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Quisquiliibacterium transsilvanicum gen. nov., sp. nov., a novel betaproteobacterium isolated from a waste-treating bioreactor

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Abstract

A new betaproteobacterium, CGI-09^T, was isolated from an activated sludge bioreactor which treated landfill leachate. Based on 16S rRNA gene sequence analysis, the new strain shared the highest pairwise similarity values with members of the order *Burkholderiales*: *Derxia gummosa* IAM 13946^T (family *Alcaligenaceae*), 93.7 % and *Lautropia mirabilis* DSM 11362^T (family *Burkholderiaceae*), 93.6 %. Cells of strain CGI-09^T were rod-shaped and non-motile. The new strain was oxidase and catalase positive and capable of reducing nitrate to nitrite. The predominant fatty acids were $C_{16:1}\omega7c$, $C_{16:0}$, cyclo$C_{17:0}$ and $C_{18:1}\omega7c$, the major respiratory quinone was Q-8, and the detected polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and an unknown phospholipid. The G+C content of the genomic DNA of strain CGI-09^T was 70.2 mol%. The new bacterium can be distinguished from the members of genera *Derxia* and *Lautropia* based on its non-motile cells, arginine dihydrolase activity, its high cyclo $C_{17:0}$ fatty acid content and the lack of hydroxy fatty acids. On the basis of the phenotypic, chemotaxonomic and molecular data, strain CGI-09^T is considered to represent a new genus and species within the family *Burkholderiaceae*, for which the name *Quisquiliibacterium transsilvanicum* gen. nov., sp. nov. is proposed. The type strain is CGI-09^T (=DSM 29781^T=JCM 31785^T).

Members of the class *Betaproteobacteria* are among the most numerous prokaryotes in biological wastewater treatment processes under aerobic conditions, including the treatment of both domestic and industrial wastewater [1–3]. A recent cultivation-based study [4] of a landfill leachate-treating bioreactor resulted in the isolation of bacterial strains representing potentially new species and genera; some of them have been already described [5, 6]. One of the new isolates, strain CGI-09^T, showed low pairwise similarity values of 16S rRNA gene sequences to species with validly published names, and the closest-related sequences represented environmental clones which were retrieved from bioreactors treating various types of waste (Fig. S1, available in the online Supplementary Material). Based on the comparative results of the polyphasic taxonomic analyses presented in this paper, this strain is supposed to represent a novel genus within the family *Burkholderiaceae* (order *Burkholderiales* of class *Betaproteobacteria*), for which the name *Quisquiliibacterium transsilvanicum*, gen. nov., sp. nov. is proposed.

Strain CGI-09^T was isolated from an activated sludge sample of a bioreactor, which treated the leachate of a landfill site located in Odorheiu Secuiesc (Harghita County, Romania), in June 2013. For isolation, a diluted R2A-based medium was used, which contained 360 ml R2A medium (DSMZ medium 830), 1.33 g $CaCl_2$ and 1.81 g NH_4Cl in 1 l final volume (pH 8.0), and was solidified with 10 g l^{-1} gellan gum (Gelzan CM, Sigma). The standard dilution plating technique was applied to obtain strains by incubation at room temperature (20–22 °C) under aerobic conditions. Strain CGI-09^T could also effectively grow on TSA agar (DSM medium 545, without NaCl, pH 7) and nutrient agar (DSM medium 1, pH 7), and was subsequently cultivated on the latter medium for detailed taxonomic analysis.

Temperature and pH optima were determined based on the observed growth intensity at 4, 10, 20, 25, 30, 37 and 45 °C, and at pH from 4 to 11 (at intervals of 1), respectively. Salt tolerance was studied in nutrient broth from 0 to 10 % (w/v) NaCl concentration (with intervals of 1 %). Colony morphology was tested by direct observation of single colonies.

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Abbreviation: BLAST, Basic Local Alignment Search Tool.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CGI-09^T is KM083133.
Two supplementary figures and two supplementary tables are available with the online Supplementary Material.

