

## *Egibacter rhizosphaerae* gen. nov., sp. nov., an obligately halophilic, facultatively alkaliphilic actinobacterium and proposal of *Egibacteraceae* fam. nov. and *Egibacterales* ord. nov.

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A novel obligately halophilic, facultatively alkaliphilic actinobacterium, designated EGI 80759<sup>T</sup>, was isolated from the rhizosphere of *Tamarix hispida* Willd, Karamay, Xinjiang province, north-west China. Cells of strain EGI 80759<sup>T</sup> were Gram-stain-positive, non-motile and non-endospore-forming rods. Strain EGI 80759<sup>T</sup> showed obligately halophilic growth with a tolerance to 8–25 % (w/v) NaCl (optimum growth at 10–12 %, w/v) and facultatively alkaliphilic growth within the pH range 7.0–11.0 (optimum growth at pH 9.0–10.0). Cell-wall hydrolysates of the isolate contained meso-diaminopimelic acid (peptidoglycan type A1<sub>7</sub>), with glucose, glucosamine, ribose and mannose as the major sugars. The major fatty acids identified were 10-methyl-C<sub>17:0</sub>, C<sub>17:1</sub>ω8c and C<sub>17:0</sub>. The predominant menaquinone was MK-9(H<sub>4</sub>). The G + C content of the genomic DNA was 72.1 mol%. Phylogenetic analysis, based on 16S rRNA gene sequences, revealed that strain EGI 80759<sup>T</sup> clustered with members of the class *Nitriliruptoria* and showed highest 16S rRNA gene sequence similarities with *Euzebya tangerina* F10<sup>T</sup> (90.3 %) and *Nitriliruptor alkaliphilus* ANL-iso2<sup>T</sup> (88.1 %). On the basis of the data obtained from phenotypic and chemotaxonomic studies and the phylogenetic analysis, the isolate is proposed to be a representative of a novel genus and a novel species, *Egibacter rhizosphaerae* gen. nov., sp. nov., of a proposed novel family, *Egibacteraceae* fam. nov., and order, *Egibacterales* ord. nov., within the class *Nitriliruptoria*. The type strain of the type species, *Egibacter rhizosphaerae*, is EGI 80759<sup>T</sup> (=CGMCC 1.14997<sup>T</sup>=KCTC 39588<sup>T</sup>).

The class *Actinobacteria* was first proposed by Stackebrandt *et al.* (1997), based on phylogenetic and signature nucleotide analysis of 16S rRNA gene sequences, and subsequently emended by Hugenholtz & Stackebrandt (2004), Zhi *et al.* (2009) and Kurahashi *et al.* (2010). With the aims of keeping consistency with the rank of other prokaryotes and developing a unified classification across all bacteria and archaea, the class *Actinobacteria* containing five subclasses (Goodfellow &

Fiedler, 2010) was elevated to the rank of phylum *Actinobacteria* phyl. nov., which includes the classes *Actinobacteria*, *Acidimicrobiia*, *Coriobacteriia*, *Nitriliruptoria*, *Rubrobacteria* and *Thermoleophilia* (Ludwig *et al.*, 2012). At the time of writing, the class *Nitriliruptoria* comprises two orders, *Euzebyales* (Kurahashi *et al.*, 2010) and *Nitriliruptorales* (Sorokin *et al.*, 2009), and two species with validly published names, *Nitriliruptor alkaliphilus* (Sorokin *et al.*, 2009) and *Euzebya tangerina* (Kurahashi *et al.*, 2010). *N. alkaliphilus* was isolated from soda lake sediments of the Kulunda steppe (Sorokin *et al.*, 2009), while *E. tangerina* was from the abdominal epidermis of a sea cucumber, *Holothuria edulis* (Kurahashi *et al.*, 2010). The class *Nitriliruptoria* has a

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EGI 80759<sup>T</sup> is KR605111.

One supplementary figure and two supplementary tables are available with the online Supplementary Material.

distinct phyletic lineage and signature nucleotides of 16S rRNA gene sequence, as well as other characteristics, such as Gram-staining-positive rods and the cell-wall peptidoglycan A1 $\gamma$  type (Sorokin *et al.*, 2009; Kurahashi *et al.*, 2010).

