Nesterenkonia sandarakina sp. nov. and *Nesterenkonia lutea* sp. nov., novel actinobacteria, and emended description of the genus *Nesterenkonia*

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Two novel actinobacteria isolates, designated YIM 70009^T and YIM 70081^T, were characterized in order to determine their taxonomic position. Cells of strains YIM 70009^T and YIM 70081^T were cocci, although only the latter were motile. The G+C contents of their DNAs were 64·0 and 64·5 mol%, respectively. On the basis of chemotaxonomic characteristics and 16S rRNA gene sequence analysis, the two isolates were classified in the genus *Nesterenkonia*. DNA–DNA hybridization and comparison of phenotypic characteristics revealed that strains YIM 70009^T and YIM 70081^T differed from each other and from known species. Therefore, it is proposed that they represent two separate novel species of the genus *Nesterenkonia*: *Nesterenkonia sandarakina* sp. nov. (type strain, YIM 70009^T=CCTCC AA 203007^T=DSM 15664^T=KCTC 19011^T) and *Nesterenkonia lutea* sp. nov. (type strain, YIM 70081^T=CCTCC AA 203010^T=DSM 15666^T=KCTC 19013^T).

The genus *Nesterenkonia* was first proposed by Stackebrandt *et al.* (1995) in the reclassification of *Micrococcus halobius* Onishi and Kamekura 1972 as *Nesterenkonia halobia* (Stackebrandt *et al.*, 1995). At present, the genus comprises four valid species: *N. halobia* (Stackebrandt *et al.*, 1995), *Nesterenkonia lacusekhoensis* (Collins *et al.*, 2002), and *Nesterenkonia halotolerans* and *Nesterenkonia xinjiangensis* (Li *et al.*, 2004). In this report, two novel species of the genus *Nesterenkonia* that were discovered during our taxonomic study of extremophilic actinomycetes are described.

Strain YIM 70009^T was isolated from a soil sample collected from the eastern desert of Egypt using modified medium A (supplemented with 15 % NaCl, w/v, pH 10·0–10·5), as described previously (Hozzein *et al.*, 2004). Strain YIM 70081^T was isolated from a saline soil sample from China using a modified glycerol/asparagine agar medium (ISP 5) (Shirling & Gottlieb, 1966) supplemented with 15 % (w/v) $MgCl_2.6H_2O$. Isolation plates were incubated at 28 °C for 2 weeks. The purified strains were cultivated and maintained on modified TSA medium containing 5–10 % NaCl (w/v), pH 8·0–9·0 for strain YIM 70009^T or 5–10 % $MgCl_2.6H_2O$ (w/v), pH 7·0–8·0 for strain YIM 70081^T. Biomass for chemical and molecular systematic studies was grown in shaken flasks (about 150 r.p.m.) at 28 °C for 1 week using the same media as above without agar. Morphological properties were examined by light microscopy (Olympus microscope BH-2) and electron microscopy (JEOL JEM-1010).

The G+C contents of isolates YIM 70009^T and YIM 70081^T, determined using the thermal denaturation method of Marmur & Doty (1962), were 64·0 and 64·5 mol%, respectively. 16S rRNA genes were analysed as described previously (Li *et al.*, 2004). Phylogenetic analysis was performed using the software packages PHYLIP (Felsenstein, 1993) and MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar *et al.*, 2001) after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances were calculated using distance options according to the Kimura two-parameter model (Kimura, 1980, 1983) and clustering was performed using the neighbour-joining

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YIM 70009^T and YIM 70081^T are AY588277 and AY588278, respectively.

Electron micrographs of strains YIM 70009^T and YIM 70081^T are available as supplementary material in IJSEM Online.

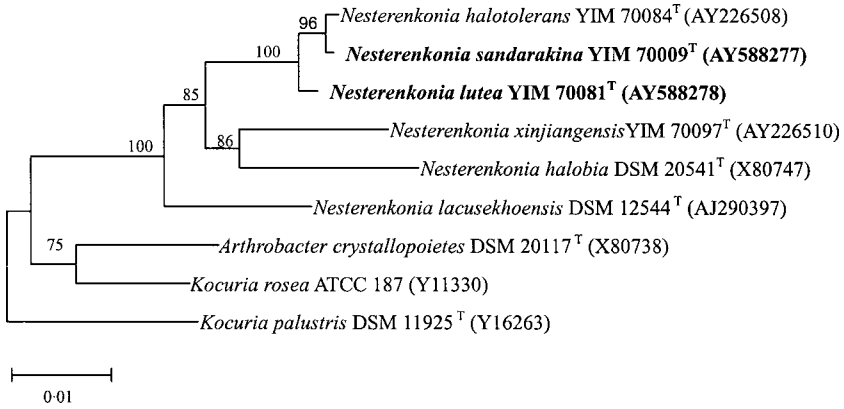

Fig. 1. Phylogenetic dendrogram, obtained by distance matrix analysis of 16S rRNA gene sequences, showing the position of strains YIM 70009^T and YIM 70081^T among phylogenetic neighbours. Numbers on branch nodes are bootstrap values (1000 resamplings). The sequence of *Streptomyces megasporus* DSM 41476^T (GenBank/EMBL/DDBJ accession no. Z68100) was used as a root. Bar, 1 % sequence divergence.

