Egicoccus halophilus gen. nov., sp. nov., a halophilic, alkalitolerant actinobacterium and proposal of *Egicoccaceae* fam. nov. and *Egicoccales* ord. nov.

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A novel Gram-stain-positive, non-motile, moderately halophilic and alkalitolerant actinobacterium, designated EGI 80432^T, was isolated from a saline–alkaline soil of Xinjiang province, north-west China. Cells were non-endospore-forming cocci with a diameter of 0.5–0.8 μm. Strain EGI 80432^T grew in the presence of 0–9 % (w/v) NaCl (optimum at 3–5 %), and also grew within the pH range 6.0–10.0 (optimum at pH 8.0–9.0) on marine 2216E medium. The peptidoglycan type was A1γ. The whole-cell hydrolysates contained glucose, galactose, mannose and three unknown sugars as major sugars. The predominant menaquinone was MK-9(H_4). The major fatty acids were $C_{17:1}\omega8c$, summed feature 3 ($C_{16:1}\omega7c$/$C_{16:1}\omega6c$), $C_{18:1}\omega9c$ and iso-$C_{15:0}$ The polar lipids comprised diphosphatidylglycerol, phosphatidylglycerol, one unknown phosphoglycolipid, three unknown phospholipids and four unknown polar lipids. The genomic DNA G+C content was 75.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain EGI 80432^T clustered within the radius of the class *Nitriliruptoria*. Levels of sequence similarity between strain EGI 80432^T and its phylogenetic neighbours *Nitriliruptor alkaliphilus* ANL-iso2^T and *Euzebya tangerina* F10^T were 94.1 and 88.1 %, respectively. Based on morphological, physiological and chemotaxonomic characteristics and phylogenetic analysis, a novel species of a new genus, *Egicoccus halophilus* gen. nov., sp. nov., is proposed, within the new family and new order *Egicoccaceae* fam. nov. and *Egicoccales* ord. nov. in the class *Nitriliruptoria*. The type strain of *Egicoccus halophilus* is EGI 80432^T (=CGMCC 1.14988^T=KCTC 33612^T).

The class *Nitriliruptoria* was originally proposed by Kurahashi *et al.* (2010) as the subclass *Nitriliruptoridae* based on its distinct phyletic lineage and signature nucleotides of the 16S rRNA gene, and it was later elevated to the rank of the class (Ludwig *et al.*, 2012). At the time of writing, the class comprises only two orders, *Nitriliruptorales* (Sorokin *et al.*, 2009) and *Euzebyales* (Kurahashi *et al.*, 2010), and two validly published species names, *Nitriliruptor alkaliphilus* (Sorokin *et al.*, 2009) and *Euzebya tangerina* (Kurahashi *et al.*, 2010), which are from a soda

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EGI 80432^T is KR605110.

One supplementary figure and one supplementary table are available with the online Supplementary Material.

lake sediment and a sea cucumber, respectively. The characteristics that distinguish *Nitriliruptoria* from other classes of the phylum '*Actinobacteria*' are its phyletic lineage and signature nucleotides of the 16S rRNA gene sequence, Gram-stain-positive rod-shaped cells and cell-wall peptidoglycan type A1γ (Sorokin *et al.*, 2009; Kurahashi *et al.*, 2010). Beside the orders described above, few taxa have been reported due to limited knowledge on nutrient requirements and the physiology of the class.

Soda deserts are naturally occurring alkaline environments, whose alkalinities are generally caused by the formation of Na_2CO_3 or $NaHCO_3$ (Horikoshi *et al.*, 2011). Diverse prokaryotes have been isolated from these biotopes and characterized because of their specific adaptive mechanisms to the extreme conditions and their potential in biotechnology (Horikoshi, 1999; Horikoshi *et al.*, 2011; Sarethy *et al.*, 2011). Actinobacteria inhabiting alkaline environments are of great interest for their ability to produce alkaline enzymes and pharmaceutical agents (Horikoshi, 1999; Horikoshi *et al.*, 2011; Sarethy *et al.*, 2011). Soda desert containing alkaline soil and saline–alkaline soil is widely distributed in Xinjiang province, north-west China. During a programme investigating the diversity of actinobacteria in unexplored soda desert, a novel halophilic, alkalitolerant strain, designated EGI 80432^T, was isolated that formed a deep phyletic lineage within the class *Nitriliruptoria*. Thus, strain EGI 80432^T was subjected to a polyphasic characterization, and a novel species of a new genus, *Egicoccus halophilus* gen. nov., sp. nov., is proposed. Accordingly, a new family *Egicoccaceae* fam. nov. and a new order *Egicoccales* ord. nov. are proposed.

