Kocuria flava sp. nov. and *Kocuria turfandensis* sp. nov., airborne actinobacteria isolated from Xinjiang, China

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Two coloured bacteria were isolated from the same plate for detecting cultivable bacteria from the air of Xinjiang in China. Phylogenetic analyses based on 16S rRNA gene sequences revealed that the isolates were members of the genus *Kocuria*, in which they represented two novel lineages. Although the two strains presented high 16S rRNA gene sequence similarity (above 97 %), their DNA G + C contents were very different (6 mol%). The G + C contents of strains HO-9041^T and HO-9042^T are 71 and 65 mol%, respectively. DNA relatedness analysis and other taxonomic evidence supports the placement of the two isolates in the genus *Kocuria*. The diagnostic diamino acid of the cell-wall peptidoglycan is L-lysine and both strains contain MK-8(H₂) and MK-9(H₂) as major menaquinones. In addition, they share similar fatty acid patterns containing straight-chain saturated and iso- and anteiso-branched acids, with a major component being anteiso-C_{15:0}. Genotypic, morphological and physiological characteristics are used to describe two novel species of *Kocuria*, for which the names *Kocuria flava* sp. nov. (type strain HO-9041^T = CCTCC AB 206106^T = KCTC 19306^T) and *Kocuria turfandensis* sp. nov. (type strain HO-9042^T = CCTCC AB 206107^T = KCTC 19307^T) are proposed.

At the time of writing, the genus *Kocuria* Stackebrandt *et al.* 1995 contains 11 species with validly published names: *Kocuria rosea* (the type species), *K. varians*, *K. kristinae* (Stackebrandt *et al.*, 1995), *K. erythromyxa* (Rainey *et al.*, 1997; regarded as a later synonym of *K. rosea* by Schumann *et al.*, 1999), *K. palustris*, *K. rhizophila* (Kovács *et al.*, 1999), *K. polaris* (Reddy *et al.*, 2003), *K. marina* (Kim *et al.*, 2004), *K. carniphila* (Tvrzová *et al.*, 2005), *K. aegyptia* (Li *et al.*, 2006) and *K. himachalensis* (Mayilraj *et al.*, 2006). These species were recovered from such varied environments as a saline, alkaline desert (*K. aegyptia*), meat (*K. carniphila*), marine sediment (*K. marina*), the rhizoplane of narrow-leaved cattail (*K. palustris*, *K. rhizophila*) and an Antarctic cyanobacterial mat sample (*K. polaris*). The members of this genus are Gram-positive, aerobic, coccoid, non-encapsulated, non-halophilic and non-endospore-forming, with the presence of the fatty acid anteiso-C_{15:0} and MK-7(H₂) and MK-8(H₂) as the major menaquinones. In the

present study, strains HO-9041^T and HO-9042^T were identified as members of novel species of the genus *Kocuria* on the basis of a polyphasic taxonomy study.

The two strains were isolated from a TGYA plate containing (l⁻¹) 5.0 g tryptone, 3.0 g yeast extract, 1.0 g glucose and 16.0 g agar. Colonies of the two strains were different; colonies of HO-9041^T were yellow, whereas HO-9042^T formed orange (after 48 h on TGYA plates) to red (after 4 days) colonies.

Isolation of genomic DNA of the two strains was carried out according to the method described by Yoon *et al.* (1996). Fragments comprising nearly the full-length 16S rRNA gene were amplified by a PCR using primers described by Rainey *et al.* (1996). The PCR products were then sequenced by the Invitrogen Corporation. Phylogenetic analysis revealed that both strains were closely related to the type strains of *K. rosea*, *K. polaris* and *K. aegyptia* (Fig. 1), with 16S rRNA gene similarities higher than 97 % (sequence similarity calculated by NCBI align two sequences and listed in Table 1). Multiple alignments with sequences of the most closely related actinobacteria were carried out using CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic trees were constructed using the neighbour-joining method (Fig. 1) and maximum-parsimony method by using MEGA version 3.1 (Kumar *et al.*, 2004). The

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains HO-9041^T and HO-9042^T are EF602041 and DQ531634.

Detailed fatty acid profiles of the novel strains and related type strains are available as supplementary material with the online version of this paper.

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on 16S rRNA gene sequences, showing the phylogenetic relationship between strains HO-9041^T and HO-9042^T and other members of the genus *Kocuria*. *Micrococcus luteus* DSM 20030^T (Wieser *et al.*, 2002) was used as an outgroup. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are given at nodes. Bar, 0.5 % sequence variation.

topology of the phylogenetic tree by the neighbour-joining method was evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The DNA G + C contents were 71 mol% (HO-9041^T) and 65 mol% (HO-9042^T) as determined by the HPLC method (Kumagai *et al.*, 1988). DNA–DNA hybridizations were carried out with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989). All of the determined DNA–DNA hybridization values were less than 70 % (Table 1) (Stackebrandt & Goebel, 1994).

