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Nesterenkonia populi sp. nov., an actinobacterium isolated from *Populus euphratica*

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An alkaliphilic and moderately halophilic actinobacterium, designated strain GP10-3^T, was isolated from *Populus euphratica* collected from the southern edge of Taklimakan desert, Xinjiang, China. Cells of this strain were Gram-stain-positive, non-motile and non-spore-forming short rods. Strain GP10-3^T grew optimally at 37 °C on LB agar media in the presence of 5–10 % (w/v) NaCl at pH 9.0. The menaquinones were MK-7, MK-8 and MK-9. The major fatty acids (>10 %) were anteiso-$C_{17:0}$, anteiso-$C_{15:0}$ and iso-$C_{16:0}$. The peptidoglycan type was variation A4α, L-Lys-L-Glu. The polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylglycolipid, phosphatidylcholine, phosphatidylinositol, glycolipid and an unidentified phospholipid. The DNA G+C content was 67.4 mol%. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain GP10-3^T belonged to the genus *Nesterenkonia*, sharing 94.6–96.9 % sequence similarity with the type strains of species within this genus with validly published names. Based on the evidence of the polyphasic taxonomic study, strain GP10-3^T represents a novel species of the genus *Nesterenkonia*, for which the name *Nesterenkonia populi* sp. nov. is proposed. The type strain is GP10-3^T (=DSM 27959^T=KCTC 29119^T).

The genus *Nesterenkonia* was first proposed by Stackebrandt *et al.* (1995) with the reclassification of *Micrococcus halobius* (Onishi & Kamekura, 1972) as *Nesterenkonia halobia*. The description of the genus was later emended by Li *et al.* (2005), and indicated that members of the genus *Nesterenkonia* are moderately halophilic or halotolerant, some are alkaliphilic or alkalitolerant. At present, the genus comprises 13 species with validly published names, 11 of which were isolated from saline-alkaline environments or deserts, including *Nesterenkonia halobia* (Stackebrandt *et al.*, 1995; Mota *et al.*, 1997); *Nesterenkonia lacusekhoensis* (Collins *et al.*, 2002); *Nesterenkonia halotolerans*, *Nesterenkonia xinjiangensis* (Li *et al.*, 2004); *Nesterenkonia lutea*, *Nesterenkonia sandarakina* (Li *et al.*, 2005); *Nesterenkonia jeotgali* (Yoon *et al.*, 2006); *Nesterenkonia aethiopica* (Delgado *et al.*, 2006); *Nesterenkonia halophila* (Li *et al.*, 2008); *Nesterenkonia suensis* (Govender *et al.*, 2013) and *Nesterenkonia rhizosphaerae* (Wang *et al.*, 2014). During a study on cultivable actinobacterial diversity associated with psammophytes, strain GP10-3^T, an alkaliphilic and moderately halophilic actinobacterium, was isolated from a saline-alkaline niche. Based on phylogenetic analysis, isolate GP10-3^T showed relatively high levels of 16S rRNA gene sequence similarities to members of the genus *Nesterenkonia*. Results of a polyphasic taxonomic study showed that strain GP10-3^T could be distinguished from previously described species of the genus *Nesterenkonia* and represented a novel species. In this paper, the taxonomic position of this strain is reported.

Strain GP10-3^T was isolated from a piece of bark on *Populus euphratica* collected from the southern edge of Taklimakan desert. The bark was covered by a thin layer of white salt and located near a wound, where briny water leaked out. The brackish bark was ground into powder by using a micromill and distributed on GP agar media (containing 1^{-1}: 10.0 g glycerol, 0.5 g yeast extract, 1.0 g proline, 1.0 g asparaginate, 0.5 g betaine, 1.25 g sodium pyruvate, 1.25 g KNO_3, 20.0 g agar) adjusted to pH 10.0 and supplemented with 1 % (v/v) plant tissue extract. Incubation was carried out at 37 °C for 6 weeks. Colonies were transferred onto ISP 2 agar (Shirling & Gottlieb, 1966) supplemented with 5 % (w/v) NaCl at pH 9.0 by the plating technique and were incubated at 37 °C until a pure isolate was obtained. The purified isolate was maintained at 4 °C on Luria–Bertani agar slants (Miller, 1972) supplemented with 5 % (w/v) NaCl at pH 9.0 (LB-NaCl) and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GP10-3^T is KP057085.

