Antonie van Leeuwenhoek (2018) 111:485–491
https://doi.org/10.1007/s10482-017-0969-5

ORIGINAL PAPER

Polyphasic taxonomic characterisation of a novel strain as *Pararhizobium haloflavum* sp. nov., isolated from soil samples near a sewage treatment tank

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Received: 11 April 2017 / Accepted: 24 October 2017 / Published online: 13 November 2017

Abstract A Gram-stain negative, aerobic, motile and ovoid- to rod-shaped bacteria strain, designated XC0140^T, was isolated from soil samples near the sewage treatment tank of a chemical factory in Zhejiang Province, China, and subjected to polyphasic taxonomic investigation. Strain XC0140^T grew at 10–37 °C and pH 6.0–9.0 (optimum, 35 °C and pH 7.5) and with 0–17% (w/v) NaCl (optimum, 1%). According to phylogenetic analysis based on 16S rRNA gene sequences, strain XC0140^T was assigned to the genus *Pararhizobium* with high 16S rRNA gene sequence similarity of 95.97% to "*Pararhizobium helanshanense* CCNWQTX14^T", followed by *Pararhizobium sphaerophysae* CCNWGS0238^T (95.95%). Chemotaxonomic analysis showed that strain XC0140^T contains ubiquinone-10 as the predominant respiratory quinone and possessed summed feature 8 (comprising $C_{18:1}$ ω7c and/or ω6c), 11-methyl $C_{18:1}$ ω7c, $C_{18:0}$ and $C_{16:0}$ as predominant forms of fatty acids. The polar lipids of strain XC0140^T consisted of seven phospholipids (PL), two aminolipids (AL), one glycolipid (GL) and three unidentified lipids (L1, L2 and L3). The DNA G+C content was 62.7 mol%. Based on the polyphasic taxonomic characterization, strain XC0140^T is considered to represent a novel species of the genus *Pararhizobium*, for which the name *Pararhizobium haloflavum* sp. nov. is proposed. (type strain XC0140^T = MCCC 1K03228^T = KCTC 52582^T).

Keywords *Pararhizobium haloflavum* · Soil samples · Sewage treatment tank · Taxonomy

Electronic supplementary material The online version of this article (http://doi.org/10.1007/s10482-017-0969-5) contains supplementary material, which is available to authorized users.

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Rhizobium is a large genus belonging to the bacterial family *Rhizobiaceae*, within the order *Rhizobiales* of the class *Alphaproteobacteria* (Sheu et al. 2015), first described by Frank (1889). Members of this genus are typically characterised as being Gram-stain negative, non-spore-forming, rod-shaped, aerobic and chemo-organotrophic, with $C_{18:1}$ ω7c as the most abundant fatty acid and a DNA G+C content between 57 and

66 mol % (Tighe et al. 2000; Young et al. 2001). However, some apparently *Rhizobium* species did not exhibit robust phylogenetic positions in family *Rhizobiaceae* before 2015. Then, in 2015, a phylogenetic examination by multilocus sequence analysis (MLSA) of four housekeeping genes among 100 strains of the family *Rhizobiaceae* was studied by Mousavi et al. As a result, a new genus, *Pararhizobium*, was proposed (Mousavi et al. 2015). At the time of writing, the genus *Pararhizobium* comprises 4 species with valid names (http://www.bacterio.net/pararhizobium.html).

In December 2015, in order to obtain halotolerant bacteria for wastewater treatment, our lab investigated the biodiversity of bacteria in a soil sample near the sewage treatment tank in a chemical factory. Then, a novel strain, named XC0140^T was isolated and selected for detailed polyphasic taxonomic analysis. In this study, the polyphasic taxonomic identification of the novel *Pararhizobium* strain XC0140^T is described in detail.