Saline–alkaline soil was collected in Shihezi district, Xinjiang province, north-west China, during April 2013. The pH and total salts of the sample were 9.1 and 12.0 % (g kg^{-1}), respectively. Two grams of the sample was transferred to an aseptic flask containing 18 ml distilled water and vibrated in a shaker at 150 r.p.m. at 30 °C for 30 min. The soil suspension was then diluted to concentrations of 10^{-3} and 10^{-4} (w/v), and 0.1 ml of each was spread on modified marine agar 2216E with pH adjusted to 10.0 with autoclaved 10 M NaOH. After 4 weeks of incubation at 30 °C, strain EGI 80432^T was picked and maintained on the medium described above except with 3 % (w/v) NaCl added. The isolate was preserved as glycerol suspensions (20 %, w/v) at −80 °C. Biomass for chemical and molecular studies was obtained by cultivation in shake flasks (about 150 r.p.m.) using marine broth 2216E modified with 3 % (w/v) NaCl added and pH adjusted to 9.0 at 30 °C for 2 weeks.

Gram staining was carried out using a standard Gram stain kit. For determination of colony characteristics, strain EGI 80432^T was cultured on marine agar 2216E supplemented with 3 % (w/v) NaCl and pH adjusted to 9.0. Colours of colonies were determined with the ISCC–NBS colour charts (Kelly, 1964). For determination of morphological characteristics, strain EGI 80432^T was inoculated on marine agar 2216E modified as described above at 30 °C for 7–14 days, then observed by light microscopy (BH-2; Olympus) and scanning electron microscopy (Quanta 200; FEI). Growth temperature range (5–60 °C, at intervals of 5 °C) was tested by growing the strain on marine agar 2216E supplemented with 3 % (w/v) NaCl and pH adjusted to 9.0 with NaOH. Salt tolerance was tested on 2216E agar supplemented with various concentrations of NaCl (0–20 %, w/v, at intervals of 1 %) and pH adjusted to 9.0 with NaOH. The pH growth range was tested between 4.0 and 12.0, at intervals of 1.0 pH units, in marine broth 2216E supplemented with 3 % (w/v) NaCl by using the buffer system described by Xu *et al.* (2005). Carbon source utilization tests were performed according to the methods described by Shirling & Gottlieb (1966) and nitrogen source utilization tests were analysed as described by Williams *et al.* (1983). Catalase activity was determined using 3 % H_2O_2, and gas production was identified as a positive reaction. Physiological and biochemical characteristics were examined as described previously (Goodfellow, 1971; Williams *et al.*, 1983). All the media above were supplemented with 3 % (w/v) NaCl and adjusted to pH 9.0 with NaOH except where otherwise described.

Cells of strain EGI 80432^T were Gram-stain-positive, non-motile, non-endospore- forming cocci with a diameter of 0.5–0.8 μm (Fig. 1). Colonies of the isolate were dry, rough and circular with a diameter of <2 mm after 14 days of incubation on marine agar 2216E at 30 °C. Colonies were light yellow, and no diffusible pigments

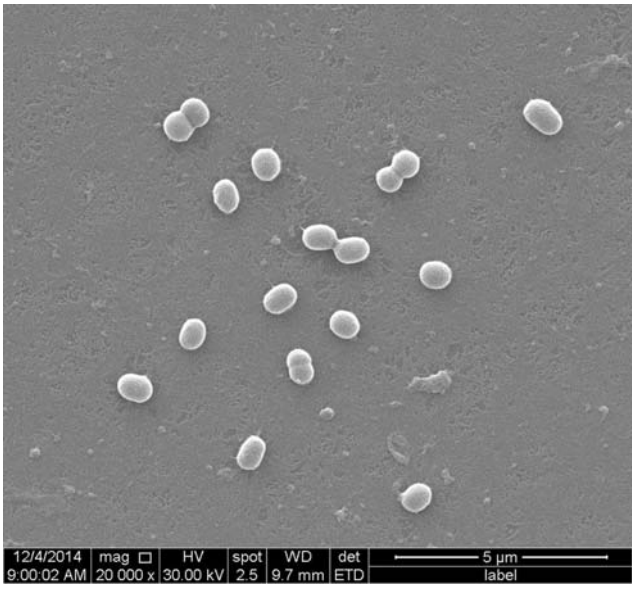

Fig. 1. Scanning electron micrograph of cells of strain EGI 80432^T grown on marine agar 2216E supplemented with 3 % (w/v) NaCl and pH adjusted to 10 with NaOH for 2 weeks at 30 °C. Bar, 5 μm.