Two supplementary tables and three supplementary figures are available with the online Supplementary Material.

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preserved as aqueous glycerol suspensions (20 %, v/v) at −80 °C.

Growth characteristics, and physiological and biochemical characteristics of strain GP10-3^T were tested alongside those of the phylogenetically closest strain, *Nesterenkonia alba* DSM 19423^T from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany), as reference strain under the same conditions. Growth characteristics were recorded after 2 weeks of incubation at 37 °C on Bennett's agar (Jones, 1949), Czapek's agar (Waksman, 1961), ISP 2, ISP 3, ISP 4, ISP 5, ISP 7 agars (Shirling & Gottlieb, 1966), LB agar, marine agar (Difco), nutrient agar (Difco), PYA agar (Luo *et al.*, 2009), R2A agar (Difco) and TSA (Difco); all media were supplemented with 5 % (w/v) NaCl. The colours of colonies and soluble pigments were determined by comparison with chips from the RAL colour charts (RAL Deutsches Institut für Gütesicherung und Kennzeichnung). Strain GP10-3^T grew well on most of the media tested except Czapek's agar, ISP 3 agar and ISP 4 agar. No growth was observed on ISP 5 agar or ISP 7 agar. Colonies on LB-NaCl agar were sulfur-yellow in colour at 37 °C (Table S1, available in the online Supplementary Material). The isolate did not produce diffusible pigments on the media tested. Morphological characteristics were observed after incubation at 37 °C for 5 days on LB-NaCl agar by light microscopy (BH2; Olympus) and scanning electron microscopy (Quanta 200; FEI). The Gram-staining test was performed as described by Magee *et al.* (1975). Cell motility was studied on LB-NaCl swarming agar (0.4 %, w/v). The NaCl tolerance was tested on LB agar with NaCl concentrations at 0, 1, 3, 5, 7, 10, 15 and 20 % (w/v). The pH range for growth was determined within the range of pH 4.0–12.0 (at intervals of 1 pH unit) in buffered LB broth (supplemented with 5 %, w/v, NaCl) using the buffer system described by Wang *et al.* (2010) and Luo *et al.* (2008). The optimum growth temperature was determined on LB-NaCl agar at 0, 4, 10, 18, 28, 37, 42 and 50 °C. Biomass for physiological and biochemical studies was obtained by cultivation on PYA (pH 9.0) plates supplemented with 5 % (w/v) NaCl at 37 °C for 5 days. Carbon utilization and acid production were determined by Biolog GEN III Microplates and the API 50CH system (bioMeriéux) according to the manufacturers' instructions, respectively. Catalase and oxidase activities were tested in 3 % (v/v) H_2O_2 and 1 % (w/v) tetramethyl-*p*-phenylenediamine, respectively. Hydrolysis of starch, gelatin and Tween 80 was determined as described by Cowan & Steel (1965). Nitrate reduction was determined according to the method of Lányí (1987). Other physiological and biochemical tests were performed with the methods described by Williams *et al.* (1983) and Kämpfer *et al.* (1991).

Cells of strain GP10-3^T were Gram-stain-positive, non-spore-forming and non-motile short rods of 0.5–0.6 μm in width and 0.8–1.3 μm in length (Fig. S1). Strain GP10-3^T could grow with 3–15 % (w/v) NaCl, but no growth occurred with 0, 1 or 20 % (w/v) NaCl; the optimum concentration of NaCl for growth was 5–10 % (w/v). Growth was observed at pH 8.0–12.0. No growth occurred at pH 4.0–7.0. The optimum pH for growth was pH 9.0. Strain GP10-3^T could grow at 10–42 °C and grew well at 18–37 °C, but no growth occurred at 0, 4 or 50 °C. The optimum growth temperature was 37 °C. Strain GP10-3^T could be distinguished from the closely related species of the genus *Nesterenkonia* by morphological, physiological and biochemical properties (Table 1). The detailed characteristics of GP10-3^T are given in the species description.