Materials and methods

Strains and culture conditions

In December 2015, a study of microbial diversity in a soil sample led to the isolation of a novel *alphaproteobacterium*. The soil sample was collected from the side of a sewage treatment tank in a chemical factory of Zhejiang province, China (30°14′ N, 120°86′ E). The soil sample was diluted, using a tenfold dilution series method, spread on modified marine 2216 agar medium and incubated at 35 °C. The modified marine 2216 agar medium contained (l^{-1}): 19.45 g NaCl, 12.6 g $MgCl_2{\cdot}6H_2O$, 6.64 g $MgSO_4{\cdot}7H_2O$, 0.55 g KCl, 1.8 g $CaCl_2$, 0.16 g $NaHCO_3$, 0.0016 g NH_4NO_3, 0.08 g KBr, 0.008 g Na_2HPO_4, 0.004 g Na_2SiO_3, 0.0024 g NaF, 0.034 g $SrCl_2{\cdot}6H_2O$, 0.022 g H_3BO_3, 0.1 g Ferric citrate, 5.0 g tryptone (Oxoid), 1.0 g yeast extract (Oxoid) and 20 g Agar (pH7.0). After 48 h of incubation, a yellow-coloured colony was collected and named as XC0140^T.

Strain XC0140^T was routinely cultured on modified marine 2216 agar medium after repeated purifying. For long-term preservation, purified strains were preserved at −80 °C with 25% (v/v) glycerol and also by lyophilization with 20% (w/v) skimmed milk. Strain XC0140^T has been deposited at the MCCC (Marine Culture Collection of China) and the KCTC (Korean Collection for Type Cultures).

Determination of 16S rRNA gene sequence and phylogenetic analysis

Amplification and sequencing of the 16S rRNA gene, as well as sequence alignment and phylogenetic analysis based on 16S rRNA gene sequences were all performed as previously established (Zhang et al. 2015).

Amplification of *nif*H and *nod* genes

In this study, the sequences of the *nod* and *nif*H genes for Strain XC0140^T were amplified as described in Elliott et al. (2007) and Laguerre et al. (2001).

Genome sequencing, annotation and local blast

The genome of XC0140^T was sequenced using an Illumina HiSeq 4000 system (Illumina, San Diego, CA, USA) at the Beijing Genomics Institute (Shenzhen, China). The paired-end fragment libraries were sequenced according to the Illumina HiSeq 4000 system's protocol. Raw reads of low quality from paired-end sequencing (those with consecutive bases covered by fewer than five reads) were discarded. The sequenced reads were assembled using ABySS software. Rapid Annotation System Technology (RAST) did genome annotation (Aziz et al. 2008). And local blast was achieved by using of the tool of ncbi-blast-2.6.0 + (Camacho et al. 2009).

Determination of chromosomal DNA G+C content

The DNA G+C content was determined by reversed-phase HPLC (Mesbah et al. 1989) using the genomic DNA of *Escherichia* coli K-12 and salmon sperm DNA (Sigma) as calibration standards.

Phenotypic characterisation

The temperature for optimal growth was tested at 4–45 °C(4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 42 and 45 °C) in duplicate. The pH range for growth was measured from pH 4.5 to pH 10.0, with an interval of 0.5 units, making use of appropriate biological buffers

to maintain the stability of each pH system (30 mM MES for pH 4.5–6.0, PIPES for pH 6.5–7.5, Tricine for pH 8.0–8.5 and CAPSO for pH 9.0–10.0). The salt tolerance was determined in Na^+ and Cl^- ion free modified marine 2216 broth with various NaCl concentrations (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10 12, 14, 15, 16, 17 and 18%, w/v); cultures incubated for 2 days were used to determine the optimal growth of strain XC0140^T and those incubated for 14 days were utilized to determine the growth limits of the strain. Growth of strain XC0140^T was observed at 10-37 °C (optimum 35 °C), at pH6.0–9.0 (optimum pH7.5), with 0–17% (w/v) NaCl (optimum 1%). No anaerobic growth was detected in the modified 2216 medium supplemented with 10 mg l^{-1} ferric citrate, NH_4NO_3, $NaNO_2$ and Na_2SO_3, 0.2 g l^{-1} sodium thioglycolate and cysteine, 3 g l^{-1} $Na_2S_2O_3$ and 1 g l^{-1} glucose by the Hungate tube method.

