or C_{18:1}ω7c), as occurs in the remaining species of the genus *Herbaspirillum* (Table S1). The fatty acid profiles of the three strains isolated in this study were similar, although C_{10:0} and 11-methyl C_{18:1}ω7c, present in small amounts in SUEMI08^T and SUEMI10^T, were not detected in strain SUEMI03^T. The latter strain also contained larger amounts of C_{18:0} than the other two strains. The fatty acids iso-C_{16:0} and C_{17:0}

cyclo, present in small amounts in SUEMI08^T, were not detected in SUEMI03^T and SUEMI10^T. Finally, strain SUEMI10^T differed from the other two strains in the amounts of C_{18:0}, C_{10:0} 3-OH and summed feature 8. The three strains differed from *H. seropedicae* DSM 6445^T in the absence of C_{12:0} 2-OH and C_{12:0} 3-OH and in the amounts of C_{12:0}, C_{14:0}, C_{10:0} 3-OH and summed feature 3 (Table 1). Strain SUEMI03^T also presented slight differences in the amounts of C_{18:0}, strain SUEMI08^T also differed in the presence (in small amounts) of iso-C_{16:0} and 11-methyl C_{18:1}ω7c and, finally, strain SUEMI10^T also presented slight differences in the amounts of C_{18:0}, C_{16:0}, 11-methyl C_{18:1}ω7c and summed feature 8.

The strains were cultivated for 3 days on TSA at 28 °C to obtain the cell mass required for isoprenoid quinone analysis, which was carried out by the Identification Service and Dr Brian Tindall at the DSMZ (Braunschweig, Germany). The three strains contained Q-8 as the predominant ubiquinone, as has been reported for other species of the genus *Herbaspirillum* (Im *et al.*, 2004; Ding & Yokota, 2004). Strains SUEMI03^T and SUEMI08^T also contained Q-7 (4 %) and Q-9 (3 %), respectively, whereas only Q-8 was detected in strain SUEMI10^T.

Table 1. Cellular fatty acid composition of the strains isolated in this study and their closest relative, *H. seropedicae* DSM 6445^T

Strains: 1, *H. canariense* sp. nov. SUEMI03^T; 2, *H. aurantiacum* sp. nov. SUEMI08^T; 3, *H. soli* sp. nov. SUEMI10^T; 4, *H. seropedicae* DSM 6445^T. ND, Not detected.

Fatty acid	1	2	3	4
Saturated straight-chain				
C _{10:0}	ND	0.5	0.5	0.2
C _{12:0}	3.3	3.2	4.3	0.8
C _{14:0}	1.1	1.1	1.3	4.1
C _{16:0}	21.8	20.9	19.0	23.3
C _{17:0}	ND	ND	ND	0.2
C _{18:0}	2.3	1.2	0.5	1.2
Saturated iso-branched				
iso-C _{16:0}	ND	0.4	ND	ND
Hydroxy fatty acids				
C _{10:0} 3-OH	5.7	5.3	8.0	1.9
C _{12:0} 2-OH	ND	ND	ND	1.8
C _{12:0} 3-OH	ND	ND	ND	4.6
C _{14:0} 2-OH	2.4	2.4	2.7	1.4
Cyclopropane fatty acids				
C _{17:0} cyclo	ND	0.4	ND	0.4
Unsaturated				
11-Methyl C _{18:1} ω7c	ND	0.4	1.5	ND
Summed feature 3*	42.5	44.3	45.5	38.9
Summed feature 8*	20.9	19.3	16.8	21.2

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI System. Summed feature 3 contains C_{16:1}ω6c and/or C_{16:1}ω7c; summed feature 8 contains C_{18:1}ω6c and/or C_{18:1}ω7c.