Cell morphology was examined with Gram-staining according to Claus [7], while the presence of flagella was checked as described by Heimbrook *et al.* [8]. Oxidase activity and catalase reaction was determined as described by Tarrand and Gröschel [9], and Cowan and Steel [10], respectively. Acid production from D-glucose was checked by using the oxidative and fermentative test of Hugh and Leifson [11]. Additional metabolic tests were performed with API 20NE, API 50CH and API ZYM (bioMérieux) systems according to instructions of the manufacturer.

Composition of isoprenoid quinones, cellular fatty acids, polar lipids and the determination of DNA base composition were performed as described in our previous work [12].

The 16S rRNA gene sequence of strain CGI-09^T was amplified with primers 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) using the PCR protocol described in detail by Máthé *et al.* [13]. PCR product purification and direct Sanger sequencing (on an Applied Biosystems 3730xl platform) were performed with primers 27F, 519F (5′- CAG CAG CCG CGG TAA TAC-3′), 928R (5′-CCG TCA ATT CCT TTG AGT TT-3′) and 1492R using the service provided by the LGC Genomics GmbH (Berlin, Germany). Closest-related species (represented by type strains) were identified by EzBioCloud's online service [14] and highly similar environmental clones by BLAST [15]. Their 16S rRNA gene sequences were obtained from GenBank. Sequence alignment was performed by the SINA Alignment Service [16]. Phylogenetic analyses, including the search for the best-fit model parameters, was conducted using MEGA7 software [17].

Sequencing the 16S rRNA gene of strain CGI-09^T resulted in a stretch of 1453 nucleotides. The closest type strains of bacterial species were identified as *Derxia gummosa* IAM 13946^T (=LMG 3977^T) showing 93.7 % pairwise similarity and *Lautropia mirabilis* DSM 11362^T (=ATCC 51599^T) showing 93.6 % pairwise similarity, based on the 16S rRNA gene, while members of *Noviherbaspirillum*, *Pigmentiphaga* and all other genera showed lower than 93 % similarities to strain CGI-09^T. These values are lower than the genus level threshold (~95 %) suggested by Tindall *et al.* [18] and lower than the value (94.5 %) proposed more recently by Yilmaz *et al.* [19]. The phylogenetic analysis based on the 16S rRNA gene also supported that the new strain is a member of a new genus, since it clustered only with low bootstrap support to *L. mirabilis* ATCC 51599^T and was placed on a completely different lineage than *D. gummosa* IAM 13946^T (Fig. 1). Additionally, the new strain was separated from its

