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Nesterenkonia aethiopica sp. nov., an alkaliphilic, moderate halophile isolated from an Ethiopian soda lake

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Strain DSM 17733^T, isolated from the shore of Lake Abjata in Ethiopia, is a heterotrophic, alkaliphilic, moderately halophilic, Gram-positive, strictly aerobic, non-motile, non-endospore-forming bacterium. The organism grows optimally at 30–37 °C, pH 9 and 3 % (w/v) NaCl. Analysis of the cell wall showed the presence of murein of the type L-Lys–Gly–L-Glu, variation A4α. The G+C content of the genomic DNA was 69·0 mol%. Sequence analysis of 16S rRNA gene sequence of strain DSM 17733^T placed the isolate in the genus *Nesterenkonia*. DNA–DNA hybridization of DSM 17733^T with those organisms with the closest phylogenetic affiliation, i.e. *Nesterenkonia halobia*, *Nesterenkonia lacusekhoensis* and *Nesterenkonia xinjiangensis*, gave relatedness values of 48·5 %, 63·7 % (repetition, 57·2 %) and 35·7 % (repetition, 29·3 %), respectively. On the basis of both phenotypic and phylogenetic criteria and the low levels of DNA–DNA relatedness with the phylogenetically closest species *N. xinjiangensis* and *N. halobia*, it is proposed that the isolate be classified in a novel species, *Nesterenkonia aethiopica* sp. nov. The type strain is DSM 17733^T (=CCUG 48939^T).

The genus *Nesterenkonia* was proposed by Stackebrandt *et al.* (1995) and initially included only one species, *Nesterenkonia halobia*, originally classified as *Micrococcus halobius* (Onishi & Kamekura, 1972). The type strain of this species was isolated from unrefined solar salt from Noda, Japan (Onishi & Kamekura, 1972). Recently, the species *Nesterenkonia lacusekhoensis*, isolated from a hypersaline lake in eastern Antarctica (Collins *et al.*, 2002), *Nesterenkonia xinjiangensis* and *Nesterenkonia halotolerans*, from hypersaline soil in China (Li *et al.*, 2004), *Nesterenkonia sandarakina*, from soil in the eastern desert of Egypt, and *Nesterenkonia lutea*, from saline soil in China (Li *et al.*, 2005), have been described. All *Nesterenkonia* species are Gram-positive, strictly aerobic and moderately halophilic or halotolerant (and some species are alkaliphilic or alkalitolerant), contain peptidoglycan of the A4α type and have DNA G+C contents in the range 64–72 mol% (Li *et al.*, 2005).

In this paper, we describe the characterization and classification of the alkaliphilic, moderately halophilic strain DSM 17733^T, which was isolated from a soiled feather sample collected on the shore of Lake Abjata in Ethiopia (7° 60′ N 38° 62′ E).

Strain DSM 17733^T was grown at 37 °C, with shaking at 200 r.p.m., in complex YP medium (Mota *et al.*, 1997). Catalase and oxidase activities and sensitivity to antibiotics were tested, according to Smibert & Krieg (1994), using YP medium, utilized previously for the taxonomic characterization of *N. halobia* (Mota *et al.*, 1997). The colony characteristics of strain DSM 17733^T were studied on solid Luria–Bertani (LB) medium at pH 8 at 30 °C (Li *et al.*, 2005). The optimum temperature for growth was determined by using incubation at 20, 30, 37, 40 and 45 °C in liquid ISP 5 medium (Tang *et al.*, 2003). The pH tolerance was also investigated in ISP 5 medium at pH values ranging from 5 to 12, according to the method of Li *et al.* (2004, 2005). Halotolerance was tested in ISP 5 medium with 0–20 % (w/v) NaCl at 37 °C. Hydrolysis of xylan and dextran was tested as described by Smibert & Krieg (1994). Hydrolysis of starch and casein was tested in solid YP medium containing 1 % (w/v) soluble starch or 1·5 % (w/v) casein, respectively. Carbon-source utilization by strain DSM 17733^T was determined as described previously (Li *et al.*, 2004). Other biochemical characteristics were screened by using the API 20E system (bioMérieux), as described by Logan & Berkeley (1984).