method (Saitou & Nei, 1987). Bootstrap analysis was used to evaluate tree topology of the neighbour-joining data by performing 1000 resamplings (Felsenstein, 1985). The neighbour-joining tree (Fig. 1) indicated that strains YIM 70009^T and YIM 70081^T were highly related to each other (99·4 % sequence similarity) and to *N. halotolerans* YIM 70084^T (99·8 and 99·4 % sequence similarity, respectively), forming a distinct branch with this species. Sequence similarities between strains YIM 70009^T and YIM 70081^T and other members of the genus *Nesterenkonia* were no more than 97·2 %.

Growth at various pH values and salt concentrations was tested as reported by Tang *et al.* (2003), except that TSA medium was used as the basic medium. Strains were grown on TSA medium containing 0, 1, 3, 5, 10, 15, 20, 25 or 30 % (w/v) total salts (NaCl, KCl or $MgSO_4.6H_2O$) and at initial pH values of 4·0, 5·0, 6·0, 7·0, 8·0, 9·0, 10·0, 11·0,12·0 and 13·0 for 30 days at 28 °C. Cell morphology and motility, physiological and biochemical test results, and chemotaxonomic properties, determined using previously described methods (Li *et al.*, 2004), are given in detail in Table 1 and the species description.

DNA–DNA relatedness tests were performed between YIM 70009^T, YIM 70081^T and *N. halotolerans* YIM 70084^T using the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992). DNA–DNA reassociation values of *N. halotolerans* YIM 70084^T with strains YIM 70009^T and YIM 70081^T were 43·3 and 39·1 %, respectively, and DNA–DNA hybridization between the two isolates was 45·2 % (repeated twice). DNA–DNA relatedness data provide decisive evidence that isolates YIM 70009^T and YIM 70081^T, and the related type strain *N. halotolerans* YIM 70084^T, are members of different genomic species (Wayne *et al.*, 1987). Genomic distinctness was also revealed by differences in a number of phenotypic characteristics (Table 1). It is therefore proposed that strains YIM 70009^T and YIM 70081^T should be classified as representatives of two novel *Nesterenkonia* species, *Nesterenkonia sandarakina* sp. nov. and *Nesterenkonia lutea* sp. nov., respectively.

Emended description of the genus *Nesterenkonia* Stackebrandt *et al.* 1995 emend. Collins *et al.* 2002

The description of the genus *Nesterenkonia* is as given previously (Stackebrandt *et al.*, 1995; Collins *et al.*, 2002), but with the following amendments. Moderately halophilic or halotolerant. Some species are alkaliphilic or alkalitolerant. Peptidoglycan is of the A4α type, L-Lys–Gly–L-Glu, L-Lys–L-Glu or Lys–Gly–D-Asp. The G+C content of the DNA is 64–72 mol%.

Description of *Nesterenkonia sandarakina* sp. nov.

Nesterenkonia sandarakina [san.da.ra′ki.na. N.L. fem. adj. *sandarakina* (from Gr. fem. adj. *sandarakinê*) of orange colour].

Cells are Gram-positive, non-spore-forming cocci (see electron micrograph available as supplementary material in IJSEM Online). Colony colour on most tested media is orange–yellow. Colonies are circular, opaque and approximately 0·5–1·0 mm in diameter after 24 h at 28 °C. Growth occurs at 1–15 % (w/v) NaCl (optimum at 5 %, w/v) and pH 5·0–12·0 (optimum at 8·0–9·0). Results of some physiological and biochemical characteristics, and metabolic and enzymic properties are indicated in Table 1. Peptidoglycan type is A4α, Lys–Gly–D-Asp. Cell wall sugars are ribose, xylose and arabinose. Main polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and an unidentified glycolipid. Predominant menaquinones are MK-7 and MK-8. Cellular fatty acids

Table 1. Phenotypic characteristics of strains YIM 70009^T, YIM 70081^T and *N. halotolerans* YIM 70084^T

Strains: 1, YIM 70009^T; 2, YIM 70081^T; 3, *N. halotolerans* YIM 70084^T. All three strains are Gram-positive, non-spore-forming coccoid cells. All are positive for catalase, milk coagulation, melanin production, growth in cellulose, and lysine decarboxylase, β-glucosidase, β-galactosidase and α-maltosidase activities. All are negative for oxidase, H_2S and indole production, decomposition of Tween 20 and Tween 80, casein and starch, and ornithine decarboxylase, arginine dihydrolase and *N*-acetylglucosaminidase activities. Maltose, sucrose, mannose, mannitol, fructose, salicin and galactose are utilized by the three strains as sole carbon sources for growth; rhamnose, acetamide, inositol, adonitol and sorbitol are not utilized. +, Positive; −, negative; W, weak reaction.