After incubation at 35 °C for 2 days, cells were harvested for morphology observation by transmission electron microscopy (JEM- 1230; Jeol) after uranyl acetate staining and by optical microscopy (BX40; Olympus) after Gram staining. The Gram-staining reaction was carried out according to Claus (1992). Furthermore, motility was determined by microscopic observation and inoculation in Semi-solid agar medium.

Generally, strains investigated in all the following tests were cultured at their optimal temperatures. Hydrolysis activities of CM-cellulose, gelatin and starch were determined as previously described (Zhang et al. 2015). Catalase and Oxidase activities were tested by the method as described by Wu et al. (2010) and Kovacs (1956), respectively. Hydrogen sulfide production, methyl red (MR) and Voges-Proskauer (VP) tests were assayed according to Zhang et al. (2013). Tweens 20, 40, 60 and 80 were examined as described by Sun et al. (2015). Nitrate and nitrite reduction was determined by Griess A solution (0.5 g *p*-aminobenzenesulfonic acid with 150 ml (10.0%, v/v) acetic acid), Griess B solution (0.1 g α-naphthylamine with 150 ml (10.0%, v/v) acetic acid and 20 ml distilled water) and diphenylamine solution (0.5 g with 100 ml concentrated sulfuric acid and 20 ml distilled water) following cultivation in liquid medium added with 0.5% (w/v) nitrate or 0.1‰ (w/v) nitrite for 7 days.

API 20NE and API ZYM miniaturized systems were performed to test enzyme activities and additional phenotypic characteristics according to the manufacturer (bioMérieux). Susceptibility to antibiotics was determined by two-layer plate method with placing antibiotics disks (Hangzhou Microbial Reagent Co. Ltd, Hangwei^{TM}) on the upper medium, and considered positive when the radius of the inhibition zone was over 2.0 mm. The antibiotics (μg per disk except stated) used were listed as follows: nalidixic acid (30), lincomycin (2), sulfamethoxazole (25), rifampicin (5), chloramphenicol (30), gentamicin (10), kanamycin (30), tetracycline (30), novobiocin (30), penicillin (10), streptomycin (10), erythromycin (15).

Acid production tests were performed in modified marine oxidation-fermentation medium added with 2.0% (w/v) NaCl and 1.0% (w/v) filter-sterilized sugars or alcohols (Leifson 1963; Xu et al. 2008). Different concentrations of yeast extract and tryptone were tested to determine the optimal basal medium and tryptone was found to be essential for growth, but not yeast extract. Basal medium containing modified upper medium supplemented with 0.05‰ (w/v) tryptone and devoid of yeast extract, was used in subsequent testing. To characterise the utilization of carbon sources, bacteria was inoculated in basal medium containing 0.4% of varying carbon source, such as sugar, alcohol and organic acid. In the test, the basal medium with substrates but without inoculation was blank control and the growth in the basal medium with inoculation but without substrates was negative control. Growth was measured with OD_{600nm}. Results were considered negative when OD_{600nm} measured in the test was equal to or less than negative control; positive when the test absorbance is higher than the control by two fold (weak positive) or higher (strong positive).

Determination of fatty acids, polar lipids and isoprenoid quinones

A series of tests were performed to characterise the isoprenoid quinones and fatty acid profiles of the bacterial strain. After incubation in the modified marine 2216 broth at 35 °C and 140 rpm for 2 days, cells were collected for extraction of isoprenoid quinones which were subsequently purified by TLC and identified using a HPLC–MS system (Agilent) (Komagata and Suzuki 1988). Isoprenoid quinones were analysed as described by Minnikin et al. (1984).