were detected when cultured on marine agar 2216E. Strain EGI 80432^T was able to grow at 20–40 °C, with 0–9 % (w/v) NaCl and at pH 7.0–10.0, and optimally at 30 °C, with 3–5 % (w/v) NaCl and at pH 8.0–9.0. Interestingly, the isolate was unable to grow on the medium formulated according to that for marine agar 2216E without NaCl added. These results suggest that the strain is moderately halophilic and alkalitolerant. Other physiological characteristics of strain EGI 80432^T are given in Table 1 and the species description below.

Diaminopimelic acid isomers in whole-cell hydrolysates were analysed by TLC as described by Staneck & 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). Cell-wall sugars were determined 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 prepared according to Collins *et al.* (1977). The purified menaquinones were dissolved in methanol and separated by atmospheric pressure photo-ionization LC-MS. The chromatographic system consisted of an AB SCIEX API 4000+TM LC/MS/MS system and a column oven (ABI). The chromatography and ionization conditions were set as described by Tang *et al.* (2008). For fatty acid analysis, strain EGI 80432^T was cultured in tripticase soy broth medium supplemented with 5 % (w/v) NaCl and pH adjusted to 9.0 with NaOH at 30 °C, and biomass was harvested at the mid-exponential growth phase (4 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. For detection of G+C content, the genomic DNA of EGI 80432^T was prepared according to Marmur (1961), and was determined by the HPLC method (Mesbah *et al.*, 1989).

The cell-wall peptidoglycan of strain EGI 80432^T contained *meso*-diaminopimelic acid, alanine and glutamic acid as major amino acids, which indicated the presence peptidoglycan type A1γ (Schleifer & Kandler, 1972; Sorokin *et al.*, 2009; Kurahashi *et al.*, 2010). Strain EGI 80432^T had a complex cell-wall sugar composition, which contained glucose, galactose, mannose and three unknown sugars as major sugars, and fucose, arabinose, xylose, ribose and rhamnose as minor sugars. The predominant menaquinone detected was MK-9(H_4) (96.3 %), and the minor menaquinone was MK-7 (3.7 %), while the polar lipids were diphosphatidylglycerol, phosphatidylglycerol, one unknown phosphoglycolipid, three unknown phospholipids and four unknown polar lipids (Fig. S1, available in the online Supplementary Material). Strain EGI 80432^T contained unsaturated straight-chain fatty acids as the major cellular fatty acids. The main fatty acids (>10 %) of the isolate were $C_{17:1}\omega8c$, summed feature 3, $C_{18:1}\omega9c$ and iso-$C_{15:0}$. The detailed fatty acid profile is given in Table S1. The genomic DNA G+C content of strain EGI 80432^T was 75.2 mol%.

Genomic DNA extraction and PCR amplification of the 16S rRNA gene were performed as described by Li *et al.* (2007). Multiple alignments with sequences of the type species belonging to the phylum '*Actinobacteria*' and

Table 1. Characteristics that distinguish strain EGI 80432^T from its closest phylogenetic neighbours *N. alkaliphilus* ANL-iso2^T and *E. tangerina* F10^T

Strains: 1, EGI 80432^T; 2, *N. alkaliphilus* ANL-iso2^T; 3, *E. tangerina* F10^T. Data for strain EGI 80432^T are from this study, data for *N. alkaliphilus* ANL-iso2^T are from Sorokin *et al.* (2009) and data for *E. tangerina* F10^T are from Kurahashi *et al.* (2010). All have cell-wall peptidoglycan type A1γ and MK-9(H_4) as the predominant menaquinone. +, Positive, utilized; −, negative, not utilized; ND, no data; Glc, glucose; Gal, galactose; Man, mannose; US, unknown sugar; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PGL, unknown phosphoglycolipid; PL, unknown phospholipid.