Actinobacteria are distributed ubiquitously in the biosphere, including in extreme environments, such as salt lakes, soda lakes and desert soil (Bull, 2011). These extreme environments are usually made up of more than one extreme condition. For example, salinized soil is commonly found in temperate deserts and is characterized by high levels of salt, solar radiation and/or pH. However, limited knowledge of the physiologies and the adaptive mechanisms to such conditions has resulted in the discovery of less extremophilic actinobacterial taxa compared to other bacteria. Recently, researchers have started formulating new culture methods to cultivate more novel taxa (Tang *et al.*, 2008; Pham & Kim, 2012). Here, we report on the isolation of a novel halo-alkaliphilic actinobacterium, designated EGI 80759<sup>T</sup>, from a rhizospheric soil sample of *Tamarix hispida* Willd that formed a deep phyletic lineage within the class *Nitriliruptoria*. Based on the results of the polyphasic characterizations, strain EGI 80759<sup>T</sup> was characterized as a novel taxon within the class *Nitriliruptoria* of the phylum *Actinobacteria* for which the name *Egibacter rhizosphaerae*, gen. nov., sp. nov. is proposed; there is also a proposal for a new family, *Egibacteraceae* fam. nov., and a new order, *Egibacterales* ord. nov.

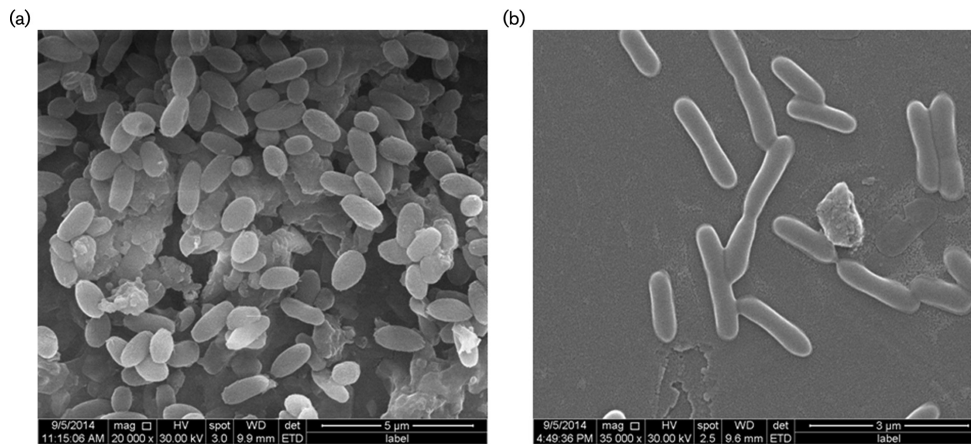
The soil sample (pH 8.7; total salts, 5.3 %, g kg<sup>-1</sup>) used in the current study was collected from the rhizosphere of *Tamarix hispida* Willd grown at Karamay, Xinjiang province, north-west China during July 2013, and stored aseptically in a refrigerator at 4 °C until processed for isolation. The soil sample was diluted to a concentration of 10<sup>-3</sup> and 10<sup>-4</sup> (w/v) with sterile distilled water, and 0.1 ml of each were spread onto modified R<sub>2</sub>A agar (Reasoner & Geldreich, 1985) plates supplemented with 10 % NaCl (w/v) and the pH was adjusted to 10.0. Colonies appearing after 28 days incubation at 30 °C were picked, purified, cultivated and maintained on the same medium. These strains were also preserved as 20 % (v/v) glycerol/10 % (w/v) NaCl suspensions at -80 °C. Among them, a pale yellow-pink, extremely halophilic and facultatively alkaliphilic strain, designated EGI 80759<sup>T</sup>, was selected for further characterization. Biomass for chemical and molecular studies was obtained by cultivating in modified R<sub>2</sub>A broth under shaking conditions (~150 r.p.m., 30 °C, 21 days) unless otherwise stated. All media used for biochemical and physiological studies were supplemented with 10 % NaCl (w/v) and pH was adjusted to 10 with NaOH, unless stated otherwise.