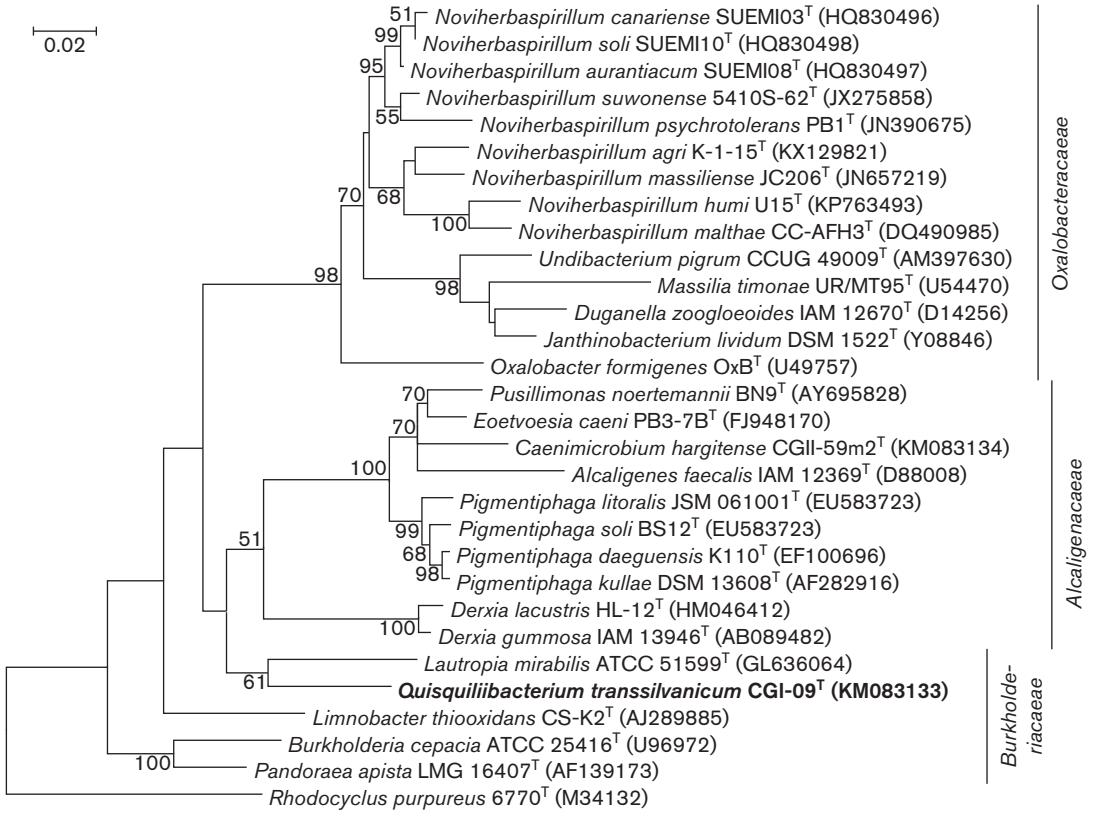

Fig. 1. Phylogenetic position of CGI-09^T and related type strains based on the 16S rRNA gene. The phylogenetic tree has been reconstructed based on 1312 positions using the maximum-likelihood method. Bootstrap values >50 % are shown at the nodes.

closest-related genera which are members of families *Alcaligenaceae* (*Derxia* and *Pigmentiphaga*) and *Oxalobacteraceae* (*Noviherbaspirillum*) and was affiliated with members of the family *Burkholderiaceae* (within order *Burkholderiales*, class *Betaproteobacteria*); however, the polyphyletic character of this family based on 16S rRNA gene sequences reported previously [20] was also observed in our analysis.

Cells of strain CGI-09^T were Gram-stain-negative, non-motile, aerobic and mesophilic with a characteristic heterotrophic metabolism (Table 1). Based on the enzyme activities and substrates tested for utilization, the new strain favours proteins rather than carbohydrates for its metabolism (Table S1). Strain CGI-09^T was capable of reducing nitrate to nitrite, and the respiratory metabolism of the new species using nitrate was supported with that of closely related environmental clone sequences (showing 98.0–99.3 % pairwise similarity values based on the 16S rRNA gene) originating from denitrifying bioreactors (Fig. S1).

The isoprenoid quinone of CGI-09^T was ubiquinone Q-8, which is also characteristic for families *Burkholderiaceae*, *Alcaligenaceae* and *Oxalobacteraceae* [20, 21]. The fatty acid pattern of strain CGI-09^T was dominated by $C_{16:1}\,\omega7c$ (32.3 %), $C_{16:0}$ (21.2 %), cyclo$C_{17:0}$ (20.5 %) and $C_{18:1}\,\omega7c$ (14.5 %) (Table S2). The high ratio of cyclo$C_{17:0}$ distinguishes the new strain from the closely related type strains, since this fatty acid was not detectable in *L. mirabilis* DSM 11362^T (Table S2), nor in *D. gummosa* LMG 3977^T and *D. gummosa* LMG 3975 [22], and was present only in low amount (1.2 %) in *Derxia lacustris* HL-12^T [22]. The new strain could be distinguished from any related genera based on on the fact that it lacks hydroxy fatty acids (Table 1). The polar lipid profile of strain CGI-09^T was rather simple:

Table 1. Differential characteristics of *Quisquiliibacterium* and related genera

1, *Quisquiliibacterium* (this study); 2, *Derxia* [22, 23]; 3, *Lautropia* (this study; [24, 25]); 4, *Noviherbaspirillum* [26–30]; 5, *Pigmentiphaga* [31–34]. Fatty acids shown in parentheses were detected only in one type strain, only fatty acids >1 % are considered. Note that summed feature components are not considered in this comparison.