Characteristic	1	2	3
Cell morphology	Cocci	Rods	Rods
Cell size (μm)	0.5–0.8	0.4 × 1.5–3.0	0.6–0.8 × 1.5–6.0
Growth:			
pH range	6–10	8.2–10.6	7–9
Optimal pH	8–9	9.0–9.5	7–9
NaCl range (%, w/v)	0–9*	0.1–11.6	0.5–12
Optimal NaCl (%, w/v)	3–5*	1.2–1.7	ND
Temperature range (°C)	20–40	Mesophilic	25
Optimal temperature (°C)	30	32	20–28
Whole-cell sugars	Glc, Gal, Man, three USs	Gal, Glc	Rha, Gal
Major polar lipids	DPG, PGL, PLs	ND	PG
DNA G+C content (mol%)	75.2	70.8	68.3

*Data are from parallel tests on marine agar 2216E with pH adjusted to 9.0 and incubated at 30 °C for 4 weeks.

calculation of levels of sequence similarity were carried out using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012) on the basis of 16S rRNA gene sequence data. Phylogenetic analysis was carried out with three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods, by using the software MEGA 6 (Tamura *et al.*, 2013). The topologies of the resultant trees were evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

An almost-complete 16S rRNA gene sequence (1512 bp) was determined for strain EGI 80432^T. Phylogenetic analysis based on the 16S rRNA gene sequences revealed that the novel strain fell within the radius of the class *Nitriliruptoria*. Levels of sequence similarity between strain EGI 80432^T and *N. alkaliphilus* ANL-iso2^{T}and *E. tangerina* F10^T were 94.1 and 88.1 %, respectively. Sequence similarities between strain EGI 80432^T and members of other classes of the phylum '*Actinobacteria*' were below 88.0 %. In the phylogenetic tree based on the neighbour-joining algorithm, strain EGI 80432^T formed a clade with *N. alkaliphilus* ANL-iso2^T with a high bootstrap value (100 %), while *E. tangerina* F10^T clustered in a separate clade (Fig. 2). This relationship was supported by the other tree-making methods used in this study (Fig. 2).

Based on the phenotypic, chemotaxonomic and phylogenetic data described above, strain EGI 80432^T had characteristics typical of the class *Nitriliruptoria*: peptidoglycan type A1γ, MK-9(H$_4$) as the predominant menaquinone and similar phylogenetic lineage, which suggest that the strain should be assigned to this class. However, strain EGI 80432^T also exhibited features distinct from those of *N. alkaliphilus* ANL-iso2^T and *E. tangerina* F10^T (Tables 1 and S1). Compared with the closest phylogenetic neighbour *N. alkaliphilus* ANL-iso2^T, strain EGI 80432^T had the following distinct phenotypic characteristics: coccus morphology, and halophic and alkalitolerant growth features, while its closest neighbour has a rod morphology, and halotolerant and obligately alkaliphilic growth