Gram staining was carried out using the standard Gram reaction (Gram, 1884). Colony characteristics were determined by growing the strain on the media described by Shirling & Gottlieb (1966) at 30 °C for 28 days. The colours of colonies were determined with ISCC-NBS colour charts (Kelly, 1964). Morphological characteristics of strain EGI 80759<sup>T</sup> were observed by light microscopy (Olympus; BH-2) and scanning electron

microscopy (Quanta 200; FEI) after incubation on modified R<sub>2</sub>A agar at 30 °C for 14–28 days. Strain EGI 80759<sup>T</sup> grew well on potato-glucose agar (PDA) and R<sub>2</sub>A agar, moderately on oatmeal agar (ISP 3), glycerol-asparagine agar (ISP 5), Czapek's agar and weakly on nutrient agar, but no growth was observed on yeast extract-malt extract agar (ISP 2), inorganic starch agar (ISP 4) or Gause No. 1. The strain formed pale yellow-pink colonies on R<sub>2</sub>A, but strong yellow-pink colonies on PDA plates. Colonies were opaque, wrinkled, unevenly sized (below 3 mm diameter), and had middle humps and irregular edges after 28 days incubation on R<sub>2</sub>A plates at 30 °C. No diffusible pigments were detected in tested media. Details are described in Table S1 (available in the online Supplementary Material). The strain was Gram-stain-positive, non-motile and non-endospore-forming rods (Fig. 1). Non-desalted cells of strain EGI 80759<sup>T</sup> were more elliptical and mature grape-shaped (Fig. 1a), while desalted cells were rod-shaped (Fig. 1b). The variation in cell morphology in the presence of extracellular salt may relate to the adaptive mechanism of strain EGI 80759<sup>T</sup>. Cell sizes ranged between 0.3–0.4  $\mu$ m in diameter and 1.0–1.3  $\mu$ m in length.

Growth at different temperatures (5–60 °C, at intervals of 5 °C) was examined by growing the strain on R<sub>2</sub>A agar modified by the addition of 10 % (w/v) NaCl and adjusting the pH to 10.0 with autoclaved 10M NaOH. Salt tolerance was determined on R<sub>2</sub>A agar modified with the addition of various concentrations of NaCl (0–20 %, at intervals of 1 %; 22, 24, 25, 26, 28, 30 %, w/v) and adjusting the pH to 10.0 with autoclaved 10M NaOH. The pH range for growth was tested between 4.0 and 12.0, at intervals of 1.0 pH unit, on R<sub>2</sub>A broth supplemented with 10 % (w/v) NaCl by using the buffer system described by Xu *et al.* (2005). Sole carbon and nitrogen-source utilization tests were performed by the methods described by Shirling & Gottlieb (1966) and Williams *et al.* (1983), respectively. Catalase activity was determined from the production of gas bubbles on the addition of a drop of 3 % (v/v) H<sub>2</sub>O<sub>2</sub>. Other physiological and biochemical characteristics were examined as described previously (Goodfellow, 1971; Williams *et al.*, 1983). Strain EGI 80759<sup>T</sup> could grow at 25–50 °C, pH 7.0–11.0 and 8.0–25.0 % (w/v) NaCl. Optimal growth occurred at 30 °C, pH 9.0–10.0 and 10–12.0 % (w/v) NaCl. Other physiological characteristics of strain EGI 80759<sup>T</sup> are given in Table 1 and the species description below.

Diaminopimelic acid isomers in cell-wall hydrolysates were analysed by TLC as described by Stanek & Roberts (1974). A purified cell-wall preparation was obtained and hydrolysed as described by Schleifer & Kandler (1972) and analysed according to the method of Tang *et al.* (2009). Whole-cell sugars were detected according to the method used by Tang *et al.* (2009). Polar lipids were extracted and identified by two-dimensional TLC following the method of Minnikin *et al.* (1984). Menaquinones were extracted and purified according to Collins *et al.* (1977) and were analysed by HPLC (Groth *et al.*, 1996). For fatty acid analysis, strain EGI 80759<sup>T</sup> was cultured on modified TSB medium (supplemented with 10 % (w/v) NaCl) at 30 °C and pH 10.0