For analysis of menaquinones, polar lipids and DNA G + C content, strain GP10-3^T and the reference type strain *N. alba* DSM 19423^T were cultured in LB-NaCl liquid medium at 37 °C for 5 days on a rotary shaker (180 r.p.m). Menaquinones were extracted as described by Collins *et al.* (1977), separated by HPLC (Groth *et al.* 1997) and then confirmed by using a single quadrupole mass spectrometer (LCMS-2020; Shimadzu) as described by Guo *et al.* (2015). Polar lipids were extracted and analysed by two-dimensional TLC on silica gel 60 F_{254} plates (Merck) as described by Minnikin *et al.* (1984). Solvent systems were modified by using chloroform/methanol/water (64 : 27 : 5, v/v) as the first dimension and chloroform/methanol/acetic acid/water (80 : 18 : 12 : 5, v/v) as the second dimension. To calculate the G + C content, DNA was prepared by the method described by Marmur (1961). The DNA G + C content was determined by reverse-phase HPLC analysis and calculated from the ratio of nucleosides according to Mesbah *et al.* (1989).

For analysis of fatty acids, strain GP10-3^T and the reference strain were cultured on LB-NaCl agar medium at 37 °C for 5 days. The whole-cell fatty acids were saponified, methylated and extracted according to the method described by Kuykendall *et al.* (1988), and analysed by using an Agilent 7890A gas chromatograph coupled with an Agilent 5975C, a single quadrupole mass spectrometer equipped with a Nist08 Library software database. An HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Agilent Technologies) was used for the separation of fatty acid methyl esters. The initial temperature of 90 °C was maintained for 1 min, raised to 180 °C at a rate of 10 °C min^{-1}, then raised to 210 °C at a rate of 2 °C min^{-1}, and finally to 270 °C at a rate of 20 °C min^{-1} and kept for 2 min. Helium was used as a carrier gas with a flow rate of 1.0 ml min^{-1}. Injection (2 μl) was made in the splitless mode at an injector temperature of 270 °C. Mass spectra were obtained using electron impact (EI; 70 eV).

The analysis of the peptidoglycan structure was carried out by the Identification Services of DSMZ. The peptidoglycan was prepared and its structure was analysed according to published protocols [Schumann, 2011; isolation of peptidoglycan, protocol 7; qualitative analysis of amino acids and peptides, protocol 9; quantitative and enantiomeric analysis of amino acids, protocols 10 and 11 (CP-ChiraSil-L-Val column CP7495; Agilent Technologies); detection of N-terminal amino acids, protocol 12].

Strain GP10-3^T contained menaquinones MK-7, MK-8 and MK-9. The polar lipids were comprised of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylglycolipid,

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Table 1. Phenotypic characteristics of strain GP10-3^T and closely related species of the genus *Nesterenkonia*

Strains: 1, *Nesterenkonia populi* sp. nov. GP10-3^T; 2, *N. alba* DSM 19423^T; 3, *N. aethiopica* DSM 17733^T; 4, *N. xinjiangensis* YIM 70097^T. +, Positive; −, negative; (+), weakly positive; ND, not determined. Data for both GP10-3^T and *N. alba* DSM 19423^T were obtained in this study. Data for *N. aethiopica* DSM 17733^T are from Delgado *et al.* (2006); data for *N. xinjiangensis* YIM 70097^T are from Li *et al.* (2004); All strains are negative for H_2S production. All strains are negative for acidification of trehalose, galactose and lactose. All strains are positive for utilization of sucrose.

