Kocuria aegyptia sp. nov., a novel actinobacterium isolated from a saline, alkaline desert soil in Egypt

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A coccoid, non-motile actinobacterium, designated strain YIM 70003^T, was isolated from a saline, alkaline, desert-soil sample from Egypt. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the organism formed a distinct phyletic line within the genus *Kocuria* and was most closely related to *Kocuria polaris* DSM 14382^T (98·6 % sequence similarity) and *Kocuria rosea* DSM 20447^T (98·2 %). Chemotaxonomic data, including the Lys–Ala_3 peptidoglycan type, the presence of phosphatidylglycerol and diphosphatidylglycerol as the predominant phospholipids, the presence of MK-8(H_2) and MK-9(H_2) as the major menaquinones, the predominance of fatty acids ai-$C_{15:0}$ and i-$C_{15:0}$ and the DNA G+C content, also supported the affiliation of the isolate to the genus *Kocuria*. The low DNA–DNA relatedness with *K. polaris* DSM 14382^T (56·6 %) and *K. rosea* DSM 20447^T (15·5 %) in combination with phenotypic data show that strain YIM 70003^T should be classified as a novel species of the genus *Kocuria*. The name *Kocuria aegyptia* sp. nov. is proposed, with strain YIM 70003^T (=CCTCC AA203006^T=CIP 107966^T=KCTC 19010^T=DSM 17006^T) as the type strain.

The genus *Kocuria* was established by Stackebrandt *et al.* (1995) by taxonomic dissection of the genus *Micrococcus*, and was clearly separated from *Micrococcus* and *Micrococcus*-related taxa on the basis of phylogenetic analyses using 16S rRNA gene sequences. Members of *Kocuria* are Gram-positive, aerobic, non-encapsulated, non-halophilic, non-endospore-forming cocci characterized by the presence of menaquinones MK-7(H_2) and MK-8(H_2), lysine-based peptidoglycan variation A3α, phosphatidylglycerol and diphosphatidylglycerol as the major phospholipids, a predominance of saturated branched fatty acids such as ai-$C_{15:0}$ and a genomic DNA G+C content in the range 60–75 mol% (Stackebrandt *et al.*, 1995; Boháček *et al.*, 1969; Kloos *et al.*, 1974; Kocur *et al.*, 1971; Kovács *et al.*, 1999; Reddy *et al.*, 2003; Kim *et al.*, 2004). At the time of writing, there are eight *Kocuria* species with validly published names: *Kocuria kristinae*, *K. palustris*, *K. polaris*, *K. rhizophila*, *K. rosea*, *K. varians*, *K. marina* and *K. carniphila* (Stackebrandt *et al.*, 1995; Kovács *et al.*, 1999; Reddy *et al.*, 2003; Kim *et al.*, 2004; Tvrzová *et al.*, 2005). Of these species, only *K. marina* was isolated from a high-salinity environment (Kim *et al.*, 2004). In this paper, we report a polyphasic taxonomic study of strain YIM 70003^T isolated from a saline, alkaline, desert-soil sample collected in Egypt.

Strain YIM 70003^T was isolated on modified Horikoshi agar medium (Horikoshi & Grant, 1998) using the dilution plating method. This medium contained the following (g l^{-1}): glucose, 10·0; peptone, 5·0; yeast extract, 5·0; KH_2PO_4, 1·0; $MgSO_4.7H_2O$, 0·2; Na_2CO_3, 10·0; and agar, 15; pH, 10·0–10·5. Sodium carbonate was sterilized separately and then added to the medium. $NaHCO_3$/Na_2CO_3 buffer was used to adjust the pH. The purified strain was maintained on Horikoshi agar slants at 4 °C and as 20 % (w/v) glycerol suspensions at −20 °C. Biomass for chemical and molecular studies was obtained by cultivation using Horikoshi broth (28 °C, 1 week, 150 r.p.m.).

Gram staining was carried out by using the standard Gram reaction and was confirmed by using the KOH lysis test (Cerny, 1978). Morphology and motility were examined by using light microscopy (model BH 2; Olympus) and

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 70003^T is DQ059617.

Table 1. Differentiating characteristics of *K. aegyptia* YIM 70003^T and other *Kocuria* species

Species/strains: 1, *K. rosea*; 2, *K. varians*; 3, *K. kristinae* (data in columns 1–3 taken from Stackebrandt *et al.*, 1995); 4, *K. palustris* (Kovács *et al.*, 1999); 5, *K. rhizophila* (Kovács *et al.*, 1999); 6, *K. polaris* (Reddy *et al.*, 2003 unless indicated); 7, *K. marina* (Kim *et al.*, 2004); 8, *K. carniphila* (Tvrzová *et al.*, 2005); 9, *K. aegyptia* sp. nov. YIM 70003^T (this study). +, Positive; −, negative; V, variable; W, weak; ND, no data available. Data for all species except *K. rosea*, *K. varians* and *K. kristinae* are based on the type strains.