**Fig. 1.** Scanning electron micrographs of strain EGI 80759<sup>T</sup> grown on R<sub>2</sub>A agar supplemented with 10 % (w/v) NaCl with the pH adjusted to 10 with NaOH for 28 days at 30 °C. Cell morphology of: a, natural cells prepared by the embedded slide culture method; b, after cultures were spread on the slides and treated by serial desalination steps (40 %, 70 %, 90 % and 100 %, v/v, absolute ethyl alcohol in sterilized deionized water) Bar, a, 5 µm; b, 3 µm).

for 21 days. Cellular fatty acid analysis was performed as described by Sasser (1990) according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System. The genomic DNA of strain EGI 80759<sup>T</sup> was prepared according to Marmur (1961), and its G + C content was determined by HPLC according to Mesbah *et al.* (1989). The cell-wall peptidoglycan of strain EGI 80759<sup>T</sup> contained *meso*-diaminopimelic acid, alanine and glutamic acid as major amino acids, which indicated the presence of the A1 $\gamma$  type peptidoglycan (Schleifer & Kandler, 1972; Sorokin *et al.*, 2009; Kurahashi *et al.*, 2010). Strain EGI 80759<sup>T</sup> contained glucose, glucosamine, ribose, mannose and one unknown sugar as the major cell-wall sugars. The

predominant menaquinone detected was MK-9(H<sub>4</sub>), while the polar lipids were diphosphatidylglycerol, one unknown phosphoglycolipid, six unknown phospholipids, two unknown glycolipids and five unknown polar lipids (Fig. S1). Strain EGI 80759<sup>T</sup> had a cellular fatty acid profile containing major amounts of branched fatty acids, unsaturated straight-chain fatty acids and minor amounts of saturated straight-chain fatty acids: 10-methyl-C<sub>17:0</sub> (32.0 %), C<sub>17:1</sub> $\omega$ 8c (22.1 %), C<sub>17:0</sub> (11.1 %), C<sub>16:0</sub> (7.9 %), summed feature 9 (iso-C<sub>17:1</sub> $\omega$ 9c or 10-methyl-C<sub>16:0</sub>) (4.7 %), iso-C<sub>16:0</sub> (4.2 %), C<sub>17:1</sub> $\omega$ 6c (3.7 %), summed feature 3 (C<sub>16:1</sub> $\omega$ 7c/C<sub>16:1</sub> $\omega$ 6c, or C<sub>16:1</sub> $\omega$ 6c/C<sub>16:1</sub> $\omega$ 7c) (2.0 %), C<sub>15:1</sub> $\omega$ 8c (1.7 %), C<sub>16:1</sub> $\omega$ 9c (1.4 %), iso-C<sub>17:0</sub> (2.0 %).

**Table 1.** Phenotypic and chemotypic features that distinguish strain EGI 80759<sup>T</sup> from its closest phylogenetic neighbours *E. tangerina* F10<sup>T</sup> and *N. alkaliphilus* ANL-iso2<sup>T</sup>

Taxa: 1, strain EGI 80759<sup>T</sup>; 2, *E. tangerina* F10<sup>T</sup>; 3, *N. alkaliphilus* ANL-iso2<sup>T</sup>. Cells of all strains are rods. All strains can utilize L-arabinose, myo-inositol, D-mannose and sucrose as carbon sources, and have the same cell-wall peptidoglycan type (A1 $\gamma$ ) and MK-9(H<sub>4</sub>) as predominant menaquinone. ND, No data; Gal, galactose; Glc, glucose; GlcN, glucosamine; Man, mannose; Rha, rhamnose; Rib, ribose; US, unknown sugar; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PGL, phosphoglycolipid; PL, unknown phospholipid.

Characteristic	1*	2†	3‡
Cell size (µm)	0.3–0.4 × 1.0–1.3	0.6–0.8 × 1.5–6.0	0.4 × 1.5–3.0
Growth at pH range (optimum)	7–11 (9–10)	7–9 (ND)	8.2–10.6 (9.0–9.50)
NaCl concn range for growth (% w/v), (optimum)	8–25 (10–12)	0.5–12 (ND)	0.1–11.6 (1.2–1.7)
Temperature range (°C) (optimum)	25–50 (30–35)	15–35 (20–28)	Mesophilic (32)
Cell-wall sugars	Glc, GlcN, Rib, Man, US	Rha, Gal	Glc, Gal
Major phospholipids	DPG, PGL, PL	PG	ND
G + C mol%	72.1	68.3	70.8

\*Data from this study.