Characteristic	1	2	3	4	5
Number of species*	1	2	1	9	4
Valid species names	*Q. transsilvanicum*	*D. gummosa, D. lacustris*	*L. mirabilis*	*N. agri, N. aurantiacum, N. canariense, N. humi, N. malthae, N. massiliense, N. psychrotolerans, N. soli, N. suwonense**	*P. daeguensis, P. kullae, P. litoralis, P. soli*
Motility	−	+	+†	+	+/−
Nitrate reduction to nitrite	+	−	+	−	−‡
Urease activity	+	−	+	−/+§	−
Arginine dihydrolase	+	−	−‖	−‖	−‖
Major hydroxy fatty acids	−	$C_{12:0}$ 3-OH, $C_{14:0}$ 2-OH, $C_{14:0}$ 3-OH	$C_{10:0}$ 3-OH	$C_{10:0}$ 3-OH, $C_{14:0}$ 2-OH, ($C_{12:0}$ 2-OH, $C_{12:0}$ 3-OH)¶	$C_{10:0}$ 3-OH, $C_{14:0}$ 2-OH, $C_{16:0}$ 2-OH, $C_{18:1}$ 2-OH, ($C_{12:0}$ 2-OH, $C_{16:1}$ 2-OH)¶
Major cyclic fatty acids	cyclo$C_{17:0}$	(cyclo$C_{17:0}$)#	−	cyclo$C_{17:0}$#	cyclo$C_{17:0}$, cyclo$C_{19:0}\,\omega8c$
DNA G+C content (mol%)	70.2	69.2–72.0	64.6–65.4	62.5–66.7**	65.5–68.5

*Based on a search performed using Prokaryotic Nomenclature Up-To-Date [35] on 17 June 2017. Two *Noviherbaspirillum* species are currently not on this list, since the paper [28] describing *N. agri* and *N. massiliense* was recently accepted for publication in the *International Journal of Systematic and Evolutionary Microbiology*.

†Non-motile forms may also occur.

‡Yoon *et al.* [32] found a negative reaction for *P. kullae* DSM 13608^T, while Lee *et al.* [34] observed a positive reaction for this type strain (=KACC 11572^T).

§Positive urease activity was detected for *N. aurantiacum* LMG 26150^T, *N. suwonense* KACC 16657^T and *N. soli* LMG 26149^T [26, 29].

‖Variable reaction was observed by Rossmann *et al.* [25] in the case of non-type strains of *L. mirabilis*. *N. canariense* LMG 26151^T was positive for arginine dihydrolase in the study of Lin *et al.* [26], but was negative, similarly to other *Noviherbasprillum* strains, in the original description of this species [30]. All four type strains of *Pigmentiphaga* showed negative reaction in the study of Lee *et al.* [34], while a weak positive reaction was observed for *P. daeguensis* JCM 14330^T by Yoon *et al.* [32] and a positive reaction for *P. litoralis* KCTC 22165^T by Chen *et al.* [33].

¶Fatty acid $C_{14:0}$ 2-OH was not detected in *N. agri* JCM 31463^T and in *N. massiliense* DSM 25712^T, while $C_{12:0}$ 2-OH and $C_{12:0}$ 3-OH were detected only in *N. massiliense* DSM 25712^T [27, 28]. Additionally, iso fatty acids were detected by Lin *et at.* [26] for some *Noviherbaspirillum* strains, but the presence of these fatty acids were not confirmed by others [27, 28]. In the case of *P. soli* JCM 17666^T, $C_{14:0}$ 2-OH was not detected, while fatty acids $C_{12:0}$ 2-OH and $C_{16:1}$ 2-OH were present [34]. Fatty acids $C_{16:0}$ 2-OH and $C_{18:1}$ 2-OH were not detected in *P. litoralis* KCTC 22165^T [33].