Characteristic	1	2	3	4	5	6	7	8	9
Pigment colour	Pink or red	Yellow	Pale cream to pale orange	Pale yellow	Yellow	Orange	Orange	Yellow	Pink
Oxidase reaction	−	−	+	−	−	+	−	−	−
Phosphatase	−	−	−	−	+	−	−	−	ND
Nitrate reduction	+	+	−	+	−	+	+	+	−
H_2S production	−	−	−	W	W	ND	−	ND	−
Hydrolysis of:									
Gelatin	−	V	−	−	+	−*	+	−	−
Starch	V	V	−	−	−	+	−	−	−
Tween 80	V	−	−	−	+	+*	−	−	−
Urea	−	+	V	+	−	−	+	−	−
Growth at/in:									
37 °C	+	+	+	−	+	−	+	+	+
0 % NaCl	+	+	+	+	+	+	+	+	−
5 % NaCl	+	+	+	+	+	−	+	+	+
7·0/7·5 % NaCl	+	+	+	+	+	−	+	+	−
10 % NaCl	−	+	+	−	+	−	+	W	−
Chemotaxonomy									
Major menaquinone(s)	MK-8(H_2)	MK-7(H_2)	MK-7(H_2), MK-8(H_2)	MK-7(H_2)	MK-7(H_2), MK-8(H_2)	MK-7(H_2), MK-8(H_2)	ND	MK-7(H_2)	MK-8(H_2), MK-9(H_2)
Major fatty acid(s)	ai-$C_{15:0}$	ai-$C_{15:0}$	ai-$C_{15:0}$	ai-$C_{15:0}$	ai-$C_{17:0}$, ai-$C_{15:0}$, i-$C_{15:0}$	ai-$C_{15:0}$	ai-$C_{15:0}$	ai-$C_{15:0}$	ai-$C_{15:0}$, i-$C_{15:0}$
DNA G+C content (mol%)	66–75	66–72	67·0	69·6	69·4	72·5	60·0	71·0	73·0

*Based on the report of Tvrzová *et al.* (2005).

electron microscopy (JEM-1010; JEOL) with cells from exponentially growing cultures. Colony morphology was observed on Horikoshi medium after incubation at 28 °C for 3 days. The colony colour was determined using ISCC–NBS colour charts (Kelly, 1964). Growth was tested in Horikoshi broth at 4, 10, 20, 28, 37, 40, 45 and 55 °C. The pH growth range and optimum were investigated at pH 4·0–13·0 using the buffer system described by Xu *et al.* (2005). Liquid cultures were cultivated in tubes at 28 °C for 2–3 weeks using ISP 2 as the basic medium. Growth at different concentrations (0, 1, 3, 7, 10, 15 and 20 %) of sodium, potassium, magnesium and calcium chlorides was tested, again in ISP 2 basic medium. Metabolic properties were determined using API ID 32E test kits (bioMérieux) according to the manufacturer's instructions. Other physiological and biochemical tests were performed as described previously (Gonzalez *et al.*, 1978).

The cells of strain YIM 70003^T were Gram-positive, aerobic, non-motile, non-endospore-forming, coccoid and about 0·8–1·1 μm in diameter. The colonies were pink, circular, slightly convex, opaque and had a maximum diameter of about 2 mm after incubation at 28 °C for 48 h on Horikoshi agar medium. No diffusible pigments were produced on any of the media. The isolate was catalase-positive and gave a negative oxidase reaction. Detailed physiological and biochemical properties are given in Table 1 and in the species description.

The chemotaxonomic properties, including peptidoglycan type, cell-wall sugars, phospholipids, menaquinones and whole-cell fatty acid pattern, were analysed as described previously (Li *et al.*, 2004). Strain YIM 70003^T possessed peptidoglycan type Lys–Ala_3, variation A3α. Galactose and minor amounts of glucose were detected in the purified cell wall. The phospholipids consisted of phosphatidylglycerol and diphosphatidylglycerol. The menaquinones were represented by MK-8(H_2), MK-9(H_2), MK-7(H_2), MK-6(H_2) and MK-10(H_2) (27 : 8·5 : 2·5 : 1·5 : 1, respectively). The major fatty acids were ai-$C_{15:0}$ (55·3 %) and i-$C_{15:0}$ (20·4 %).

Extraction of genomic DNA and PCR amplification of 16S rRNA gene were performed as described by Xu *et al.* (2003). Multiple alignments with sequences of the most closely related actinobacteria and calculations of levels of sequence similarity were carried out using CLUSTAL X (Thompson *et al.*, 1997). A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987) from K_{nuc} values (Kimura, 1980, 1983) by using MEGA version 2.1 (Kumar *et al.*, 2001). The topology of the phylogenetic tree was evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The almost-complete 16S rRNA gene sequence (1492 bp) for strain YIM 70003^T was determined. Phylogenetic analysis revealed that the strain's closest relative were *K. polaris* DSM 14382^T and *K. rosea* DSM 20447^T, showing respective 16S rRNA gene sequence similarities of 98·6 and 98·2 % (Fig. 1). The DNA G+C content was determined as 73·0 mol% by using the thermal denaturation method of Marmur & Doty (1962). DNA–DNA relatedness was studied using the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992) on a UV-Vis spectrophotometer (model UV1601; Shimadzu). The DNA–DNA hybridization values for strain YIM 70003^T with respect to *K. polaris* DSM 14382^T and *K. rosea* DSM 20447^T were 56·6 and 15·5 %, respectively.