†Data from Kurahashi *et al.* (2010).

‡Data from Sorokin *et al.* (2009) and Kurahashi *et al.* (2010).

(1.4 %), C<sub>15:1</sub>ω6c (1.3 %), anteiso-C<sub>17:0</sub> (1.2 %), 10-methyl-C<sub>18:0</sub>, TBSA (1.0 %), iso-C<sub>16:1</sub> H (1.0 %), C<sub>18:0</sub> (0.6 %), iso-C<sub>15:0</sub> (0.5 %), C<sub>18:1</sub>ω9c (0.5 %), summed feature 8 (C<sub>18:1</sub>ω7c or C<sub>18:1</sub>ω6c) (0.6 %), C<sub>18:3</sub>ω6c (6, 9, 12) (0.4 %), C<sub>14:0</sub> (0.4 %), summed feature 5 (C<sub>18:2</sub>ω6,9c/anteiso-C<sub>18:0</sub> or anteiso-C<sub>18:0</sub>/C<sub>18:2</sub>ω6,9c (0.4 %) and anteiso-C<sub>15:0</sub> (0.2 %). The genomic DNA G + C content of strain EGI 80759<sup>T</sup> was 72.1 mol%.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were carried out as described by Li *et al.* (2007). Multiple alignments with sequences of representative type species of the phylum *Actinobacteria* and calculations of levels of sequence similarity were carried out using the EzTaxon-e server (<http://eztaxon-e.ezbiocloud.net/>; Kim *et al.*, 2012). Phylogenetic analysis was performed using three tree-making algorithms: the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods using MEGA 6 software (Tamura *et al.*, 2013). The topologies of the resultant trees were evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

An almost complete 16S rRNA gene sequence (1517 bp) was determined for strain EGI 80759<sup>T</sup>. Phylogenetic analysis, based on the 16S rRNA gene sequences, revealed that the strain clustered with members of the class *Nitriliruptoria*. The 16S rRNA gene sequence similarities between the novel isolate, EGI 80759<sup>T</sup>, and strains *E. tangerina* F10<sup>T</sup> and *N. alkaliphilus* ANL-iso2<sup>T</sup> were 90.3 % and 88.1 %, respectively. In the phylogenetic tree based on the neighbour-joining algorithm, strains EGI 80759<sup>T</sup> and *E. tangerina* F10<sup>T</sup> formed a single clade with a high bootstrap value (95 %), while *N. alkaliphilus* ANL-iso2<sup>T</sup> formed another clade (Fig. 2). The relationship was also supported by the other tree-making methods used in this study (Fig. 2).

The data above confirm that the novel isolate should be assigned to the class *Nitriliruptoria*. Although strain EGI 80759<sup>T</sup> exhibited similar characteristics to *E. tangerina* F10<sup>T</sup> and *N. alkaliphilus* ANL-iso2<sup>T</sup> in having rod-shaped morphology, a type (A1γ) cell-wall peptidoglycan, MK-9(H<sub>4</sub>) as the major menaquinone, C<sub>17:1</sub>ω8c as one major fatty acid and a separate phyletic lineage, it had some features distinguishing it from *E. tangerina* F10<sup>T</sup> and *N. alkaliphilus* ANL-iso2<sup>T</sup> (Tables 1 and S2). Despite showing the highest 16S rRNA gene sequence similarity to *E. tangerina* F10<sup>T</sup>, strain EGI 80759<sup>T</sup> differed to *E. tangerina* on the basis of several characteristics: 1) cell size (0.3–0.4 × 1.0–1.3 μm) was smaller than that of *E. tangerina* F10<sup>T</sup>; 2) obligately halophilic (8–25 %, NaCl, w/v) and facultatively alkaliphilic (pH 7–11) growth; a wider growth temperature range (22–50 °C); 3) cell-wall sugars were glucose, glucosamine, ribose and mannose for strain EGI 80759<sup>T</sup>, while they were rhamnose and galactose for *E. tangerina* F10<sup>T</sup>; saturated fatty acids for strain EGI 80759<sup>T</sup> (Table S2) but non-saturated fatty acids for *E. tangerina* F10<sup>T</sup> (Kurahashi *et al.*, 2010) as major fatty acid composition; diphosphatidylglycerol, one unknown phosphoglycolipid and one unknown glycolipid as major polar lipids for strain EGI 80759<sup>T</sup>, while