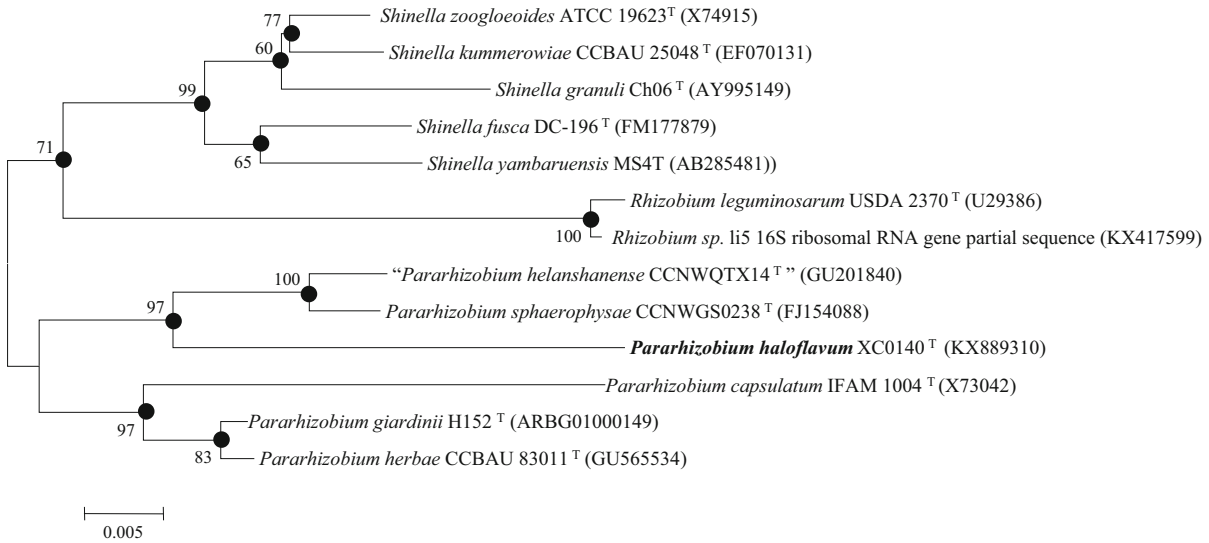

Fig. 1 Neighbour-joining phylogenetic tree of strain XC0140^T and some closely related taxa, based on 16S rRNA gene sequences (1541 bp). Numbers at branching points represent bootstrap values (%) from 1000 replicates. Solid circles indicate that the corresponding nodes were also recovered in maximum-likelihood and minimum-evolution trees. Bar, 0.005 substitutions per nucleotide position

Cells used for the analysis of fatty acids and polar lipids were harvested from the third quadrants of the modified marine 2216 agar plates. Fatty acids were saponified, methylated and extracted according to the standard protocol of the Sherlock Microbial Identification System (version 6.2B, MIDI) and identified based on the RTSBA6 method. Polar lipids were extracted as described by Kates (1986) and separated by two-dimensional TLC on silica gel 60 F254 plates (Merck) as described by Tindall (1990). Five kinds of spray reagents were used to visualize corresponding lipids, including molybdophosphoric acid for total lipids, a-naphthol/sulphuric acid and anisaldehyde for glycolipids, molybdenum blue for phospholipids and ninhydrin for aminolipids.

Results and discussion

Pairwise alignment based on the nearly full-length 16S rRNA gene sequence (1541 bp) of strain XC0140^T indicated that this isolate shares high sequence similarity with "*Pararhizobium helanshanense* CCNWQTX14^T" (invalid name, 95.97% 16S rRNA gene sequence similarity) and *Pararhizobium sphaerophysae* CCNWGS0238^T (95.96%). Topologies exhibited in both neighbour-joining tree, maximum-likelihood tree and minimum-evolution tree (Fig. 1) illustrated that strain XC0140^T clustered with the members of genus *Pararhizobium* under the support of a high bootstrap value, which suggested that it should belong to this genus. The DNA G+C content of strain XC0140^T was 62.7 mol%, which is very similar to the other two strains compared in our study (Table 1). Moreover, the DNA G+C content is in line with the description of genus *Pararhizobium* (Mousavi et al. 2015).