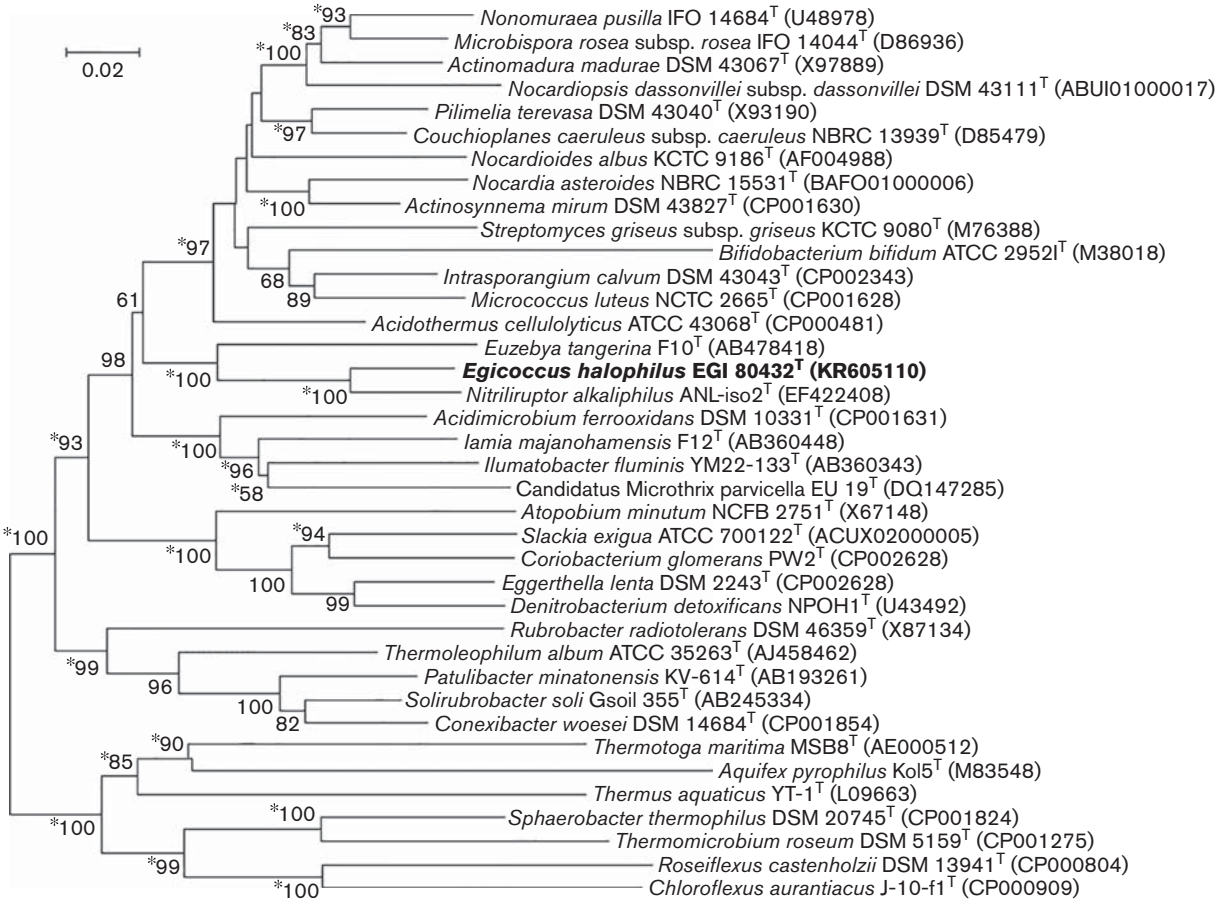

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain EGI 80432^T. Bootstrap values (expressed as percentages of 1000 replications) of above 50 % are shown at branch nodes. Asterisks indicate that the clades were conserved when the maximum-parsimony and maximum-likelihood methods were used to reconstruct the phylogenetic trees. Bar, 2 % sequence divergence.

properties (Sorokin *et al.*, 2009). Furthermore, strain EGI 80432^T differs greatly from *N. alkaliphilus* ANL-iso2^T by the following chemotaxonomic characteristics: (1) whole-cell sugars (glucose, galactose, mannose and three unknown sugars for strain EGI 80432^T, glucose and galactose for *N. alkaliphilus* ANL-iso2^T); and (2) fatty acid profile (Table S1) [$C_{18:1}\omega9c$ and iso-$C_{15:0}$ for strain EGI 80432^T, $C_{16:0}$ and iso-$C_{14:0}$ as major fatty acids (>10 %) for *N. alkaliphilus* NBRC 105792^T], although both have $C_{17:1}\omega8c$ and summed feature 3 as main components (>10 %). The fatty acid profile of *N. alkaliphilus* NBRC 105792^T determined herein differs from previous reports (Sorokin *et al.*, 2009; Kurahashi *et al.*, 2010), which indicates that different media and temperatures for growth greatly influence the fatty acid compositions of this strain. In addition, strain EGI 80432^T had distinct signature nucleotides of the 16S rRNA gene sequence from those of *N. alkaliphilus* ANL-iso2^T and *E. tangerina* F10^T at positions 47 : 396 (U–C) and 841 : 845 (G–A), as given in the description of the family. Clearly, strain EGI 80432^T should not be affiliated to the order *Nitriliruptorales*. In comparison with *E. tangerina* F10^T, the new isolate has a coccus morphology and alkalitolerant growth properties, whereas *E. tangerina* F10^T exhibits a rod morphology and neutrophilc growth properties; furthermore, some chemotaxonomic characteristics differ greatly between strain EGI 80432^T and *E. tangerina* F10^T, such as whole-cell sugars, major polar lipids and genomic DNA G+C content (Table 1). Clearly, strain EGI 80432^T cannot be assigned to the order *Euzebyales*. In conclusion, strain EGI 80432^T does not fall into any order of the class *Nitriliruptoria*.