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DSM 17733^T is AY574575.

Electron micrographs of strain DSM 17733^T are available as supplementary material in IJSEM Online.

Cell size and morphology were examined under a Nixon Optiphot-2 phase-contrast microscope at × 1000 magnification during various different stages of cell growth in YP and ISP 5 media at 37 °C. Gram staining was performed using a Difco Gram-stain set. Scanning electron microscopy (JSM-5600 LV; JEOL) was performed at × 10 000–15 000 magnification, using cells grown in YP medium at 37 °C for 12–18 h. Cells were then harvested, washed twice with water and dehydrated with increasing concentrations of ethanol (from 40 to 80 %, v/v) followed by increasing concentrations of isopropanol (20–100 %, v/v), mounted on 12 mm cover slips, dried in a vacuum desiccator for 12 h and, finally, coated with gold/palladium (80 : 20).

Peptidoglycan was purified from the cell wall and its composition was determined at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) as described by Schleifer & Kandler (1972) and Schleifer (1985).

Genomic DNA was extracted and purified as described by Sambrook *et al.* (1989). Universal primers corresponding to positions (*Escherichia coli* numbering) 8–27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492–1509R (5′-GGTTACCTTGTTACGACTT-3′) were used to amplify the 16S rRNA gene sequence of the novel isolate by PCR (Weisburg *et al.*, 1991). The PCR products were purified and sequenced as described previously (Quillaguamán *et al.*, 2004).

An almost complete sequence (1449 bp) of the 16S rRNA gene of strain DSM 17733^T was obtained. Sequences belonging to type strains of the genus *Nesterenkonia* available through the public databases (GenBank and Ribosomal Database Project II) were aligned and a similarity matrix was calculated (Maidak *et al.*, 2000). 16S rRNA gene sequence analysis was performed with the aid of the DNAMAN 4.03 software package by using the neighbour-joining method and the Jukes–Cantor distance correction method (Saitou & Nei, 1987); the stability of the grouping was estimated by bootstrap analyses (500 replications). Only unambiguously aligned positions from all sequences were used, and gaps were not included in the match/mismatch count.

In order to calculate G+C content, DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion *et al.* (1977). The G+C content was calculated from the ratio of deoxyguanosine to thymidine, according to the method of Mesbah *et al.* (1989), by the Identification Service of the DSMZ.

DNA–DNA hybridization was carried out at the DSMZ, as described by De Ley *et al.* (1970) with the modification described by Huß *et al.* (1983) and Escara & Hutton (1980), using a Gilford System model 2600 spectrometer equipped with a Gilford model 2527-R thermoprogrammer and plotter. Renaturation rates were computed with the TRANSFER.BAS program (Jahnke, 1992).

16S rRNA gene sequence analysis placed the novel isolate in the family *Micrococcaceae* with a close relationship to members of *Nesterenkonia* (Fig. 1). The closest sequence similarities were found with *N. xinjiangensis* YIM 70097^T (98 %; AY226510) and *N. halobia* DSM 20541^T (97 %; X80747).

The phenotypic features of strain DSM 17733^T were investigated and compared with those of other species of the genus *Nesterenkonia*; differential characteristics are listed in Table 1. Strain DSM 17733^T produced circular, convex, smooth, entire, opaque colonies that were white in colour but turned yellow in old cultures grown on solid LB medium. We have observed a similar change in the colour of colonies of *N. xinjiangensis* grown on LB medium. Microscopic examination of strain DSM 17733^T at various stages of growth showed the cells to be non-motile, non-endospore-forming, non-capsule-forming, short rods around 0·7 μm in width and 1·2 μm in length (see Supplementary Fig. S1 available in IJSEM Online). In unstained preparations, cells occur singly, in pairs and in irregular clumps (Supplementary Fig. S1).