#Detected only in *D. lacustris* HL-12^T in low amounts (1.2 %) [22]. Detected only in traces in *N. psychrotolerans* DSM 26001^T and in *N. agri* JCM 31463^T [26, 28].

**No genomic DNA G+C content data is available for *N. suwonense* KACC 16657^T.

phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and an unknown phospholipid were detected (Fig. S2). The G+C content of the genomic DNA of strain CGI-09^T was 70.2 mol%.

In conclusion, strain CGI-09^T is considered to represent a novel genus within the family *Burkholderiaceae* (order *Burkholderiales*, class *Betaproteobacteria*), for which the name *Quisquiliibacterium transsilvanicum*, gen. nov., sp. nov. is proposed. The new genus could be distinguished from the closest related genera, *Derxia* and *Lautropia*, based on its non-motile cells and positive arginine dihydrolase activity. Further distinguishing characters are presented in Table 1.

DESCRIPTION OF *QUISQUILIIBACTERIUM* GEN. NOV.

Quisquiliibacterium (Qius.qui.li.i.bac.te′ri.um. L. neut. pl. n. *quisquilia* waste, rubbish, referring to the isolation of the type strain from activated sludge; N.L. neut. n. *bacterium*. a rod; N.L. neut. n. *Quisquiliibacterium*, rod from waste).

Cells are Gram-stain-negative, non-motile short rods. Aerobic. Oxidase- and catalase-positive. The respiratory quinone is Q-8 (no other quinones are detected). The major cellular fatty acids are $C_{16:1}\,\omega7c$, $C_{16:0}$, cyclo$C_{17:0}$ and $C_{18:1}\,\omega7c$. Polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and an unknown phospholipid.

The type species is *Quisquiliibacterium transsilvanicum*.

DESCRIPTION OF *QUISQUILIIBACTERIUM TRANSSILVANICUM* SP. NOV.

Quisquiliibacterium transsilvanicum (trans.sil.va′ni.cum. M. L. neut. adj. *transsilvanicum* of, or belonging to Transylvania, referring to the historical region located in the central part of Romania, from where the landfill leachate originated which was treated by the bioreactor and gave rise to the isolation of the type strain).

Cells are rod-shaped (0.6–0.7×1.0–1.2 µm) and non-motile. Colonies on nutrient medium are beige-coloured, circular and raised with a diameter of 1 mm. Growth occurs after 4–6 days of incubation, at pH 7–8, at 10–45 °C (with an optimum between 25 and 30 °C) and 0–2 % NaCl concentration (optimum 0–1 %). Negative for oxidative and fermentative acid production from D-glucose. Positive for nitrate reduction to nitrite, while negative for the reduction of nitrate to nitrogen gas, indole production, glucose fermentation and hydrolysis of aesculin and gelatine. Positive for acid phosphatase (weak), alkaline phosphatase, arginine dihydrolase, α-chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and urease activities; negative for α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, lipase (C14), α-mannosidase, *N*-acetyl-β-glucosaminidase, trypsin and valine arylamidase activities. Positive for the assimilation of adipic acid. Negative for the assimilation of D-adonitol, aesculin, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, capric acid, cellobiose, citrate, dulcitol, erythritol, D-fructose, D-fucose, L-fucose, D-galactose, gentiobiose, gluconate, D-glucose, glycerol, glycogen, inositol, inulin, 2-ketogluconate, 5-ketogluconate, lactose, D-lyxose, malic acid, maltose, D-mannitol, D-mannose, melezitose, melibiose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, *N*-acetyl-glucosamine, phenylacetic acid, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, starch, sucrose, D-tagatose, trehalose, turanose, xylitol, D-xylose and L-xylose. Polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and an unknown phospholipid.

The G+C content of the genomic DNA is 70.2 mol%.

The type strain is CGI-09^T (=DSM 29781^T=JCM 31785^T) which was isolated from activated sludge.