Morphological analyses were performed by using a standardized method (Murray *et al.*, 1994). Physiological tests, such as growth at different temperatures (5, 10, 28, 30, 42 and 45 °C), pH (5.5, 6, 7, 8, 9, 9.5, 10, 10.5, 11, 11.5 and 12) and NaCl concentrations (1, 3, 5, 8, 10, 15 and 17 %), were performed by growing the strains in TGY broth under appropriate test conditions. Catalase activity, indole production, the methyl red and Voges–Proskauer

tests, oxidase and gelatinase activities, starch hydrolysis (amylase), nitrate reduction and urease, phosphatase, β -galactosidase and β -glucuronidase activities were assessed as described by Smibert & Krieg (1994) (Table 2). Low concentrations (20 μ g ml⁻¹) of antibiotics were used to detect resistance of the strains; both strains were sensitive to kanamycin, ampicillin, carbenicillin, chlortetracycline and erythromycin.

The utilization of various carbon compounds as sole carbon sources was tested using Biolog GP2 MicroPlates in accordance with the manufacturer's instructions with no adjustments. Strains were cultivated on BUG agar (Biolog catalogue no. 71101) with 5 % sheep blood at 30 °C for 24–48 h. Inoculated plates were incubated for 24 h and the results were read with a MicroPlate Reader using the Microlog 4.2 computer software to perform automated readings. Some results are listed in Table 2, and the others are shown in the species description

A cell wall hydrolysate was prepared by the method of Komagata & Suzuki (1987). The peptidoglycan of both strains contained L-lysine as the diagnostic diamino acid, as analysed by two-dimensional TLC on cellulose plates using the solvent systems of Schleifer & Kandler (1972). In addition, extraction of menaquinones and determination of menaquinone profiles by HPLC was done as described previously (Xie & Yokota, 2003). Strain HO-9041^T contained MK-8(H₂) (69 %), MK-9(H₂) (18 %) and MK-7(H₂) (13 %) and HO-9042^T possessed MK-8(H₂) (46 %), MK-9(H₂) (31 %) and MK-7(H₂) (27 %).

For cellular fatty acid analysis, the strains were grown on TSA at 30 °C for 24 h and cellular fatty acids were extracted, methylated and analysed by using the Sherlock Microbial Identification System (MIDI) according to the manufacturer as described previously (Pandey *et al.*, 2002); detailed fatty acid profiles are given in Supplementary Table S1, available in IJSEM Online.

Based on the results of phylogenetic analysis and other properties, strains HO-9041^T and HO-9042^T are proposed as the type strains of two novel *Kocuria* species, respectively named *Kocuria flava* sp. nov. and *Kocuria turfanensis* sp. nov.

Table 1. 16S rRNA gene sequence similarity and DNA–DNA hybridization results

Values are percentages of 16S rRNA gene sequence similarity/DNA–DNA relatedness. 16S rRNA gene sequences of reference type strains were obtained from previous studies and some reference strains for hybridization were obtained from the DSMZ and CCTCC. ND, Not determined.

Strain	HO-9041 ^T	HO-9042 ^T
Strain HO-9041 ^T	(100)/(100)	–
Strain HO-9042 ^T	97.9/35.2	(100)/(100)
<i>K. rosea</i> DSM 20447 ^T	97.8/58.3	98.2/34.5
<i>K. polaris</i> MTCC3702 ^T	97.6/45.3	98.1/52.9
<i>K. aegyptia</i> YIM 70003 ^T	97.1/60.8	98.3/53.8
<i>K. palustris</i> DSM 11925 ^T	96.8/ND	96.5/ND
<i>K. himachalensis</i> DSM 44905 ^T	96.5/ND	96.8/ND
<i>K. carniphila</i> CCM 132 ^T	96.6/ND	96.7/ND
<i>K. marina</i> KMM 3905 ^T	96.2/ND	96.5/ND
<i>K. rhizophila</i> DSM 11926 ^T	96.2/ND	96.5/ND
<i>K. varians</i> DSM 20033 ^T	96.5/ND	96.1/ND
<i>K. kristinae</i> DSM 20032 ^T	95.4/ND	95.8/ND

Table 2. Differential physiological characteristics of strains HO-9041^T and HO-9042^T and type strains of closely related species

Strains: 1, HO-9041^T; 2, HO-9042^T; 3, *K. rosea* DSM 20447^T; 4, *K. polaris* MTCC3702^T; 5, *K. aegyptia* YIM 70003^T. All data are from the present study. +, Positive; −, negative; w, weakly positive. All strains were negative for gelatinase, indole production and the methyl red and Voges–Proskauer tests and positive for catalase. In Biolog GP2 tests, all strains were positive for the utilization of dextrin, glycogen, Tweens 40 and 80, D-fructose, α-D-glucose, maltose, maltotriose, D-mannose, D-psicose, D-ribose, D-sorbitol, sucrose, turanose, D-xylose, acetic acid, α-, β- and γ-hydroxybutyric acids, α-ketovaleic acid, D-lactic acid methyl ester, L-lactic acid, L-malic acid, succinic acid monomethyl ester, pyruvic acid, glycerol, adenosine, thymidine and uridine and negative for mannan, N-acetyl-D-glucosamine, D-arabitol, L-fucose, methyl β-D-galactoside, methyl α-D-mannoside, salicin, sedoheptulosan, glycyl L-glutamic acid and thymidine 5'-monophosphate.