The strains isolated in this study were grown on TSA plates (Difco) for 24 h for catalase and oxidase production analysis. Catalase production was assayed by using 0.3 % hydrogen peroxide with a single colony taken from a TSA plate. Oxidase activity was detected by using *N,N,N',N'*-tetramethyl-1,4-phenylenediamine dihydrochloride. The ability to grow at different temperatures (from 4 to 37 °C) was determined on TSA plates. The ability to grow at pH 4.5–8 was determined on SA1 medium (Trujillo *et al.*, 2005); PCA buffer (0.4 M Na₂HPO₄ and 0.2 M citric acid) was used to adjust the pH from 4.5 to 6.5 and 0.2 M TE buffer was used for pH 8. The ability to grow in the presence of NaCl was analysed in TSB supplemented with NaCl to obtain final concentrations of 0.5–2 %. Nitrate reduction was tested in nitrate broth adjusted to pH 7 (Claus & Berkeley, 1986) containing (l⁻¹) 5 g peptone, 3 g beef extract and 1 g potassium nitrate. Tween hydrolysis was analysed on Sierra basal medium (Sierra, 1957) containing 1 g Tween 20 or Tween 80 l⁻¹, which was autoclaved separately. Urease production was checked in urea broth (Fluka). Other physiological and biochemical tests were done using API 20NE, API ID32GN and API ZYM strips (bioMérieux) following the manufacturer's instructions. Although the isolates were inoculated on API 50CH strips using the API CHB medium recommended for *Herbaspirillum* species, they did not produce acid from any of the glucidic substrates included in API 50CH and, therefore, API ID32GN was used for phenotypic characterization of our isolates. This was a clear difference from *H. seropedicae* DSM 6445^T, which produced acid from many carbohydrates included in API 50CH. The results were read after 3 and 5 days of incubation at 28 °C. To test for natural antibiotic resistance, discs (Becton Dickinson) containing antibiotics at the following amounts were used: ampicillin (2 µg), erythromycin (2 µg), ciprofloxacin (5 µg), penicillin (10 IU), polymyxin B (300 IU), cloxacillin (1 µg), oxytetracycline (30 µg), gentamicin (10 µg), cefuroxime (30 µg) and neomycin (5 µg). The disc diffusion method was used on TSA medium (Becton Dickinson). The type strain of the most closely related species, *H. seropedicae* DSM 6445^T, was included in the phenotypic study as a reference. Phenotypic characteristics of the novel strains are reported in the species descriptions, and differences from *H. seropedicae* DSM 6445^T are recorded in Table 2. In Table S2, the phenotypic characteristics of all species of the genus *Herbaspirillum* are recorded. Although the phenotypic profiles of the strains isolated in this study seem to be similar overall in comparison to that of *H. seropedicae* DSM 6445^T, SUEMI03^T presented 11 differences with respect to SUEMI08^T and nine differences with respect to SUEMI10^T, and these two strains differed in the results of six tests. These differences were comparable to those found between *H. hiltneri* and *H. lusitanum*, with eight differences (Rothballer *et al.*, 2006), and between *H. aquaticum* and *H. huttiense*, with four differences (Dobritsa *et al.*, 2010).

Therefore, considering the current accepted criteria for species definition within the genus *Herbaspirillum*, the

phylogenetic, chemotaxonomic and phenotypic characteristics of strains SUEMI03^T, SUEMI08^T and SUEMI10^T showed that they represent three novel species, for which the names *Herbaspirillum canariense* sp. nov., *Herbaspirillum aurantiacum* sp. nov. and *Herbaspirillum soli* sp. nov., respectively, are proposed.

Description of *Herbaspirillum canariense* sp. nov.

Herbaspirillum canariense [ca.na.ri.en'se. N.L. neut. adj. *canariense* pertaining to the Canary Islands (Islas Canarias), where the type strain was isolated].

Gram-negative, aerobic, non-spore-forming, slightly curved rods, 1.9–1.6 µm long and 1.0–1.3 µm in diameter. Motile by a polar flagellum. Colonies on TSA are circular, convex, white-cream and opaque, usually 1–2 mm in diameter within 2 days at 28 °C. Catalase- and oxidase-positive. Growth occurs at 20 and 30 °C and occurs slowly at 15 and 34 °C, with optimum growth at 28 °C. No growth occurs at pH 4.5. Grows at pH 8, with optimal growth at pH 6–8. No growth occurs in presence of 2 % NaCl, but it does grow in the presence of 1.5 % NaCl. The predominant quinone is Q-8. The major cellular fatty acids (>10 % of total fatty acids) are summed feature 3 (C_{16:1ω6c} and/or C_{16:1ω7c}), summed feature 8 (C_{18:1ω6c} and/or C_{18:1ω7c}) and C_{16:0}. Hydrolyses Tween 20 but not Tween 80. Nitrate reduction is negative. β-Galactosidase, arginine dihydrolase, indole, urease and gelatinase are not produced. Aesculin and arbutin are hydrolysed. Urease is positive after 4 weeks of incubation in urea broth. In the API 20NE system, glucose, gluconate, adipate, malate and citrate are used as carbon sources. Assimilation of L-arabinose, mannitol, mannose, N-acetylglucosamine, maltose, malate and caprate is negative. Assimilation of phenylacetate is weak. In the API ID32GN system, assimilation of glucose, sucrose, sorbitol, itaconate, suberate, acetate, lactate, 3-hydroxybenzoate, propionate, valerate, citrate, 3-hydroxybutyrate and 4-hydroxybenzoate is positive. Assimilation of maltose and L-alanine is weak. Assimilation of L-rhamnose, D-ribose, N-acetylglucosamine, salicin, melibiose, L-fucose, L-arabinose, mannitol, inositol, glycogen, malonate, 5-ketogluconate, 2-ketogluconate, caprate, L-serine, L-histidine and L-proline is negative. In the API ZYM system, production of alkaline and acid phosphatases, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase and phosphohydrolase is positive. Production of lipase (C14), cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase is negative. Sensitive to cefuroxime, ciprofloxacin, erythromycin, gentamicin, neomycin, polymyxin B and tetracycline. Resistant to ampicillin, penicillin and cloxacillin. The DNA G+C content of the type strain is 61.6 mol%.