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

References

1. Cydzik-Kwiatkowska A, Zielińska M. Bacterial communities in full-scale wastewater treatment systems. *World J Microbiol Biotechnol* 2016;32:66.
2. Saunders AM, Albertsen M, Vollertsen J, Nielsen PH. The activated sludge ecosystem contains a core community of abundant organisms. *ISME J* 2016;10:11–20.
3. Zhu S, Wu H, Wei C, Zhou L, Xie J. Contrasting microbial community composition and function perspective in sections of a full-scale coking wastewater treatment system. *Appl Microbiol Biotechnol* 2016;100:949–960.
4. Felföldi T, Kovács E, Fikó DR, Tankó G, Szabó A *et al.* Unconventional strategies for the cultivation of new bacterial strains from aquatic environments. *Acta Microbiol Immunol Hung* 2015; 62:150–151.
5. Felföldi T, Mentes A, Schumann P, Kéki Z, Máthé I *et al.* *Rufibacter quisquiliarum* sp. nov., a new member of the phylum *Bacteroidetes* isolated from a bioreactor treating landfill leachate. *Int J Syst Evol Microbiol* 2016;66:5150–5154.
6. Felföldi T, Schumann P, Mentes A, Kéki Z, Máthé I *et al.* *Caenimicrobium hargitense* gen. nov., sp. nov., a new member of the family *Alcaligenaceae* (*Betaproteobacteria*) isolated from activated sludge. *Int J Syst Evol Microbiol* 2017;67:627–632.
7. Claus D. A standardized Gram staining procedure. *World J Microbiol Biotechnol* 1992;8:451–452.
8. Heimbrook ME, Wang WL, Campbell G. Staining bacterial flagella easily. *J Clin Microbiol* 1989;27:2612–2615.
9. Tarrand JJ, Gröschel DH. Rapid, modified oxidase test for oxidase-variable bacterial isolates. *J Clin Microbiol* 1982;16:772–774.
10. Cowan ST, Steel KJ. *Manual for the Identification of Medical Bacteria*, 2nd ed. Cambridge: Cambridge University Press; 1974.