In this study, PCR products for the *nod* and *nif*H genes of strain XC0140^T could not be obtained. Furthermore, genome annotation and local blast results showed that sequences of *nod*A, *nod*C and *nif*H genes could not be found in strain XC0140^T.

As is shown in Fig. S1, strain XC0140^T in ovoid- to rod-shaped with one polar flagellum. Strain XC0140^T and the members of the genus of *Pararhizobium* share several common morphology characteristics, including Gram-stain negative and non-spore-forming (Fig. S1) (Mousavi et al. 2015).

In chemotaxonomic analysis, the major fatty acids were summed feature 8 ($C_{18:\ 1}$ ω7c/ω6c), 11-methyl $C_{18:1}$ ω7c,$C_{18:\ 0}$ and $C_{16:\ 0}$, which is very similar with the description of the genus *Pararhizobium* (Mousavi et al. 2015.). In addition, ubiquinone-10 (Q-10) was the predominant respiratory quinone of strain

Table 1 Differential characteristics between strain XC0140^T and related members of the genus *Pararhizobium*. Strains: 1, XC0140^T; 2, "*P. helanshanense* CCNWQTX14^T"; 3, *P. sphaerophysae* CCNWGS0238^T +, Positive reaction; -, negative reaction; ND, not determined. All strains were negative for indole production and Voges- Prokauer test

Characteristic	1[a]	2	3
Cell size (width × length; μm)	0.41–0.82 × 0.88–1.43	0.3–0.6 × 1.0–2.5	0.3–0.6 × 2–2.5
Motility	Motile	Motile	Motile
Type of metabolism	Aerobic	Aerobic	Aerobic
Color	Light yellow	White	White
Temperature range (optimum)(°C)	10–37 (35)	> 4, < 60 (25–30)	20–45 (28)
pH range (optimum)	6.0–9.0 (7.5)	9.0–11.0 (6.0–8.0)	7.0–11.0 (8.0)
NaCl tolerance (%, w/v)	< 18.0	< 6.0	< 5.0
G + C content (mol %)	62.7	60.3	63.5
Catalase activity	+	+	–
Oxidase activity	–	ND	–
Nitrate reduction	–	–	+
H_2S production	–	–	ND
Nitrite reduction	+	–	+
Hydrolysis of			
Gelatin	+	–	+
Starch	–	–	ND
Tween 80	–	–	+
Utilization of:			
D-Mannose	–	+	ND
D-Xylose	+	–	+
D-Sorbitol	+	ND	–
Sucrose	–	ND	+

[a]Data generated from this study. Data for 2 and 3 were taken from Qin et al. (2012) and Xu et al. (2011), respectively

XC0140^T. As is shown in Fig. S2, the polar lipid profile of strain XC0140^T was composed of seven phospholipids (PL), two aminolipids (AL), one glycolipid (GL) and three unidentified lipids (L1, L2 and L3).

Despite the overall similarities, strain XC0140^T displayed certain distinct characteristics from the two reference strains. Notably, NaCl tolerance of strain XC0140^T was significantly higher than the two reference strains. In addition, the optimal culture temperature of strain XC0140^T was higher than the reference strains. Strain XC0140^T differed from other strains in features such as catalase activity as depicted in Table 1.

Additionally, strain XC0140^T was resistant to nalidixic acid,lincomycin, and sensitive to sulfamethoxazole,trimethoprim, rifampicin, chloramphenicol, gentamicin, kanamycin, tetracycline, novobiocin, penicillin, streptomycin and erythromycin.

On the basis of the phylogenetic, genomic, chemotaxonomic and phenotypic characteristics, we propose that strain XC0140^T represents a novel species of the genus *Pararhizobium*, named *Pararhizobium haloflavum* sp. nov.

Description of *Pararhizobium haloflavum* sp. nov.

Pararhizobium haloflavum (ha.lo.fla'vum. Gr. n. hals, halos salt; L. adj. flavus yellow: N.L. neut. adj. *haloflavum* salty and yellow).

