Note

Kocuria polaris sp. nov., an orange-pigmented psychrophilic bacterium isolated from an Antarctic cyanobacterial mat sample

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Strain CMS 76or^T, an orange-pigmented bacterium, was isolated from a cyanobacterial mat sample from a pond located in McMurdo Dry Valley, Antarctica. On the basis of chemotaxonomic and phylogenetic properties, strain CMS 76or^T was identified as a member of the genus *Kocuria*. It exhibited a 16S rDNA similarity of 99.8 % and DNA–DNA similarity of 71 % with *Kocuria rosea* (ATCC 186^T). Phenotypic traits confirmed that strain CMS 76or^T and *K. rosea* were well differentiated. Furthermore, strain CMS 76or^T could be differentiated from the other reported species of *Kocuria*, namely *Kocuria kristinae* (ATCC 27570^T), *Kocuria varians* (ATCC 15306^T), *Kocuria rhizophila* (DSM 11926^T) and *Kocuria palustris* (DSM 11025^T), on the basis of a number of phenotypic features. Therefore, it is proposed that strain CMS 76or^T (=MTCC 3702^T = DSM 14382^T) be assigned to a novel species of the genus *Kocuria*, as *Kocuria polaris*.

The genus *Kocuria* was created from the genus *Micrococcus* on the basis of the phylogenetic and chemotaxonomic dissection of the genus *Micrococcus* (Stackebrandt *et al.*, 1995). At the time of writing, the genus *Kocuria* contained five species, i.e. *Kocuria rosea*, *Kocuria varians*, *Kocuria kristinae*, *Kocuria palustris* and *Kocuria rhizophila*. All of the recognized species of *Kocuria* are coccoid, Gram-positive, non-endospore-forming, aerobic, non-halophilic micro-organisms that can be differentiated from other genera in the order *Actinomycetales* on the basis of their peptidoglycan type (L-Lys–Ala_{3/4}), the presence of galactosamine and glucosamine as their major cell-wall amino sugars, the presence of MK-7(H₂) and MK-8(H₂) as their major menaquinones, the presence of diphosphatidylglycerol and phosphatidylglycerol, the presence of the fatty acid anteiso-C_{15:0} and the G + C content of their DNA, which ranges from 66 to 75 mol%. In addition, all of the reported species have been observed to be mesophilic (Stackebrandt *et al.*, 1995). In the present study, a psychrophilic bacterium

(strain CMS 76or^T) isolated from a cyanobacterial mat sample originating from a pond in Antarctica was identified as a novel species of the genus *Kocuria* on the basis of its morphological, chemotaxonomic and phylogenetic properties. We propose the name *Kocuria polaris* for this novel species.

Strain CMS 76or^T was isolated from a cyanobacterial mat sample (predominantly containing *Phormidium* sp.) collected from pond L9 (77°33'3"S, 160°38'E) located in Wright Valley, McMurdo, Antarctica (Matsumoto, 1993). The mat sample (200 mg) was processed, plated onto Antarctic bacterial medium (ABM) plates containing 0.5 % (w/v) peptone, 0.2 % (w/v) yeast extract and 1.5 % (w/v) agar (pH 6.9) and incubated at 5 °C (Shivaji *et al.*, 1992) for 15 days. The colonies that appeared were predominantly white in colour, except those of strains CMS 76or^T and CMS 90r^T, which were orange ('or' suffix) and red ('r' suffix), respectively. Strain CMS 90r^T has subsequently been described as *Arthrobacter roseus* (Reddy *et al.*, 2002a).

The shape and motility of cultures from the lag, exponential and stationary phases of growth were observed by phase-contrast microscopy (magnification, ×1000). Motility was determined by the hanging drop method, and staining of the flagellum was done by the silver impregnation method (Blenden & Goldberg, 1965). All enzyme tests were performed by growing strains at 20 °C in the appropriate

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Abbreviation: ABM, Antarctic bacterial medium.

The EMBL accession number for the 16S rDNA sequence of *Kocuria polaris* CMS 76or^T is AJ278868.

Pigment characteristics of strain CMS 76or^T are available as supplementary data in IJSEM Online (<http://ijs.sgmjournals.org>).

medium according to standard methods (Holding & Collee, 1971). The production of indole, the methyl red test, the Voges-Proskauer test, levan formation, reduction of nitrate to nitrite, hydrolysis of starch and aesculin, and the formation of acid and gas were also monitored according to standard methods (Hugh & Leifson, 1953; Stanier *et al.*, 1966; Holding & Collee, 1971; Stolp & Gadkari, 1981). The abilities of strain CMS 76or^T, *K. rosea* ATCC 186^T and *K. rosea* ATCC 187 (formerly the type strain of *Kocuria erythromyxa*) to utilize various carbon compounds as sole carbon sources were tested using minimal medium [1.05 % K₂HPO₄, 0.45 % KH₂PO₄, 0.1 % (NH₄)₂SO₄, 0.5 % carbon source]. Determinations of sensitivity to different antibiotics and of the optimal temperature, pH and salt values for growth were done on ABM plates. *K. rosea* ATCC 186^T, *K. rosea* ATCC 187 and *Sphingobacterium antarcticum* ATCC 51969^T were used as controls in this study.