The results of comparative 16S rRNA sequence analysis clearly demonstrate that strain YIM 70003^T is a member of the genus *Kocuria*. The chemotaxonomic characteristics of strain YIM 70003^T, such as peptidoglycan type, major fatty acids and DNA G+C content, were consistent with its assignment to the genus *Kocuria*.

The DNA–DNA relatedness among strains YIM 70003^T, *K. polaris* DSM 14382^T and *K. rosea* DSM 20447^T is below 70 %, which indicates that the novel isolate represents a distinct genospecies (Wayne *et al.*, 1987) of the genus *Kocuria*. The relatively large proportion of menaquinone MK-9(H_2) (21 %), the fatty acid profile and some other phenotypic properties (e.g. pigmentation, NaCl and temperature tolerance, oxidase reaction, nitrate reduction and hydrolysis results) of strain YIM 70003^T differentiate it from other members of the genus *Kocuria* at the phenotypic level (Table 1).

On the basis of its phenotypic and genotypic properties, it is proposed that strain YIM 70003^T represents a novel species of the genus *Kocuria*, for which the name *Kocuria aegyptia* sp. nov. is proposed.

Description of *Kocuria aegyptia* sp. nov.

Kocuria aegyptia (ae.gyp′ti.a. L. fem. adj. *aegyptia* from Egypt, referring to the country of isolation of the type strain).

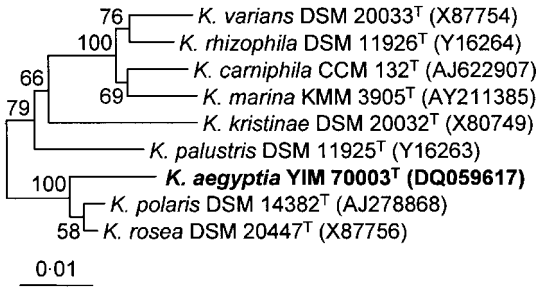

Fig. 1. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequences, showing the position of strain YIM 70003^T among its phylogenetic neighbours. Numbers on branch nodes are bootstrap percentages (1000 resamplings); only values over 50 % are shown. The sequence of *Streptomyces megasporus* DSM 41476^T (Z68100) was used as a root (not shown). Bar, 1 % sequence divergence.

Cells are Gram-positive, coccoid, occur in pairs, tetrads or clusters, are non-motile and do not form endospores. Colonies are pink, circular, opaque and approximately 2 mm in diameter. Cannot grow in ISP 2 medium without salt, but can grow in ISP 2 medium containing 1–5 % NaCl, 1–10 % KCl, 1–5 % $MgCl_2.6H_2O$ or 1–5 % $CaCl_2$; optimum growth occurs at 3 %, but at 5 % in the case of KCl. The temperature range for growth is 20–40 °C, with optimum growth occurring at 28 °C. The pH range for growth is 5·0–12·0, with optimum growth occurring between at pH 10·0–10·5. The oxidase test with tetramethyl-*p*-phenylenediamine is negative. Negative for urease, *N*-acetylglucosaminidase, L-aspartic arylamidase, β-galactosidase, α-galactosidase, α-maltosidase, β-glucuronidase, Tweens 20 and 80, esterase, tyrosinase, in methyl red and Voges–Proskauer tests, for melanin, indole and H_2S production, nitrate reduction, gelatin liquefaction, milk peptonization and coagulation and for starch hydrolysis; the catalase reaction is positive. Produces ornithine decarboxylase, arginine dihydrolase, lysine decarboxylase, lipase, β-glucosidase and ammonia. Maltose, D-glucose, D-cellobiose, D-trehalose, D-sorbitol, D-fructose, D-mannose and dextrin can each be utilized as a sole carbon source; acid is produced only from D-fructose. The peptidoglycan type is Lys–Ala_3, variation A3α. The cell-wall sugars consist mainly of galactose and minor amounts of glucose. The phospholipids are phosphatidylglycerol and diphosphatidylglycerol. The predominant menaquinones are MK-8(H_2) and MK-9(H_2). The major cellular fatty acids are ai-$C_{15:0}$ (55·3 %) and i-$C_{15:0}$ (20·4 %). The G+C content of the DNA is 73·0 mol%.

The type strain is YIM 70003^T (=CCTCC AA203006^T=CIP 107966^T=KCTC 19010^T=DSM 17006^T) and was isolated from a saline, alkaline, desert-soil sample collected from Egypt.

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