Cells are Gram-stain negative, aerobic, motile, non-spore-forming and ovoid- to rod-shaped, approximately 0.41–0.82 μm in width and 0.88–1.43 μm in length with single polar flagella. After incubation on

modified marine 2216 agar at 35 °C for 2 days, colonies are light convex with smooth surface, light yellow-colored and uniformly circular with a diameter of approximately 0.8–1.0 mm. Cells can grow on yeast mannitol agar (YMA) medium, but can not grow well. Colonies on YMA agar are circular and white. Growth occurs at 10–37 °C (optimum, 35 °C), pH 6.0–9.0 (optimum, pH 7.5) and with 0–17% (w/v) NaCl (optimum, 1%). Negative for hydrolysis of starch, CM-cellulose, Tweens 20, 40, 60 and 80, but positive for hydrolysis of gelatin. In addition, catalase activity and nitrite reduction are positive while hydrogen sulfide production, methyl red test, Voges-Prokauer test, nitrate reduction and oxidase activity are negative. In API 20NE tests, a positive reaction for hydrolysis of urea and negative reactions for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis. In the API ZYM kit, alkaline phosphatase, C4 esterase, C8 esterase lipase, leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, α-glucosidase activities are present; however, C14 lipase, cysteine arylamidase, chymotrypsin, napthol-AS-Bi-phosphopydrase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and β-fucosidase activities are absent. In utilization of sole carbon source tests, positive for D-glucose, lactose, D-galactose, melibiose, D-ribose, L-sorbin, D-trehalose, D-maltose, D-xylose, D-cellobiose, glycerol,inositol, xylitol, mannitol, D-sorbitol, succinate, pyruvate, acetate, malonate, L-valine and negative for D-mannose, D-fructose, D-arabinose, sucrose, ethanol, erythritol, ribitol, α-ketoglutaric acid, citrate, oxalate and formic acid. Acid can be produced from D-maltose, D-xylose, D-mannose, D-fructose and D-trehalose; weakly produced from sucrose and mannitol, but not produced from D-galactose, D-sorbitol, D-glucose, D-arabinose, α-lactose, inositol and glycerol. The isoprenoid quinone is Q-10. The predominant cellular fatty acids are summed feature 8 ($C_{18:1}$ ω7c/ω6c) and 11-methyl $C_{18:1}$ ω7c. The polar lipids include seven phospholipids, two aminolipids, one glycolipid and three unidentified lipids.

The type strain is XC0140^T (= MCCC 1K03228^T = KCTC 52582^T), which was isolated from a soil sample near the sewage treatment tank of a chemical factory in Zhejiang Province, China. The DNA G+C content of the type strain is 62.7 mol%.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant No. 31470005).

Compliance with ethical standards

Conflict of Interest Authors declare that there is no conflict of interest.

References

Aziz RK, Bartels D, Best AA, Dejongh M, Disz T et al (2008) The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J et al (2009) BLAST+: architecture and applications. BMC Bioinformatics 10:421

Claus D (1992) A standardized Gram staining procedure. World J Microb Biot 8:451–452

Elliott GN, Chen WM, Bontemps C, Chou JH, Young JPW et al (2007) Nodulation of *Cyclopia* spp. (Leguminosae, Papilionoideae) by Burkholderia tuberum. Ann Bot 100:1403–1411

Frank B (1889) Ueber die Pilzsymbiose der Leguminosen. Berichte der Deutschen Botanischen Gesellschaft 7:332–346

Kates M (1986) Techniques of lipidology, 2nd edn. Elsevier, Amsterdam

Komagata K, Suzuki K (1988) Lipids and cell-wall analysis in bacterial systematics. Methods Microbiol 19:161–207

Kovacs N (1956) Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature 178:703

Laguerre G, Nour SM, Macheret V, Sanjuan J, Drouin P et al (2001) Classification of rhizobia based on *nod*C and *nif*H gene analysis reveals a close phylogenetic relationship among *Phaseolus vulgaris* symbionts. Microbiology 147:981–993