The type strain is SUEMI03^T (=LMG 26151^T =CECT 7838^T), isolated from soil on Tenerife (Canary Islands).

Table 2. Phenotypic differences among the three novel strains and their closest relative, *H. seropedicae* DSM 6445^T

Strains: 1, *H. canariense* sp. nov. SUEMI03^T; 2, *H. aurantiacum* sp. nov. SUEMI08^T; 3, *H. soli* sp. nov. SUEMI10^T; 4, *H. seropedicae* DSM 6445^T. w, Weakly positive.

Characteristic	1	2	3	4
Pigmentation	White	Orange	Pale orange	Cream brownish
Growth in/at:				
2 % NaCl	—	—	w	+
pH 4.5	—	—	—	+
Nitrate reduction	—	—	—	+
Urease (72 h)	—	—	—	+
Hydrolysis of:				
Aesculin*	+	—	—	—
Arbutin†	+	+	—	—
Growth on:				
L-Arabinose*	—	—	—	+
N-Acetylglucosamine	—	—	—	+
D-Mannose‡	—	—	—	+
D-Glucose*	+	—	—	+
Sucrose§	+	—	—	—
Salicin§	—	+	—	—
L-Rhamnose§	—	—	—	+
D-Ribose§	—	w	—	+
L-Fucose§	—	—	—	+
Mannitol§	—	—	—	+
Sorbitol§	+	—	—	+
Inositol§	—	—	—	+
L-Proline§	—	+	—	+
L-Alanine§	w	+	w	w
L-Serine§	—	—	—	+
2-Ketogluconate§	—	—	—	+
5-Ketogluconate§	—	—	—	+
Adipate‡	+	+	+	—
Caprate‡	—	—	—	+
Phenylacetate‡	w	—	—	+
Acetate§	+	+	w	w
Suberate§	+	+	+	—
3-Hydroxybenzoate§	+	+	+	—
4-Hydroxybenzoate§	+	+	w	—
Enzyme activities (API ZYM)				
Cystine arylamidase	—	—	—	+
β-Galactosidase	—	—	—	+
Resistance to:				
Ampicillin (2 µg)	+	—	+	+
Penicillin (10 IU)	+	—	—	+

*Data from both API 20NE and API ID32GN (after 3 days of incubation).

†Data from API 50CH.

‡Data from API 20NE.

§Data from API ID32GN (after 5 days of incubation).

Description of *Herbaspirillum aurantiacum* sp. nov.

Herbaspirillum aurantiacum (au.ran'ti.a'cum. N.L. neut. adj. *aurantiacum* orange-coloured).

Gram-negative, aerobic, non-spore-forming, slightly curved rods, 1.7–1.9 µm long and 0.8–1.1 µm in diameter. Motile

by a polar flagellum. Colonies on TSA are circular, convex, orange and opaque, usually 1–2 mm in diameter within 2 days at 28 °C. Catalase- and oxidase-positive. Growth occurs at 20 and 30 °C and occurs slowly at 15 and 34 °C, with optimum growth at 28 °C. No growth occurs at pH 4.5, but growth is positive at pH 8, with