Characteristic	1	2	3	4
Morphology	Short rods	Short rods[a]*	Short rods	Short rods
Optimum temperature for growth (°C)	37	42	30–37	28
Optimum NaCl for growth (%)	5–10	0–5	3	10
Optimum pH	9	10	9	8–9
Catalase activity	+	+	ND	ND
Cellulose degradation	−	−	ND	−
Indole production	−	−	−	ND
Milk coagulation	−	−	ND	−
Nitrate reduction	−	−	ND	−
ONPG test	−	+	−	ND
Oxidase activity	(+)	−	+	−
Urease activity	+	−	−	+
Hydrolysis of:				
Gelatin	−	+	+	+
Starch	−	+	+	−
Tween 80	−	+	−[b]	+[b]
Carbon utilization				
Cellobiose	+	−	ND	+
D-Fructose	+	−	+[b]	+
D-Galactose	−	−	+[b]	+
Glucose	(+)	+	ND	+
myo-Inositol	+	+	+[b]	−[b]
Lactose	−	−	−	+
Maltose	+	+	ND	+
D-Mannitol	−	+	−	−[b]
D-Mannose	−	−	ND	+
Raffinose	−	+	+[b]	+[b]
L-Rhamnose	+	+	ND	ND
D-Sorbitol	(+)	−	−[b]	−
Trehalose	+	(+)	−	−
Acid production from:				
L-Arabinose	−	+	+	ND
Fructose	+	−	+	ND
D-Glucose	−	−	+	−[b]
Rhamnose	+	−	ND	ND
D-Xylose	−	(+)	−	−
Menaquinones	MK-7 (28.5 %), MK-8 (67.4 %), MK-9 (4.1 %)	MK-7 (11.6 %), MK-8 (49.4 %), MK-9 (39.0 %)	ND	MK-7 (24.0 %), MK-8 (56.0 %), MK-9 (17.0 %)
Predominant fatty acids (>10 %)	anteiso-$C_{17:0}$ (53.3 %), anteiso-$C_{15:0}$ (18.6 %)	anteiso-$C_{17:0}$ (65.5 %), anteiso-$C_{15:0}$ (26.5 %)	ND	anteiso-$C_{17:0}$ (38.1 %), anteiso-$C_{15:0}$ (28.5 %)
Peptidoglycan type	L-Lys-L-Glu	L-Lys-Gly-D-Asp[a]	L-Lys-Gly-L-Glu	L-Lys-Gly-L-Glu
DNA G+C content (mol%)	67.4	60.2[a]	69.0	66.7

*Data were taken from: *a*, Luo *et al.* (2009); *b*, Wang *et al.* (2014).

phosphatidylinositol, phosphatidylcholine, glycolipids and an unidentified phospholipid as shown in Fig. S2. The major cellular fatty acids (>10 %) were anteiso-$C_{17:0}$ (53.3 %), anteiso-$C_{15:0}$ (18.6 %) and iso-$C_{16:0}$ (13.2 %); other fatty acids were $C_{16:0}$ (5.1 %), $C_{16:1}\omega7c$ (3.8 %), $C_{17:1}\omega10c$ (1.9 %), iso-$C_{17:0}$ (1.6 %), iso-$C_{15:0}$ (1.4 %) and

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$C_{15:0}$ (1.1 %) as shown in Table S2. The peptidoglycan type of strain GP10-3^T was L-Lys-L-Glu, variation A4α (Schleifer & Kandler, 1972; type A11.54 according to www.peptidoglycan-types.info). The DNA G+C content was 67.4 mol%.

The 16S rRNA gene was amplified from genomic DNA extracted as described by Li *et al.* (2007). The PCR amplicon was cloned by using a pEASY-T1 cloning kit (TransGen Biotech) and sequenced by using an ABI PRISM 3730XL DNA Analyser. The comparison of 16S rRNA gene sequence similarity was carried out by using the EzTaxon server (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012). Multiple alignments were performed in BioEdit (Hall, 1999). Phylogenetic analysis was performed by using maximum-parsimony (Fitch, 1971), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) methods. A neighbour-joining phylogenetic tree from the Kimura two-parameter model (Kimura, 1983) based on 16S rRNA gene sequences was reconstructed by using MEGA version 5 (Tamura *et al.*, 2011), evolutionary distances were computed with bootstrap values based on 1000 replications (Felsenstein, 1985). Comparative analyses of 16S rRNA gene sequence (1481 bp) showed that strain GP10-3^T belonged to the genus *Nesterenkonia*. Strain GP10-3^T was most closely related to *N. alba* DSM 19423^T (96.9 % 16S rRNA gene sequence similarity), *N. aethiopica* DSM 17733^T (96.3 %) and *N. xinjiangensis* YIM 70097^T (96.1 %). Levels of 16S rRNA gene sequence similarity between strain GP10-3^T and the other species of the genus *Nesterenkonia* with validly published names were less than 96.0 %. The phylogenetic trees (Fig. S1 and S3) based on 16S rRNA gene sequence indicated that strain GP10-3^T formed a distinct subclade with *N. alba* DSM 19423^T among members of genus *Nesterenkonia*.