Leifson E (1963) Determination of carbohydrate metabolism of marine bacteria. J Bacteriol 85:1183–1184

Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G+C content of deoxyribonucleic-acid by high-performance liquid-chromatography. Int J Syst Bacteriol 39:159–167

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M et al (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241

Mousavi SA, Willems A, Nesme X, de Lajudie P, Lindström K (2015) Revised phylogeny of Rhizobiaceae: proposal of the delineation of Pararhizobium gen. nov., and 13 new species combinations. Syst Appl Microbiol 38:84–90

Qin W, Deng ZS, Xu L, Wang NN, Wei GH (2012) *Rhizobium helanshanense* sp. nov., a bacterium that nodulates *Sphaerophysa salsula* (Pall.) DC. in China. Arch Microbiol 194:371–378

Sheu SY, Hsing HW, Young CC, Chen WM (2015) *Rhizobium alvei* sp. nov., isolated from a freshwater river. Int J Syst Evol Microbiol 65:472–478

Sun C, Pan J, Zhang XQ, Su Y, Wu M (2015) *Pseudoroseovarius zhejiangensis* gen. nov., sp nov., a novel *alphaproteobacterium* isolated from the chemical wastewater, and reclassification of *Roseovarius crassostreae* as *Pseudoroseovarius crassostreae* comb. nov., *Roseovarius sediminilitoris* as *Pseudoroseovarius sediminilitoris* comb. nov and *Roseovarius halocynthiae* as *Pseudoroseovarius halocynthiae* comb. nov. Antonie van Leeuwenhoe 108:291–299

Tighe SW, de Lajudie P, Dipietro K, Lindström K, Nick G et al (2000) Analysis of cellular fatty acids and phenotypic relationships of *Agrobacterium, Bradyrhizobium, Mesorhizobium, Rhizobium* and *Sinorhizobium* species using the Sherlock Microbial Identification System. Int J Syst Evol Microbiol 50:787–801

Tindall BJ (1990) Lipid composition of *Halobacterium lacusprofundi*. FEMS Microbiol Lett 66:199–202

Wu YH, Zheng G, Zhang WW, Xu XW, Wu M et al (2010) *Amphibacillus jilinensis* sp. nov., a facultatively anaerobic, alkaliphilic bacillus from a soda lake. Int J Syst Evol Microbiol 60:2540–2543

Xu XW, Wu YH, Wang CS, Yang JY, Oren A et al (2008) *Marinobacter pelagius* sp. nov., a moderately halophilic bacterium. Int J Syst Evol Microbiol 58:637–640

Xu L, Shi JF, Zhao P, Chen WM, Qin W et al (2011) *Rhizobium sphaerophysae* sp. nov., a novel species isolated from root nodules of *Sphaerophysa salsula* in China. Antonie van Leeuwenhoe 99:845–854

Young JM, Kuykendall LD, Martínez-Romero E, Kerr A, Sawada HA et al (2001) Revision of Rhizobium Frank 1889, with an emended description of the genus, and the inclusion of all species of Agrobacterium Conn 1942 and Allorhizobium undicola de Lajudie, 1998 as new combinations: *Rhizobium radiobacter, R. rhizogenes, R. rubi, R. undicola and R. vitis*. Int J Syst Evol Microbiol 51:89–103

Zhang WY, Huo YY, Zhang XQ, Zhu XF, Wu M (2013) *Halolamina salifodinae* sp. nov. and *Halolamina salina* sp. nov., two extremely halophilic archaea isolated from a salt mine. Int J Syst Evol Microbiol 63:4380–4385

Zhang XQ, Sun C, Wang CS, Zhang X, Zhou X et al (2015) *Sinimarinibacterium flocculans* gen. nov., sp. nov., a gammaproteobacterium from offshore surface seawater. Int J Syst Evol Microbiol 65:3541–3546