According to the view that 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) and the distinct phenotypic and chemotaxonomic features of strain EGI 80432^T described above, we conclude that strain EGI 80432^T represents a novel species in a new genus, *Egicoccus halophilus* gen. nov., sp. nov. We also propose the names with a new order *Egicoccales* ord. nov. and a new family *Egicoccaceae* fam. nov., based on the distinct phylogenetic position of *Egicoccus halophilus* gen. nov., sp. nov. within the class *Nitriliruptoria*.

Description of *Egicoccus halophilus* gen. nov.

Egicoccus [E.gi.coc′cus. N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos*) grain, seed; N.L. masc. n. *Egicoccus* arbitrary name formed from the acronym of the Institute of Ecology and Geography, EGI, where the first taxonomic study of this taxon was performed].

Cells are non-motile, Gram-stain-positive and coccus-shaped. Aerobic and chemo-organotrophic. Catalase-positive. The predominant menaquinone is MK-9(H_4). The cell-wall peptidoglycan is type A1γ. Moderately halophilic and alkalitolerant. The type species is *Egicoccus halophilus*.

Description of *Egicoccus halophilus* sp. nov.

Egicoccus halophilus (ha.lo′phi.lus. Gr. n. *hals* salt; Gr. adj. *philos* loving; N.L. masc. adj. *halophilus* salt-loving).

Has the following characteristics in addition to those given for the genus. Cell are non-endospore-forming cocci with a diameter of 0.5–0.8 μm. Colonies are dry, rough and circular when cultured on marine agar 2216E at 30 °C for 14 days. Colonies are light yellow. Growth occurs at 20–40 °C (optimum at 30 °C), at pH 6.0–10.0 (optimum at pH 8.0–9.0) and with 0–9 % (w/v) NaCl (optimum at 3–5 %) on marine 2216E. No growth occurs on marine 2216E medium without NaCl added. Utilizes cellobiose, D-fructose, D-galactose, D-glucose, glycerol, lactose, maltose, D-mannose, melibiose, D-sorbitol, sucrose, trehalose and D-xylitol, but not D-arabinose, L-arabinose, dulcitol, *myo*-inositol, D-mannitol, L-rhamnose, raffinose, D-ribose, D-xylose, sodium acetate or sodium citrate as sole carbon source for growth. L-Arginine, L-glutamic acid, glycine, L-leucine and L-lysine can be utilized as sole nitrogen source for growth, but not L-alanine, L-aspartic acid, L-cysteine, L-glutamine, L-histidine, L-isoleucine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, L-tryptophan, L-valine or L-hypoxanthine. Positive for nitrate reduction and catalase activity, but negative for H_2S production, and coagulation and peptonization of skimmed milk. Cells can hydrolyse cellulose and Tweens 20, 40, 60 and 80, but not casein, starch or tryphtophan. Whole-cell hydrolysates contain glucose, galactose, mannose and three unknown sugars as major sugars. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, one unknown phosphoglycolipid, three unknown phospholipids and four unknown polar lipids. The major fatty acids are $C_{17:1}\omega8c$, summed feature 3, $C_{18:1}\omega9c$ and iso-$C_{15:0}$.

The type strain is EGI 80432^T (=CGMCC 1.14988^T=KCTC 33612^T), isolated from a saline–alkaline soil collected from Shihezi district, Xinjiang province, northwest China. The G+C content of the genomic DNA of the type strain is 75.2 mol%.

Description of *Egicoccaceae* fam. nov.

Egicoccaceae (E.gi.coc.ca.ce′ae. N.L. masc. n. *Egicoccus* type genus of the family; -*aceae* ending to denote a family; N.L. fem. pl. n. *Egicoccaceae* the family of the genus *Egicoccus*).

The description is the same as that for the genus *Egicoccus*. Segregation of these organisms within a new family is justified by their distinct phyletic lineage based on 16S rRNA gene sequences. The pattern of 16S rRNA gene sequence signature nucleotides of members of the family consists of 47 : 396 (U–C) and 841 : 845 (G–A).

Description of *Egicoccales* ord. nov.

Egicoccales (E.gi.coc.ca′les. N.L. masc. n. *Egicoccus* type genus of the family; -*ales* ending to denote an order; N.L. fem. pl. n. *Egicoccales* the order of the genus *Egicoccus*).