11. **Hugh R, Leifson E.** The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. *J Bacteriol* 1953;66:24–26.
12. **Felföldi T, Kéki Z, Sipos R, Márialigeti K, Tindall BJ *et al.*** *Ottowia pentelensis* sp. nov., a floc-forming betaproteobacterium isolated from an activated sludge system treating coke plant effluent. *Int J Syst Evol Microbiol* 2011;61:2146–2150.
13. **Máthé I, Borsodi AK, Tóth EM, Felföldi T, Jurecska L *et al.*** Vertical physico-chemical gradients with distinct microbial communities in the hypersaline and heliothermal Lake Ursu (Sovata, Romania). *Extremophiles* 2014;18:501–514.
14. **Yoon SH, Ha SM, Kwon S, Lim J, Kim Y *et al.*** Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
15. **Zhang Z, Schwartz S, Wagner L, Miller W.** A greedy algorithm for aligning DNA sequences. *J Comput Biol* 2000;7:203–214.
16. **Pruesse E, Peplies J, Glöckner FO.** SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012;28:1823–1829.
17. **Kumar S, Stecher G, Tamura K.** MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
18. **Tindall BJ, Rosselló-Móra R, Busse HJ, Ludwig W, Kämpfer P.** Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* 2010;60:249–266.
19. **Yilmaz P, Yarza P, Rapp JZ, Glöckner FO.** Expanding the world of marine bacterial and archaeal clades. *Front Microbiol* 2015;6:1524.
20. **Rosenberg E, Delong EF, Lory S, Stackebrandt E, Thompson F *et al.*** *The Prokaryotes, Alphaproteobacteria and Betaproteobacteria*, 4th ed. Berlin: Springer-Verlag; 2014.
21. **Brenner DJ, Krieg NR, Staley JT.** *Bergey's Manual of Systematic Bacteriology, The Proteobacteria*, 2nd ed. New York: Springer; 2005.
22. **Chen WM, Huang WC, Sheu SY.** *Derxia lacustris* sp. nov., a nitrogen-fixing bacterium isolated from a freshwater lake. *Int J Syst Evol Microbiol* 2013;63:965–970.
23. **Jennsen HL, Petersen EJ, de PK, Bhattacharya R.** A new nitrogen-fixing bacterium: *Derxia gummosa* nov. gen. nov. spec. *Arch Mikrobiol* 1960;36:182–195.
24. **Gerner-Smidt P, Keiser-Nielsen H, Dorsch M, Stackebrandt E, Ursing J *et al.*** *Lautropia mirabilis* gen. nov., sp. nov., a gram-negative motile coccus with unusual morphology isolated from the human mouth. *Microbiology* 1994;140:1787–1797.
25. **Rossmann SN, Wilson PH, Hicks J, Carter B, Cron SG *et al.*** Isolation of *Lautropia mirabilis* from oral cavities of human immunodeficiency virus-infected children. *J Clin Microbiol* 1998;36:1756–1760.
26. **Lin SY, Hameed A, Arun AB, Liu YC, Hsu YH *et al.*** Description of *Noviherbaspirillum malthae* gen. nov., sp. nov., isolated from an oil-contaminated soil, and proposal to reclassify *Herbaspirillum soli*, *Herbaspirillum aurantiacum*, *Herbaspirillum canariense* and *Herbaspirillum psychrotolerans* as *Noviherbaspirillum soli* comb. nov., *Noviherbaspirillum aurantiacum* comb. nov., *Noviherbaspirillum canariense* comb. nov. and *Noviherbaspirillum psychrotolerans* comb. nov. based on polyphasic analysis. *Int J Syst Evol Microbiol* 2013;63:4100–4107.
27. **Sundararaman A, Srinivasan S, Lee SS.** *Noviherbaspirillum humi* sp. nov., isolated from soil. *Antonie van Leeuwenhoek* 2016;109:697–704.
28. **Chaudhary DK, Kim J.** *Noviherbaspirillum agri* sp. nov., isolated from reclaimed grassland soil, and reclassification of *Herbaspirillum massiliense* (Lagier *et al.*, 2014) as *Noviherbaspirillum massiliense* comb. nov. *Int J Syst Evol Microbiol* 2017;67:1508–1515.
29. **Kim SJ, Moon JY, Weon HY, Hong SB, Seok SJ *et al.*** *Noviherbaspirillum suwonense* sp. nov., isolated from an air sample. *Int J Syst Evol Microbiol* 2014;64:1552–1558.
30. **Carro L, Rivas R, León-Barrios M, González-Tirante M, Velázquez E *et al.*** *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*. *Int J Syst Evol Microbiol* 2012;62:1300–1306.
31. **Blümel S, Mark B, Busse HJ, Kämpfer P, Stolz A.** *Pigmentiphaga kullae* gen. nov., sp. nov., a novel member of the family *Alcaligenaceae* with the ability to decolorize azo dyes aerobically. *Int J Syst Evol Microbiol* 2001;51:1867–1871.
32. **Yoon JH, Kang SJ, Kim W, Oh TK.** *Pigmentiphaga daeguensis* sp. nov., isolated from wastewater of a dye works, and emended description of the genus *Pigmentiphaga*. *Int J Syst Evol Microbiol* 2007;57:1188–1191.
33. **Chen YG, Zhang YQ, Huang K, Tang SK, Cao Y *et al.*** *Pigmentiphaga litoralis* sp. nov., a facultatively anaerobic bacterium isolated from a tidal flat sediment. *Int J Syst Evol Microbiol* 2009;59:521–525.
34. **Lee JJ, Srinivasan S, Kim MK.** *Pigmentiphaga soli* sp. nov., a bacterium isolated from soil. *J Microbiol* 2011;49:857–861.
35. **Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Germany.** April 2017. Prokaryotic Nomenclature Up-to-date. www.dsmz.de/bacterial-diversity/prokaryotic-nomenclature-up-to-date.