Members of genus *Nesterenkonia* possess the same key properties (Stackebrandt, 2012); cells may be short rods, sometimes showing branching, or cocci. Menaquinones are MK-7, MK-8 and MK-9. The polar lipids include diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. The predominant cellular fatty acids are anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. Peptidoglycan is A4α type, L-Lys-Gly-L-Glu, L-Lys-Gly-D-Asp or L-Lys-L-Glu. DNA G+C contents are between 64 and 72 mol%. Most characteristics of strain GP10-3^T fitted the description of the genus *Nesterenkonia*. However, the peptidoglycan type of strain GP10-3^T was A4α, L-Lys-L-Glu, which differed from that of strain *N. alba* DSM 19423^T. The proportions of menaquinones MK-7, MK-8 and MK-9 of strain GP10-3^T were 28.5 %, 67.4 % and 4.1 %, respectively, which were quite different from those of strain *N. alba* DSM 19423^T [MK-7 (11.6 %), MK-8 (49.4 %) and MK-9 (39.0 %)]. Meanwhile, strain GP10-3^T contained phosphatidylcholine and an unidentified phospholipid, which were absent in strain *N. alba* DSM 19423^T. Qualitative and quantitative analysis of fatty acids performed concurrently on both strains showed differences between strain GP10-3^T and the reference strain. Strain GP10-3^T contained a large amount of iso-$C_{16:0}$ while *N. alba* DSM 19423^T contained a little, and several minor fatty acids detected in GP10-3^T could not be found in *N. alba* DSM 19423^T.

It is generally accepted that micro-organisms displaying 16S rRNA gene sequence similarity values of 97 % or less do not belong to the same species (Stackebrandt & Goebel, 1994). The genotypic and phenotypic differences observed support that strain GP10-3^T represents a novel species in the genus *Nesterenkonia*, for which the name *Nesterenkonia populi* sp. nov. is proposed.

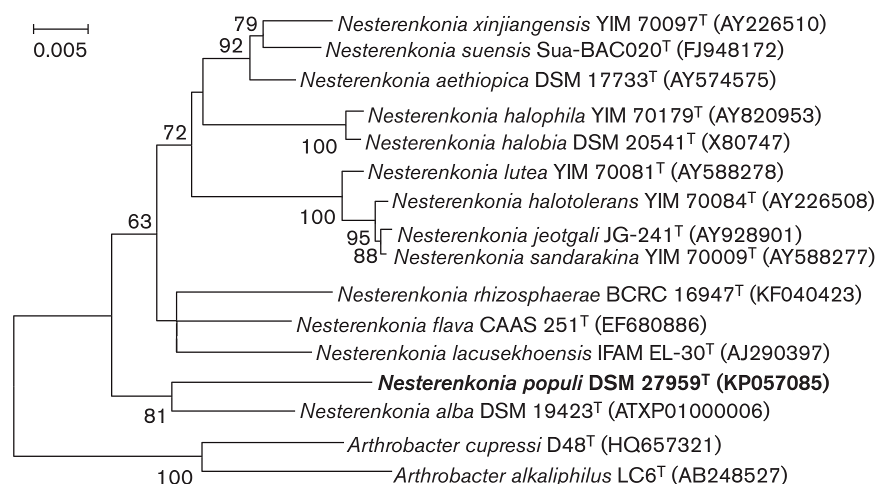

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of strain GP10-3^T and related strains in the genus *Nesterenkonia*. Numbers at nodes refer to bootstrap values (based on 1000 replicates; only values >50 % are shown). Bar, 0.005 changes per nucleotide position.

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Description of *Nesterenkonia populi*

Nesterenkonia populi (po′pu.li. L. gen. n. *populi* of poplar, pertaining to *Populus*, the Latin name for the poplars from which the strain was isolated).