only phosphatidylglycerol for strain *E. tangerina* F10<sup>T</sup>. Strain EGI 80759<sup>T</sup> was negative for the utilization of glucose as the sole carbon source and for the production of H<sub>2</sub>S, unlike *E. tangerina* F10<sup>T</sup> (Kurahashi *et al.*, 2010). Furthermore, strain EGI 80759<sup>T</sup> has some signature nucleotides of the 16S rRNA gene sequence (see the family description) that are distinct to those of *E. tangerina* F10<sup>T</sup> (Kurahashi *et al.*, 2010). These comparative features along with its distinct morphological and chemical characteristics indicate that strain EGI 80759<sup>T</sup> does not belong to the order *Euzebyales*.

Unlike *N. alkaliphilus* ANL-iso2<sup>T</sup>, strain EGI 80759<sup>T</sup> is an obligate halophile that grows only at a higher concentration of NaCl. 10-Methyl-C<sub>17:0</sub> is one of the major fatty acids of strain EGI 80759<sup>T</sup>, comprising 32.0 % of the total, but not of *N. alkaliphilus* ANL-iso2<sup>T</sup> (Kurahashi *et al.*, 2010). The phylogenetic relationship (Fig. 2) and signature nucleotides of 16S rRNA gene sequences (see the family description) between strains EGI 80759<sup>T</sup> and *N. alkaliphilus* ANL-iso2<sup>T</sup> (Sorokin *et al.*, 2009) also differs greatly, thereby indicating that strain EGI 80759<sup>T</sup> does not fall into the order *Nitriliruptoriales*. In conclusion, strain EGI 80759<sup>T</sup> does not belong to any order of the class *Nitriliruptoria*.

Higher hierarchical taxa in the class *Actinobacteria* should be based mainly on 16S rRNA gene signature nucleotide patterns and phylogenetic criteria (Stackebrandt *et al.*, 1997; Zhi *et al.*, 2009). Based on the phenotypic and phylogenetic data, we concluded that strain EGI 80759<sup>T</sup> represents a novel species in a novel genus, *Egibacter rhizosphaerae*, gen. nov., sp. nov. In addition, a new lineage, based on the distinct phylogenetic position of *Egibacter rhizosphaerae* gen. nov., sp. nov., with the novel order *Egibacterales* ord. nov. and a novel family *Egibacteraceae* fam. nov. is proposed within the class *Nitriliruptoria*.