The phenotypic properties of strain CMS 76or^T are given in the species description for *K. polaris*. Characteristics useful for distinguishing strain CMS 76or^T from its closest relatives are listed in Tables 1 and 2.

The 16S rRNA gene of strain CMS 76or^T was amplified and sequenced as described previously (Shivaji *et al.*, 2000). The almost-complete 16S rDNA sequence of strain CMS 76or^T (1451 nt) was aligned with 14 reference sequences deposited in EMBL, using CLUSTAL V (Higgins *et al.*, 1992). Furthermore, following an initial crude taxonomic affiliation, a more-detailed analysis was done using the 16S rDNA sequences of members of the genera *Kocuria*, *Micrococcus*, *Nesterenkonia*, *Dermaococcus* and *Renibacterium*. The DNADIST program with the Kimura-2 correction model (Kimura, 1980) was used to compute the pairwise evolutionary distances from the aligned sequences. To obtain the confidence values for the 16S-rDNA-based genetic affiliations, the original sequence dataset was resampled 1000 times using SEQBOOT and subjected to bootstrap analysis. Phylogenetic trees were constructed using parsimony analysis (DNAPARS) and clustering algorithms such as FITCH, KITCH and the UPGMA linkage algorithm (Felsenstein, 1993). The different algorithms gave consistent results.

On the basis of 16S rDNA sequence similarity values, strain CMS 76or^T was most closely related to members of the genus *Kocuria* (96.1–99.8 % similarity) and exhibited highest similarity values with the sequence of the type strain of *K. rosea* (99.8 %). In the 16S-rDNA-based phylogenetic tree (Fig. 1), strain CMS 76or^T was observed to be positioned closest to *K. rosea* DSM 20447^T. The statistical significance of this lineage was high (bootstrap value, 100 %). It was observed that the overall genomic relatedness between strains CMS 76or^T and *K. rosea* ATCC 186^T, based on DNA–DNA hybridization performed using the membrane filter method (Reddy *et al.*, 2000b), was 71 %.

Determinations of the type of peptidoglycan (Rosenthal & Dziarski, 1994), the fatty acid composition (Sato & Murata,

1988), the types of menaquinone (Collins *et al.*, 1977; Dunphy *et al.*, 1971), the polar lipids and the cell-wall sugars were done according to standard methods, as described previously (Komagata & Suzuki, 1987). Furthermore, pigments were extracted from lyophilized bacterial cell pellets, separated by TLC, and the absorption spectra of the individual pigments were recorded using a Hitachi 2000 spectrophotometer (Jagannadham *et al.*, 1991). The G + C content (mol%) of the DNA of strain CMS 76or^T was determined as described previously (Reddy *et al.*, 2000).

The affiliation of strain CMS 76or^T with the genus *Kocuria* was further supported by its chemotaxonomic properties, since in strain CMS 76or^T, as in other species of the genus *Kocuria*, the peptidoglycan present was L-Lys-Ala₃, of the A3α peptidoglycan type. MK-7(H₂) (48 %) and MK-8(H₂) (43 %) were the major menaquinones of strain CMS 76or^T; MK-6(H₂) (5.6 %) and MK-9(H₂) (3.4 %) were the minor menaquinones. The major fatty acid was anteiso-C_{15:0} (70.6 %). Phosphatidylglycerol, diphosphatidylglycerol (cardiolipin), phosphatidylinositol and an unidentified phospholipid were the polar lipids of strain CMS 76or^T. The DNA G + C content of strain CMS 76or^T was 72.5 mol% (Tables 1 and 2). Strain CMS 76or^T also contained six carotenoid pigments (with R_F values of 0.92, 0.78, 0.61, 0.53, 0.46 and 0.32, respectively). Pigment characteristics of strain CMS 76or^T are available as supplementary data in IJSEM Online (<http://ijs.sgmjournals.org>).