optimal growth at pH 6–8. No growth occurs in the presence of 2% NaCl, but growth is positive at 1.5% NaCl. The predominant quinone is Q-8. The major cellular fatty acids (>10% of total fatty acids) are summed feature 3 (C_{16:1}ω6c and/or C_{16:1}ω7c), summed feature 8 (C_{18:1}ω6c and/or C_{18:1}ω7c) and C_{16:0}. Hydrolyses Tween 20 and hydrolyses Tween 80 weakly. Nitrate reduction is negative. β-Galactosidase, arginine dihydrolase, indole, urease and gelatinase are not produced. Arbutin is hydrolysed but aesculin is not. Urease is positive after 4 weeks of incubation in urea broth. In the API 20NE system, gluconate, adipate, malate and citrate are used as carbon sources. Assimilation of glucose, L-arabinose, mannitol, mannose, N-acetylglucosamine, maltose, caprate and phenylacetate is negative. In the API ID32GN system, assimilation of salicin, itaconate, suberate, acetate, lactate, 3-hydroxybenzoate, propionate, valerate, citrate, 3-hydroxybutyrate, 4-hydroxybenzoate, L-proline and L-alanine is positive. Assimilation of D-ribose is weak. Assimilation of glucose, L-rhamnose, L-fucose, L-arabinose, N-acetylglucosamine, sucrose, maltose, melibiose, mannitol, inositol, sorbitol, glycogen, malonate, 5-ketogluconate, 2-ketogluconate, caprate, L-serine and L-histidine is negative. In the API ZYM system, production of alkaline and acid phosphatases, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase and phosphohydrolase is positive. Production of lipase (C14), cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase is negative. Sensitive to ampicillin, penicillin, cefuroxim, ciprofloxacin, erythromycin, gentamicin, neomycin, polymyxin B and tetracycline. Resistant to cloxacillin. The DNA G+C content of the type strain is 60.4 mol%.

The type strain is SUEMI08^T (=LMG 26150^T =CECT 7839^T), isolated from soil on Tenerife (Canary Islands).

Description of *Herbaspirillum soli* sp. nov.

Herbaspirillum soli (so'li. L. gen. n. *soli* of soil).

Gram-negative, aerobic, non-spore-forming, slightly curved rods, 1.9 µm long and 1.2 µm in diameter. Motile by a polar flagellum. Colonies on TSA are circular, convex, pale orange and opaque, usually 1–2 mm in diameter within 2 days at 28 °C. Catalase- and oxidase-positive. Growth occurs at 20 and 34 °C and is weak at 15 and 37 °C, with optimum growth at 28 °C. No growth occurs at pH 4.5, but growth is positive at pH 8 with optimal growth at pH 6–8. Growth is positive in the presence of 1.5% NaCl and weak at 2% NaCl. The predominant quinone is Q-8. The major cellular fatty acids (>10% of total fatty acids) are summed feature 3 (C_{16:1}ω6c and/or C_{16:1}ω7c), summed feature 8 (C_{18:1}ω6c and/or C_{18:1}ω7c) and C_{16:0}. Hydrolyses Tween 20 and hydrolyses Tween 80 weakly. Nitrate reduction is negative. β-Galactosidase, arginine dihydrolase, indole, urease and gelatinase are not

produced and aesculin is not hydrolysed in API 20NE after 72 h of incubation. Aesculin and arbutin are not hydrolysed. Urease is positive after 4 weeks of incubation in urea broth. In the API 20NE system, gluconate, adipate, malate and citrate are used as carbon sources. Assimilation of glucose, L-arabinose, mannitol, mannose, N-acetylglucosamine, maltose, caprate and phenylacetate is negative. In the API ID32GN system, assimilation of itaconate, suberate, lactate, 3-hydroxybenzoate, propionate, valerate and 3-hydroxybutyrate is positive. Assimilation of acetate, citrate, 4-hydroxybenzoate and L-alanine is weak. Assimilation of glucose, D-ribose, L-fucose, L-arabinose, L-rhamnose, N-acetylglucosamine, sucrose, maltose, melibiose, mannitol, inositol, sorbitol, salicin, glycogen, malonate, 5-ketogluconate, 2-ketogluconate, caprate, L-histidine, L-serine and L-proline is negative. In the API ZYM system, production of alkaline and acid phosphatases, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase and phosphohydrolase is positive and that of lipase (C14) is weak. Production of cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase is negative. Sensitive to penicillin, cefuroxim, ciprofloxacin, erythromycin, gentamicin, neomycin, polymyxin B and tetracycline. Resistant to ampicillin and cloxacillin. The DNA G+C content of the type strain is 61.9 mol%.

The type strain is SUEMI10^T (=LMG 26149^T =CECT 7840^T), isolated from soil on Tenerife (Canary Islands).

Emended description of the genus *Herbaspirillum* Baldani *et al.* 1986

The description of the genus *Herbaspirillum* is as given by Baldani *et al.* (1986), with the following amendments. Urease is positive or slowly positive. Assimilation of D-glucose, D-galactose, D-ribose, L-fucose, D-mannose, L-arabinose, D-fructose, sorbitol, mannitol, L-proline, caprate, 5-ketogluconate and 2-ketogluconate is variable.

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