Growth of the isolate in YP liquid medium was uniform, the broth was turbid and no pellicles were produced. The isolate was strictly aerobic and mesophilic, exhibiting good growth at 25–40 °C with an optimum between 30 and 37 °C. It was able to grow at pH values in the range 7–11, with optimal growth at pH 9. Variation of the NaCl concentration in the medium showed that good growth occurred at 3–8 % (w/v) salt, maximal growth being observed at 3 % (w/v) NaCl.

Like *N. halobia*, the isolate exhibited sensitivity to ampicillin, chloramphenicol, nalidixic acid, streptomycin and kanamycin, but showed resistance to erythromycin and tetracycline (Stackebrandt *et al.*, 1995). The minimal inhibitory concentrations of the antibiotics were as follows: ampicillin, 10 μg; tetracycline, 30 μg; chloramphenicol, 30 μg; nalidixic

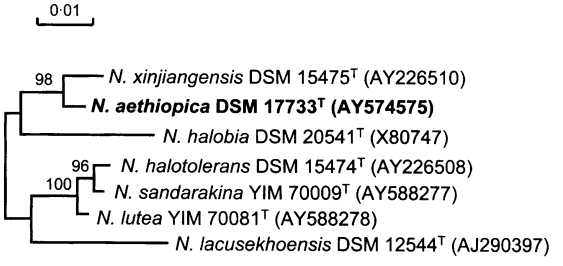

Fig. 1. Dendrogram showing phylogenetic relationships derived from 16S rRNA gene sequence analysis of strain DSM 17733^T with respect to *Nesterenkonia* species with validly published names. The tree was constructed using the neighbour-joining method; bootstrap values are expressed as percentages of 500 replications. The sequence of *Streptomyces megasporus* DSM 41476^T (Z68100) was used as the root (not shown). Accession numbers used in the phylogenetic analysis are given in parentheses.

Table 1. Comparison of the phenotypic characteristics of strain DSM 17733^T and *Nesterenkonia* species with validly published names

Strains: 1, *N. aethiopica* sp. nov. DSM 17733^T; 2, *N. halobia* DSM 20541^T; 3, *N. lacusekhoensis* DSM 12544^T; 4, *N. halotolerans* DSM 15474^T; 5, *N. xinjiangensis* DSM 15475^T; 6, *N. sandarakina* YIM 70009^T; 7, *N. lutea* YIM 70081^T. All of the strains are Gram-positive, aerobic and moderately halophilic. +, Positive; −, negative; W, weak reaction; ND, not determined. Data for reference strains were taken from Collins *et al.* (2002), Li *et al.* (2004, 2005) and Stackebrandt *et al.* (1995).

Characteristic	1	2	3	4	5	6	7
Cell shape	Short rods	Cocci	Short rods	Cocci	Short rods	Cocci	Cocci
Colony colour	Yellow	Unpigmented	Bright yellow	Deep orange–yellow	Light yellow	Orange–yellow	Light yellow to primrose yellow
Motility	−	−	−	+	−	−	+
Temperature optimum (°C)	30–37	30	27–33·5	28	28	28	28
NaCl range for growth (%, w/v)	3–12	5–23	0–15	0–25	0–25	1–15	0–20
pH range for growth	7·0–11·0	<6·0–10·0	7·5–9·5	7·0–9·0	7·0–12·0	5·0–12	6·5–10
Citrate test	−	−	W	ND	ND	ND	ND
Oxidase	+	+	−	−	−	−	−
Sulfide formation	−	−	W	−	−	−	−
ONPG test	−	+	ND	ND	ND	ND	ND
Acid production from:							
D-Galactose	−	W	−	−	−	ND	ND
D-Lactose	−	+	−	−	−	ND	ND
D-Mannitol	−	+	−	−	−	ND	ND
D-Trehalose	−	−	+	−	−	ND	ND
D-Xylose	−	+	−	−	−	ND	ND
Utilization of:							
L-Arabinose	+	+	ND	+	+	W	+
D-Mannose	−	−	+	ND	+	+	W
Glycerol	+	−	W	−	ND	ND	W
D-Xylose	−	+	ND	−	+	+	+
D-Trehalose	−	−	+	+	−	+	ND
Sucrose	W	−	+	+	+	+	+
D-Lactose	−	ND	−	ND	+	−	−
Hydrolysis of:							
Gelatin	+	−	−	+	+	+	−
Tyrosine	−	+	ND	ND	ND	ND	ND
Casein	+	+	ND	−	+	−	−
Starch	+	+	−	−	−	−	−
DNA G+C content (mol%)	69·0	71·5	66·1	64·4	66·7	64·0	64·5