Strain CMS 76or^T, like other species of the genus *Kocuria*, was an aerobic, non-halophilic, catalase-positive, indole-negative micro-organism that was sensitive to penicillin, chloramphenicol, ampicillin and erythromycin (Schleifer, 1986; Rainey *et al.*, 1997; Stackebrandt *et al.*, 1995; Kovács *et al.*, 1999). Furthermore, the chemotaxonomic characteristics of the strain and the topology of the 16S-rDNA-based phylogenetic tree (Fig. 1) clearly indicated that strain CMS 76or^T was a member of the genus *Kocuria*. At the 16S rDNA level, strain CMS 76or^T was closely related to all species of the genus *Kocuria* (96.1–99.8 % similarity), and exhibited maximum similarity with the 16S rDNA sequence of *K. rosea* ATCC 186^T. DNA–DNA hybridization data for strain CMS 76or^T and *K. rosea* ATCC 186^T indicated 71 % similarity between the two strains. Despite the similarity at the DNA level between the novel strain and *K. rosea* ATCC 186^T, strain CMS 76or^T differed from *K. rosea* ATCC 186^T, and from *K. kristinae* (ATCC 186^T), *K. varians* (ATCC 15306^T), *K. rhizophila* (DSM 11926^T) and *K. palustris* (DSM 11025^T), with respect to the following five phenotypic features: its ability to grow at 5 °C; its inability to grow in the presence of 5 % NaCl; its production of acid from D-mannitol and D-xylose; its high DNA G + C content (72.5 mol%) (Table 1). In addition to the above five differences, strain CMS 76or^T differed from *K. rosea* ATCC 186^T and *K. rosea* ATCC 187 in that it formed extremely mucoid colonies on ABM plates, produced acid from D-glucose and D-mannitol, and could utilize adonitol, D-melibiose, D-cellobiose, meso-inositol, lactose, pyruvate, inulin,

Table 1. Phenotypic characteristics useful for differentiating strain CMS 76or^T from *Kocuria* spp.

Strain: 1, CMS 76or^T; 2, *K. rosea* ATCC 186^T; 3, *K. rosea* ATCC 187; 4, *K. varians* ATCC 15306^T; 5, *K. kristinae* ATCC 27570^T; 6, *K. palustris* ATCC 11925^T; 7, *K. rhizophila* DSM 11926^T. NA, Data not available; ND, not done. +, Positive; –, negative; (+), weakly positive. *K. rosea* ATCC 187 was previously known as the type species of *K. erythromyxa*.

Characteristic	1*	2*	3*†	4‡	5‡	6§	7§
Major pigment colour	Orange	Orange/red	Orange/red	Yellow	Pale orange	Pale yellow	Yellow
Growth at:							
5 °C	+	–	–	–	–	–	–
37 °C	–	+	+	+	+	–	+
Growth in 5 % NaCl	–	+	+	+	+	(+)	+
Acid (produced aerobically) from:							
D-Glucose	+	–	–	+	+	+	+
D-Mannose	–	–	–	–	+	–	+
D-Mannitol	+	–	–	–	–	–	–
L-Rhamnose	+	–	+	–	–	–	–
D-Xylose	+	–	–	+	–	–	–
Aesculin hydrolysis	–	–	–	–	+	–	–
Starch hydrolysis	+	+	+	–	–	–	–
Gelatinase	–	–	–	(+)	–	–	+
Oxidase	+	+	+	–	+	–	–
Urease	–	–	–	+	+	+	–
Phosphatase	–	–	–	–	–	–	+
Nitrate reduction	+	+	+	+	–	+	–
Utilization of carbon source:							
D-Maltose	+	–	+	+	+	–	–
Adonitol	+	–	–	+	+	+	+
D-Melibiose	+	–	–	+	–	–	–
L-Arabinose	–	–	–	+	+	–	+
L-Alanine	–	–	–	–	+	–	–
D-Cellobiose	(+)	–	–	NA	NA	–	–
meso-Inositol	+	–	–	NA	NA	NA	NA
Lactose	+	–	–	NA	NA	NA	NA
D-Mannitol	–	–	+	–	+	–	–
Pyruvate	+	–	–	NA	NA	NA	NA
L-Arabinose	–	+	–	+	+	–	+
Inulin	+	–	–	NA	NA	NA	NA
D-Raffinose	(+)	–	–	NA	NA	NA	NA
L-Arginine	+	–	–	NA	NA	NA	NA
L-Aspartic acid	+	–	–	NA	NA	NA	NA
L-Asparagine	–	+	+	NA	NA	NA	NA
L-Glutamic acid	(+)	–	+	–	+	–	–
L-Leucine	+	–	–	NA	NA	NA	NA
L-Phenylalanine	+	–	–	NA	NA	NA	NA
Fermentation of sugar:							
Lactose	+	–	–	NA	NA	NA	NA
Sucrose	+	–	–	NA	NA	NA	NA
Menaquinones	MK-6(H ₂), MK-7(H ₂), MK-8(H ₂), MK-9(H ₂)	MK-7(H ₂)‡, MK-8(H ₂), MK-9(H ₂)	MK-6(H ₂), MK-7(H ₂), MK-8(H ₂), MK-9(H ₂)	MK-7(H ₂), MK-8(H ₂)	MK-7(H ₂), MK-8(H ₂)	MK-6(H ₂), MK-7(H ₂), MK-8(H ₂)	MK-6(H ₂), MK-7(H ₂), MK-8(H ₂), MK-9(H ₂)
DNA G + C content (mol%)	72.5	66–75†	71	66–72	67	69.6	69.4