The description is the same as that for the genus *Egicoccus*. Separation of these organisms within a new order is

justified by their distinct phyletic lineage based on 16S rRNA gene sequences. The pattern of 16S rRNA gene sequence signature nucleotides of members of the order is as for the family *Egicoccaceae*. The order contains the family *Egicoccaceae*. *Egicoccus* is the type genus.

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References

Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–789.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Goodfellow, M. (1971). Numerical taxonomy of some nocardioform bacteria. *J Gen Microbiol* **69**, 33–80.

Horikoshi, K. (1999). Alkaliphiles: some applications of their products for biotechnology. *Microbiol Mol Biol Rev* **63**, 735–750.

Horikoshi, K., Antranikian, G., Bull, A. T., Robb, F. T. & Stetter, K. O. (2011). Extremophiles: alkaliphiles. In *Extremophiles Handbook*, pp. 17–251. Edited by K. Horikoshi, G. Antranikian, A. T. Bull, F. T. Robb & K. O. Stetter. Berlin: Springer.

Kelly, K. L. (1964). *Inter-Society Color Council – National Bureau of Standards Color Name Charts Illustrated with Centroid Colors.* Washington, DC: US Government Printing Office.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Kurahashi, M., Fukunaga, Y., Sakiyama, Y., Harayama, S. & Yokota, A. (2010). *Euzebya tangerina* gen. nov., sp. nov., a deeply branching marine actinobacterium isolated from the sea cucumber *Holothuria edulis*, and proposal of *Euzebyaceae* fam. nov., *Euzebyales* ord. nov. and *Nitriliruptoridae subclassis* nov. *Int J Syst Evol Microbiol* **60**, 2314–2319.

Li, W. J., Xu, P., Schumann, P., Zhang, Y. Q., Pukall, R., Xu, L. H., Stackebrandt, E. & Jiang, C. L. (2007). *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China), and emended description of the genus *Georgenia*. *Int J Syst Evol Microbiol* **57**, 1424–1428.

Ludwig, W., Euzéby, J., Schumann, P., Busse, H.-J., Trujillo, M. E., Kämpfer, P. & Whitman, W. B. (2012). Road map of the phylum *Actinobacteria*. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 5, pp. 1–28. Edited by W. Whitman, M. Goodfellow, P. Kämpfer, H.-J. Busse, M. Trujillo, W. Ludwig & K. Suzuki. New York: Springer.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

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

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, K. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sarethy, I. P., Saxena, Y., Kapoor, A., Sharma, M., Sharma, S. K., Gupta, V. & Gupta, S. (2011). Alkaliphilic bacteria: applications in industrial biotechnology. *J Ind Microbiol Biotechnol* **38**, 769–790.

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newwark, DE: MIDI Inc.

Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.

Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.

Sorokin, D. Y., van Pelt, S., Tourova, T. P. & Evtushenko, L. I. (2009). *Nitriliruptor alkaliphilus* gen. nov., sp. nov., a deep-lineage haloalkaliphilic actinobacterium from soda lakes capable of growth on aliphatic nitriles, and proposal of *Nitriliruptoraceae* fam. nov. and *Nitriliruptorales* ord. nov. *Int J Syst Evol Microbiol* **59**, 248–253.

Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.

Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* **30**, 2725–2729.

Tang, S.-K., Tian, X.-P., Zhi, X.-Y., Cai, M., Wu, J.-Y., Yang, L.-L., Xu, L.-H. & Li, W.-J. (2008). *Haloactinospora alba* gen. nov., sp. nov., a halophilic filamentous actinomycete of the family *Nocardiopsaceae*. *Int J Syst Evol Microbiol* **58**, 2075–2080.

Tang, S. K., Wang, Y., Chen, Y., Lou, K., Cao, L. L., Xu, L. H. & Li, W. J. (2009). *Zhihengliuella alba* sp. nov., and emended description of the genus *Zhihengliuella*. *Int J Syst Evol Microbiol* **59**, 2025–2032.

Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M., Sneath, P. H. & Sackin, M. J. (1983). Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129**, 1743–1813.

Xu, P., Li, W. J., Tang, S. K., Zhang, Y. Q., Chen, G. Z., Chen, H. H., Xu, L. H. & Jiang, C. L. (2005). *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family '*Oxalobacteraceae*' isolated from China. *Int J Syst Evol Microbiol* **55**, 1149–1153.

Zhi, X. Y., Li, W. J. & Stackebrandt, E. (2009). An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class *Actinobacteria*, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. *Int J Syst Evol Microbiol* **59**, 589–608.