Cells are Gram-stain-positive, non-spore-forming and non-motile short rods of 0.5–0.6 μm in width and 0.8–1.3 μm in length. Colonies are smooth, circular and sulfur-yellow in colour on LB-NaCl agar medium. Alkaliphilic and moderately halophilic. Optimum growth occurs on LB agar supplemented with 5–10 % (w/v) NaCl at 37 °C and pH 9.0. Temperature, pH and NaCl tolerance ranges are 10–42 °C, pH 8.0–12.0 and 3–15 % (w/v), respectively. Oxidase activity, catalase activity and urease activity are positive. But milk coagulation, production of H_2S and indole, cellulose degradation, gelatin liquefaction, hydrolysis of starch and Tween 80, and nitrate reduction are negative. Cellobiose, D-fructose, glucose, *myo*-inositol, maltose, L-rhamnose, D-sorbitol, sucrose and trehalose can be utilized as carbon sources, but D-galactose, lactose, D-mannitol, D-mannose and raffinose cannot be utilized. Acid is produced from fructose, D-fucose, rhamnose and L-sorbose. The peptidoglycan structure is type A4α, L-Lys-L-Glu. The predominant menaquinones are MK-8 and MK-7; a minor menaquinone is MK-9. The main cellular fatty acids are anteiso-$C_{17:0}$, anteiso-$C_{15:0}$ and iso-$C_{16:0}$. Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylglycolipid, phosphatidylcholine, phosphatidylinositol, glycolipid and an unidentified phospholipid.

The type strain, GP10-3^T (=DSM 27959^T=KCTC 29119^T), was isolated from a bark sample of *Populus euphratica* collected on the southern edge of Taklimakan desert, Xinjiang, China. The DNA G+C content of the type strain GP10-3^T is 67.4 mol%.

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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.

Collins, M. D., Lawson, P. A., Labrenz, M., Tindall, B. J., Weiss, N. & Hirsch, P. (2002). *Nesterenkonia lacusekhoensis* sp. nov., isolated from hypersaline Ekho Lake, East Antarctica, and emended description of the genus *Nesterenkonia*. *Int J Syst Evol Microbiol* **52**, 1145–1150.

Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.

Delgado, O., Quillaguamán, J., Bakhtiar, S., Mattiasson, B., Gessesse, A. & Hatti-Kaul, R. (2006). *Nesterenkonia aethiopica* sp. nov., an alkaliphilic, moderate halophile isolated from an Ethiopian soda lake. *Int J Syst Evol Microbiol* **56**, 1229–1232.

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**, 783–791.

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

Govender, L., Naidoo, L. & Setati, M. E. (2013). *Nesterenkonia suensis* sp. nov., a haloalkaliphilic actinobacterium isolated from a salt pan. *Int J Syst Evol Microbiol* **63**, 41–46.

Groth, I., Schumann, P., Rainey, F. A., Martin, K., Schuetze, B. & Augsten, K. (1997). *Demetria terragena* gen. nov., sp. nov., a new genus of actinomycetes isolated from compost soil. *Int J Syst Bacteriol* **47**, 1129–1133.

Guo, L., Tuo, L., Habden, X., Zhang, Y., Liu, J., Jiang, Z., Liu, S., Dilbar, T. & Sun, C. (2015). *Allosalinactinospora lopnorensis* gen. nov., sp. nov., a new member of the family *Nocardiopsaceae* isolated from soil. *Int J Syst Evol Microbiol* **65**, 206–213.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.

Jones, K. L. (1949). Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. *J Bacteriol* **57**, 141–145.

Kämpfer, P., Kroppenstedt, R. M. & Dott, W. (1991). A numerical classification of the genera *Streptomyces* and *Streptoverticillium* using miniaturized physiological tests. *J Gen Microbiol* **137**, 1831–1891.

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.

Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. London: Cambridge University Press.

Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradorhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.

Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.

Li, W.-J., Chen, H.-H., Zhang, Y.-Q., Schumann, P., Stackebrandt, E., Xu, L.-H. & Jiang, C.-L. (2004). *Nesterenkonia halotolerans* sp. nov. and *Nesterenkonia xinjiangensis* sp. nov., actinobacteria from saline soils in the west of China. *Int J Syst Evol Microbiol* **54**, 837–841.

Li, W.-J., Chen, H.-H., Kim, C.-J., Zhang, Y.-Q., Park, D.-J., Lee, J.-C., Xu, L.-H. & Jiang, C.-L. (2005). *Nesterenkonia sandarakina* sp. nov. and *Nesterenkonia lutea* sp. nov., novel actinobacteria, and emended description of the genus *Nesterenkonia*. *Int J Syst Evol Microbiol* **55**, 463–466.