Characteristic	1	2	3	4	5
Colony colour*	Y	O–R	O–R	O	O–P
Ranges for growth					
NaCl (%)	0–10	0–15	0–5	0–1	1–5
Temperature (°C)	30–45	30–40	30–37	5–28	30–37
pH	7–9	6–9.5	6–9	7–12	5.5–11
Nitrate reduction	+	w	+	+	–
Urease	+	–	–	–	–
Oxidase	–	–	+	+	–
Phosphatase	+	+	–	–	w
β-Galactosidase	w	+	–	–	–
Amylase	+	+	–	+	–
Carbon-source utilization					
α-Cyclodextrin	+	+	–	–	–
L-Arabinose	+	–	w	+	–
D-Gluconic acid	+	–	w	–	–
Pyruvic acid methyl ester	–	+	–	–	+
Methyl β-D-glucoside	+	–	–	–	–
N-Acetyl-L-glutamic acid	–	+	w	w	–
α-Ketoglutaric acid	+	–	+	+	+
α-D-Lactose	+	–	+	w	–
Inulin	+	–	w	–	–
p-Hydroxyphenylacetic acid	+	+	–	–	–
DNA G + C content (mol%)	71	65	70.2	72.3	73

*O, Orange; P, pink; R, red; Y, yellow.

Description of *Kocuria flava* sp. nov.

Kocuria flava (fla'va. L. fem. adj. *flava* yellow, referring to the colony colour of the type strain).

Gram-positive, aerobic, non-motile and coccoid cells. Positive for catalase, nitrate reduction, amylase, phosphatase and urease, but negative for oxidase, gelatinase and β-glucuronidase. Production of indole and acetoin (Voges–

Proskauer reaction) is negative. Growth can occur in the absence of NaCl and in the presence of up to 10 % NaCl, at 28–45 °C and pH 7–9. Substrates utilized as sole carbon sources are given in Table 2. In addition, β-cyclodextrin, D-galactose, myo-inositol, D-mannitol, melibiose, methyl α-D-galactoside, palatinose, raffinose, stachyose, D-tagatose, trehalose, D-malic acid, propionic acid, succinamic acid, succinic acid, L-alaninamide, L-alanyl glycine, L-asparagine, L-glutamic acid, L-pyroglyutamic acid, L-serine, putrescine, 2,3-butanediol, 2'-deoxyadenosine, inosine, adenosine 5'-monophosphate, D-fructose 6-phosphate, α-D-glucose 1-phosphate, D-glucose 6-phosphate and DL-α-glycerol phosphate can be utilized, whereas N-acetyl-D-glucosamine, amygdalin, arbutin, cellobiose, D-galacturonic acid, gentiobiose, lactulose, 3-methyl D-glucose, lactamide, alanine and uridine 5'-monophosphate are not utilized. The type strain is sensitive to kanamycin, ampicillin, carbenicillin, chlortetracycline and erythromycin. Contains the major cellular fatty acid 15:0 anteiso and the predominant quinones MK-8(H₂) and MK-9(H₂) and contains L-lysine as the diagnostic diamino acid. The G + C content of the genomic DNA of the type strain is 71 mol%.

The type strain is HO-9041^T (=CCTCC AB 206106^T =KCTC 19306^T), isolated from the air.

Description of *Kocuria turfanensis* sp. nov.

Kocuria turfanensis (tur.fan.en'sis. N.L. fem. adj. *turfanensis* referring to Turfan, China, the geographical origin of the type strain).

Gram-positive, aerobic, non-motile and coccoid cells. Positive for catalase, nitrate reduction, amylase, phosphatase and β-galactosidase, but negative for oxidase, gelatinase, urease, β-glucuronidase and urease. Production of indole and acetoin (Voges–Proskauer reaction) is negative. Growth can occur in 15 % NaCl; however, NaCl is not necessary for growth. Growth is also detected at 30–40 °C and pH 6.5–9. Substrates utilized as sole carbon sources are given in Table 2. In addition, β-cyclodextrin, D-galactose, myo-inositol, D-mannitol, melibiose, methyl α-D-galactoside, palatinose, raffinose, trehalose, 2'-deoxyadenosine and inosine are also utilized. Other substrates in the Biolog GP2 plate are not utilized. The type strain is sensitive to kanamycin, ampicillin, carbenicillin, chlortetracycline and erythromycin. Contains the major cellular fatty acids 15:0 anteiso and 15:0 iso and the predominant menaquinones MK-8(H₂) and MK-9(H₂) and contains L-lysine as the diagnostic diamino acid. The G + C content of the genomic DNA of the type strain is 65 mol%.

The type strain is HO-9042^T (=CCTCC AB 206107^T =KCTC 19307^T), isolated from the air.

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