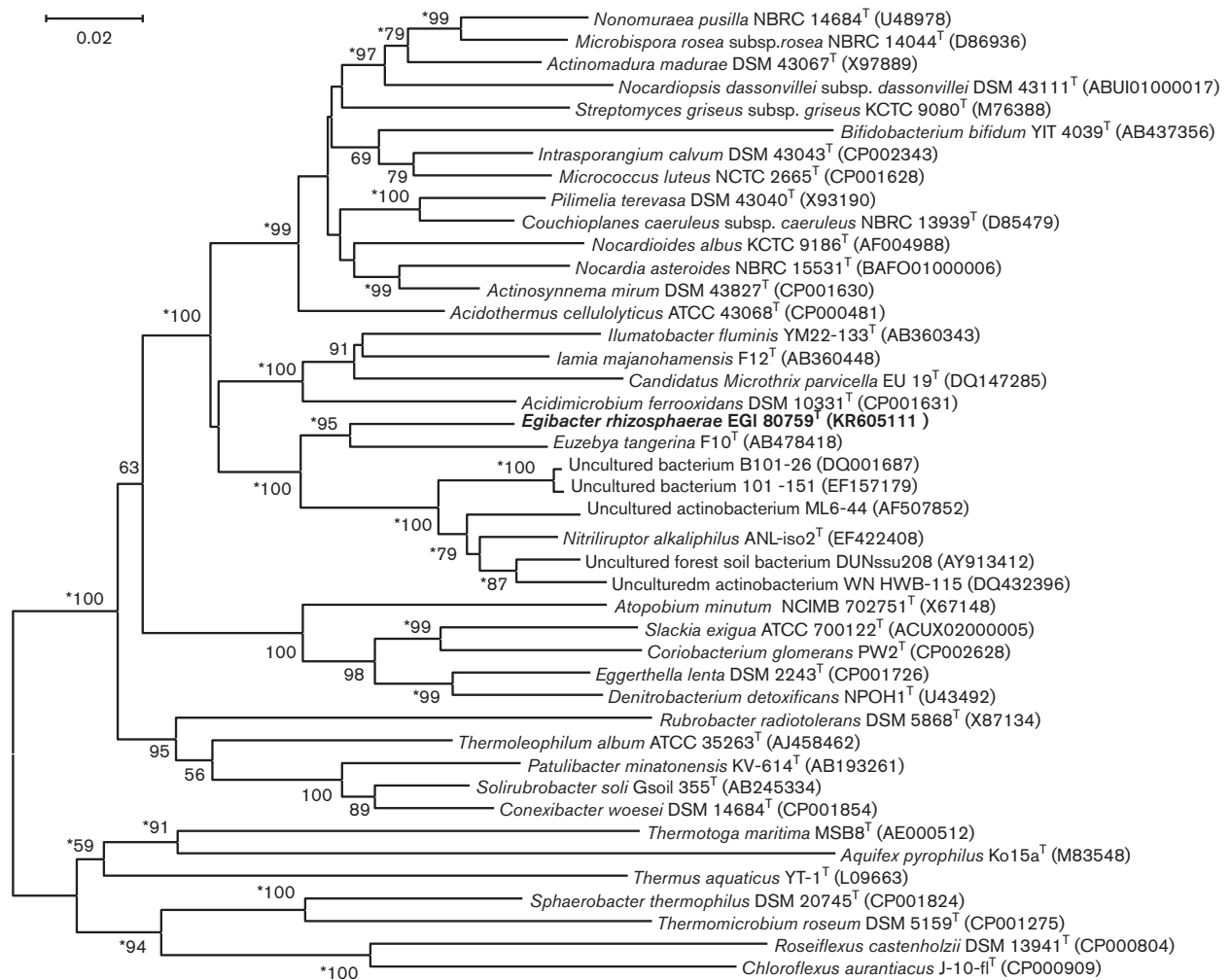
### Description of *Egibacter* gen. nov.

*Egibacter* (E.gi.bac'ter N.L. masc. n. bacter, rod; N.L. masc. n. *Egibacter* arbitrary name formed from the acronym of Institute of Ecology and Geography, EGI, where the first taxonomic studies of this taxon were performed).

Gram-stain-positive, non-motile, non-endospore-forming rods. Aerobic and chemo-organotrophic. Catalase-positive. Obligately halophilic and facultatively alkaliphilic. The menaquinone pattern is MK-9(H<sub>4</sub>). Cell wall contains meso-diaminopimelic acid as the diagnostic diamino acid. The peptidoglycan type is A1γ. Glucose, glucosamine, ribose, mannose and one unknown sugar are detected as cell-wall sugars. The major fatty acids are 10-methyl-C<sub>17:0</sub>, C<sub>17:1</sub>ω8c and C<sub>17:0</sub>. Contains diphosphatidylglycerol, one unknown phospholipid and one phosphoglycolipid as major polar lipids. The type species is *Egibacter rhizosphaerae*.

### Description of *Egibacter rhizosphaerae* sp. nov.

*Egibacter rhizosphaerae* (rhi.zo.sphae'rae. N.L. gen. n. *rhizosphaerae* of the rhizosphere).



**Fig. 2.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain EGI 80759<sup>T</sup>. Bootstrap values (expressed as percentages of 1000 replications) of above 50 % are shown at the branch nodes. Asterisks indicate that the clades were conserved when maximum-parsimony and maximum-likelihood methods were used to reconstruct the phylogenetic trees. Bar, 0.02 sequence divergence.