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.

Li, W.-J., Zhang, Y.-Q., Schumann, P., Liu, H.-Y., Yu, L.-Y., Zhang, Y.-Q., Stackebrandt, E., Xu, L.-H. & Jiang, C.-L. (2008). *Nesterenkonia halophila* sp. nov., a moderately halophilic, alkalitolerant actinobacterium isolated from a saline soil. *Int J Syst Evol Microbiol* **58**, 1359–1363.

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Luo, H.-Y., Miao, L.-H., Fang, C., Yang, P.-L., Wang, Y.-R., Shi, P.-J., Yao, B. & Fan, Y.-L. (2008). *Nesterenkonia flava* sp. nov., isolated from paper-mill effluent. *Int J Syst Evol Microbiol* **58**, 1927–1930.

Luo, H.-Y., Wang, Y.-R., Miao, L.-H., Yang, P.-L., Shi, P.-J., Fang, C.-X., Yao, B. & Fan, Y.-L. (2009). *Nesterenkonia alba* sp. nov., an alkaliphilic actinobacterium isolated from the black liquor treatment system of a cotton pulp mill. *Int J Syst Evol Microbiol* **59**, 863–868.

Magee, C. M., Rodeheaver, G., Edgerton, M. T. & Edlich, R. F. (1975). A more reliable gram staining technic for diagnosis of surgical infections. *Am J Surg* **130**, 341–346.

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.

Miller, J. H. (1972). *Experiments in Molecular Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

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

Mota, R. R., Márquez, M. C., Arahal, D. R., Mellado, E. & Ventosa, A. (1997). Polyphasic taxonomy of *Nesterenkonia halobia*. *Int J Syst Bacteriol* **47**, 1231–1235.

Onishi, H. & Kamekura, M. (1972). *Micrococcus halobius* sp. n. *Int J Syst Bacteriol* **22**, 233–236.

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

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

Schumann, P. (2011). Peptidoglycan structure. *Methods Microbiol* **38**, 101–129.

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

Stackebrandt, E. (2012). Genus VI. *Nesterenkonia* Stackebrandt, Koch, Gvozdiak & Schumann 1995, 689VP. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol 5, pp. 636–642. Edited by M Goodfellow, P Kämpfer, H.-J. Busse, M. E. Trujillo, K. Suzuki, W. Ludwig & W. B. Whitman. New York: Springer.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Stackebrandt, E., Koch, C., Gvozdiak, O. & Schumann, P. (1995). Taxonomic dissection of the genus *Micrococcus*: *Kocuria* gen. nov., *Nesterenkonia* gen. nov., *Kytococcus* gen. nov., *Dermacoccus* gen. nov., and *Micrococcus* Cohn 1872 gen. emend. *Int J Syst Bacteriol* **45**, 682–692.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Waksman, S. A. (1961). *The Actinomycetes. Classification, Identification and Description of Genera and Species*, vol. II. Baltimore, MD: Williams & Wilkins.

Wang, J., Li, Y., Bian, J., Tang, S.-K., Ren, B., Chen, M., Li, W.-J. & Zhang, L.-X. (2010). *Prauserella marina* sp. nov., isolated from ocean sediment of the South China Sea. *Int J Syst Evol Microbiol* **60**, 985–989.

Wang, H.-F., Zhang, Y.-G., Chen, J.-Y., Hozzein, W. N., Li, L., Wadaan, M. A. M., Zhang, Y.-M. & Li, W.-J. (2014). *Nesterenkonia rhizosphaerae* sp. nov., an alkaliphilic actinobacterium isolated from rhizosphere soil in saline-alkaline desert. *Int J Syst Evol Microbiol* **64**, 4021–4026.

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

Yoon, J.-H., Jung, S.-Y., Kim, W., Nam, S.-W. & Oh, T.-K. (2006). *Nesterenkonia jeotgali* sp. nov., isolated from jeotgal, a traditional Korean fermented seafood. *Int J Syst Evol Microbiol* **56**, 2587–2592.

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