acid, 30 μg; kanamycin, 10 μg; novobiocin, 30 μg; streptomycin, 10 μg; erythromycin, 50 μg.

Analysis of the cell wall showed the presence of murein of the type L-Lys–Gly–L-Glu, as in *N. halobia* (Stackebrandt *et al.*, 1995) and *N. xinjiangensis* (Li *et al.*, 2004); this differed from the L-Lys–L-Glu reported for *N. lacusekhoensis* (Collins *et al.*, 2002) and the L-Lys–Gly–D-Asp found in *N. halotolerans* (Li *et al.*, 2004), *N. lutea* and *N. sandarakina* (Li *et al.*, 2005).

As shown in Table 1, the DNA G+C content of strain DSM 17733^T was found to be 69·0 mol%, which is within the range (64–72 mol%) reported in the emended description of the genus *Nesterenkonia* (Li *et al.*, 2005). Furthermore, DNA–DNA hybridization analysis of strain DSM 17733^T revealed 48·5 % relatedness to *N. halobia* DSM 20541^T, 63·7 % (repetition, 57·2 %) to *N. lacusekhoensis* DSM 12544^T and 35·7 % (repetition, 29·3 %) to *N. xinjiangensis* DSM 15475^T. These values were below 70 %, the threshold value recommended for the delineation of genomic species (Wayne *et al.*, 1987).

On the basis of notable differences in the phenotypic and phylogenetic features, the G+C content and the relatively low levels of DNA–DNA relatedness to species belonging to the genus *Nesterenkonia*, we conclude that strain DSM 17733^T represents a novel alkaliphilic, moderately halophilic *Nesterenkonia* species, for which the name of *Nesterenkonia aethiopica* sp. nov. is proposed.

Description of *Nesterenkonia aethiopica* sp. nov.

Nesterenkonia aethiopica (L. fem. adj. *aethiopica* pertaining to Ethiopia).

Cells are Gram-positive, non-motile, non-capsule-forming, non-endospore-forming, short rods around 0·7 μm in width and 1·2 μm in length. Catalase- and oxidase-positive and urease-negative. Strictly aerobic and mesophilic: exhibits good growth at 25–40 °C, with an optimum between 30 and 37 °C; unable to grow at 45 °C. Alkaliphilic and moderately halophilic. Optimal growth occurs at initial pH 9 (range pH 7–11) and at 3 % (w/v) NaCl (range 3–12 %). Carbohydrates utilized as sole carbon sources are D-glucose, D-fructose and other sugars listed in Table 1. Acid is produced in medium supplied with D-glucose, D-fructose, L-arabinose and glycerol, but acidification is not detected with D-trehalose, D-galactose, D-lactose, D-mannitol or D-xylose. Starch, casein, tyrosine and gelatin are hydrolysed. Indole is not produced and the Voges–Proskauer test is negative. The DNA G+C content is 69·0 mol% (determined by HPLC). Cell wall has murein of the type L-Lys–Gly–L-Glu.

The type strain, DSM 17733^T (=CCUG 48939^T), was isolated from a soiled feather sample collected at the shore of Lake Abjata, Ethiopia.

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