In addition to the properties given for the genus, cells are approximately  $0.3\text{--}0.4 \times 1.0\text{--}1.3 \mu\text{m}$ . Colonies are pale yellow-pink, opaque and uneven in size, and have wrinkles, middle humps and irregular edges after 28 days incubation on R<sub>2</sub>A plates at 30 °C. Growth occurs at 25–50 °C, pH 7.0–11.0 and with 8–25.0 % (w/v) NaCl. Optimal growth occurs at 30–35 °C, pH 9.0–10.0 and with 10–12.0 % (w/v) NaCl. Grows well on R<sub>2</sub>A agar and PDA. Utilizes D-arabinose, L-arabinose, cellobiose, dulcitol, D-fructose, D-galactose, glycerol, *myo*-inositol, lactose, maltose, D-mannitol, D-mannose, L-rhamnose, D-ribose, D-sorbitol, sucrose, trehalose, D-xylose, D-xylitol, sodium acetate and sodium citrate as the sole carbon source for growth, but not D-glucose. Utilizes L-arginine, L-aspartic acid, L-histidine, L-leucine, L-isoleucine, L-phenylalanine,

L-tryptophan, L-valine, L-serine and L-hyperxanthine as the sole nitrogen source for growth, but not L-alanine, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-lysine, L-methionine, L-threonine, L-tyrosine or L-proline. Positive for the hydrolysis of Tweens 40 and 80, nitrate reduction, catalase, coagulation and peptonization of skimmed milk, while negative for the hydrolysis of Tweens 20 and 60, casein, cellulose, starch and tryptophan, and H<sub>2</sub>S production. The polar lipids are diphosphatidylglycerol, one unknown phosphoglycolipid, two unknown glycolipids, six unknown phospholipids and five unknown polar lipids. The major fatty acids are 10-methyl-C<sub>17:0</sub>, C<sub>17:1</sub>ω8c and C<sub>17:0</sub>.

The type strain is EGI 80759<sup>T</sup> (=CGMCC 1.14997<sup>T</sup>=KCTC 39588<sup>T</sup>), which was isolated from the rhizosphere soil sample of *Tamarix hispida* Willd located in Karamay,

Xinjiang province, north-west China. The G + C content of the genomic DNA is of the type strain is 72.1 mol%.

### Description of *Egibacteraceae* fam. nov.

*Egibacteraceae* (E.gi.bac.te.ra.ce'ae. N. L. masc. n. *Egibacter* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Egibacteraceae* the family of the genus *Egibacter*).

The description is the same as that for the genus *Egibacter*. Segregation of these organisms into a novel family is justified by their distinct phyletic lineage based on the 16S rRNA gene sequence. The pattern of 16S rRNA gene sequence signature nucleotides of members of the family comprises 47 : 396 (C-G), 232 (C), 241 : 285 (C-G), 294 : 303 (C-G), 361 (G), 845 (A), 1038 (C), 1031 (U).

### Description of *Egibacterales* ord. nov.

*Egibacterales* (E.gi.bac.te.ra'les. N.L. masc. n. *Egibacter* type genus of the family; *-ales*, ending to denote an order; N.L. fem. pl. n. *Egibacterales* the order of the genus *Egibacter*).

The description is the same as that for the genus *Egibacter*. Separation of these organisms into a novel order is justified by their distinct phyletic lineage based on the 16S rRNA gene sequence. The pattern of 16S rRNA gene sequence signature nucleotides of members of the order is as for the family *Egibacteraceae*. The order contains the family *Egibacteraceae*. *Egibacter rhizosphaerae* is the type genus.

### Acknowledgements

The authors are grateful to Dr Nimaichand Salam for his kind help to polish the manuscript. This research was supported by the joint fund of the National Natural Science Foundation of China (NSFC) and Xinjiang Province (U1403101) and the West Light Foundation of the Chinese Academy of Sciences (RCPY201203). W.-J. L. was also supported by the 'Hundred Talents Program' of the Chinese Academy of Sciences and Guangdong Province Higher Vocational Colleges & Schools Pearl River Scholar Funded Scheme (2014).

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