Characteristic	1	2	3
Motility	−	+	+
Colony pigmentation (PYGV medium)	Orange–yellow	Light yellow to primrose yellow	Deep orange–yellow
Optimal concn of NaCl (%, w/v) for growth	5	5–10	5–10
Range of salt concn for growth (%, w/v):			
NaCl	1–15	0–20	0–25
KCl	1–25	0–20	0–25
$MgCl_2.6H_2O$	1–25	0–20	0–25
Initial pH for growth:			
Range	5·0–12·0	6·5–10·0	6·5–9·0
Optimum	8·0–9·0	7·0–8·0	7·2
Milk peptonization	−	+	+
Nitrate reduction	−	+	−
Gelatin liquefaction	+	−	+
Ammonia production	+	−	−
Methyl red test	+	−	−
Vogues–Proskauer	−	−	+
Carbon source utilization:			
Glucose	+	W	+
Ribose	+	−	−
Xylose	+	+	−
Dextrin	W	+	+
Arabinose	W	W	−
Cellobiose	+	+	−
Trehalose	+	−	−
Starch	−	+	+
Lactose	−	−	+
Enzymic properties:			
Urease	−	−	+
α-Galactosidase	+	+	−
α-Glucosidase	−	+	+
β-Glucuronidase	+	−	−
L-Aspartate arylamidase	+	−	−

are $C_{16:0}$ (33·11 %), $aiC_{15:0}$ (27·63 %), $aiC_{17:0}$ (20·68 %), $C_{14:0}$ (0·81 %), $C_{15:0}$ (0·81 %), $C_{17:0}$ (1·46 %), $C_{18:0}$ (2·18 %), $iC_{16:0}$ (6·33 %), $iC_{17::0}$ (0·47 %), A-$aiC_{15:1}$ (4·81 %), G-$iC_{16:1}$ (0·82 %) and A-$aiC_{17:1}$ (0·88 %).

The type strain is YIM 70009^T (=CCTCC AA 203007^T=DSM 15664^T=KCTC 19011^T), isolated from a soil sample collected from the eastern desert of Egypt. The DNA G+C content of the type strain is 64·0 mol%.

Description of *Nesterenkonia lutea* sp. nov.

Nesterenkonia lutea (lu′te.a. L. fem. adj. *lutea* gold–yellow).

Cells are Gram-positive, non-spore-forming, motile cocci with flagella (see electron micrograph available as supplementary material in IJSEM Online). Colony colour on most tested media is light yellow to primrose yellow. Colonies are circular, opaque, somewhat convex and approximately 0·5–1·0 mm in diameter after 24 h at 28 °C. Growth occurs

at 0–20 % (w/v) $MgCl_2.6H_2O$ (optimum at 5–10 %, w/v) and pH 6·5–10·0 (optimum at 7·0–8·0). Results of some physiological and biochemical characteristics, and metabolic and enzymic properties are indicated in Table 1. Peptidoglycan type is A4α, Lys–Gly–D-Asp. Cell wall sugars are ribose, xylose, arabinose and glucose. Predominant menaquinones are MK-8 and MK-7. Main polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and an unidentified glycolipid. Cellular fatty acids are $aiC_{15:0}$ (10·27 %), $iC_{16:0}$ (31·40 %), $aiC_{17:0}$ (15·27 %), A-$aiC_{15:1}$ (15·12 %), G-$iC_{16:1}$ (11·09 %), G-$iC_{15:1}$ (0·84 %), $C_{14:0}$ (0·34 %), 2-OH $C_{14:0}$ (0·24 %), $C_{16:0}$ (5·75 %), $iC_{14:0}$ (0·99 %), $iC_{15:0}$ (0·57 %), $iC_{17:0}$ (0·96 %), $C_{17:1}\omega6c$ (0·36 %), A-$aiC_{17:1}$ (3·21 %), $iC_{18:0}$ (1·55 %), $C_{18:1}\omega7c$ (1·64 %) and summed feature 3 ($C_{16:1}\omega7c$ and/or 2-OH i-$C_{15:0}$; 0·40 %).

The type strain is YIM 70081^T (=CCTCC AA 203010^T= DSM 15666^T=KCTC 19013^T), isolated from a saline soil sample from the Xinjiang Province, China. The DNA G+C content of the type strain is 64·5 mol%.

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