*Data acquired in the present study.

†G + C data were taken from Brooks *et al.* (1980) and menaquinone data from Rainey *et al.* (1997).

‡Data from Stackebrandt *et al.* (1995).

§Data from Kovács *et al.* (1999).

Table 2. Fatty acid compositions of *K. rosea* ATCC 186^T and strain CMS 76or^T

Both strains were cultured in ABM at 22 °C.

Fatty acid	Fatty acid (percentage of total fatty acids)	
	<i>K. rosea</i> ATCC 186 ^T	CMS 76or ^T
iso-C _{14:0}	1.3	0.7
C _{14:0}	0.5	1.1
iso-C _{15:0}	5.1	3.2
anteiso-C _{15:0}	65.0	70.6
C _{15:0}	0.8	0.6
iso-C _{16:0}	3.0	0.9
C _{16:0}	3.2	2.4
C _{16:1}	3.5	2.2
iso-C _{17:0}	1.5	2.9
anteiso-C _{17:0}	7.1	5.6
anteiso-C _{17:1}	5.0	4.8
iso-C _{18:0}	0.9	3.0
C _{18:0}	2.9	1.2
C _{18:1}	—	0.9

Fig. 1. Phylogenetic relationship of strain CMS 76or^T with related species of the genera *Kocuria*, *Micrococcus*, *Derma-coccus* and *Nesterenkonia*, based on the 16S rDNA sequence analysis done using DNAPARS. *Escherichia coli* was used to root the tree. The bootstrap values (expressed as percentages) are given at the nodes. Branch lengths indicated in the tree are not to scale.

D-raffinose, L-arginine, L-aspartic acid, L-glutamic acid, L-leucine or L-phenylalanine as the sole carbon source. It was unable to utilize L-asparagine as the sole carbon source, but could ferment lactose and sucrose (Table 1). Furthermore, it was observed that in strain CMS 76or^T six carotenoid pigments were present, whereas in *K. rosea* ATCC 186^T only five pigments were present (supplementary data; <http://ijs.sgmjournals.org>). It was also observed in the present study that the type strain of *K. rosea* (ATCC 186^T) had MK-7(H₂) (33.4 %) and MK-8(H₂) (61.0 %) as its major menaquinones, with MK-9(H₂) (5.6 %) as a minor component. In contrast, strain CMS 76or^T had MK-7(H₂) (48 %) and MK-8(H₂) (43 %) as its major menaquinones and, in addition, small amounts of MK-6(H₂) (5.5 %) and MK-9(H₂) (3.4 %) (Table 1). It was also observed that when *K. rosea* ATCC 186^T and strain CMS 76or^T were cultured in ABM they exhibited distinct quantitative differences in their fatty acid compositions. For instance, in strain CMS 76or^T, iso-C_{14:0}, iso-C_{16:0} and C_{18:0} were decreased relative to *K. rosea* ATCC 186^T, whereas C_{14:0}, iso-C_{17:0} and iso-C_{18:0} were increased and C_{18:1} was also present (Table 2). Strain CMS 76or^T could also be differentiated from the other species of *Kocuria* on the basis of some of its phenotypic characteristics (Table 1). Thus, on the basis of the similarities of strain CMS 76or^T with respect to the generic characteristics of the genus *Kocuria*, and its distinct differences with respect to all recognized species of *Kocuria*, it is proposed that strain CMS 76or^T be assigned to a novel species of the genus *Kocuria*, as *Kocuria polaris*.

Description of *Kocuria polaris* sp. nov.

Kocuria polaris (po.la'ris. M.L. adj. *polaris* of, or pertaining to, a pole).

Cells are coccoid (1.0–1.5 µm in diameter), occurring in pairs, tetrads or clusters. Non-motile, Gram-positive and aerobic. Colonies on peptone/yeast extract medium are 0.1–2.0 mm in diameter and appear smooth, round, uniformly edged, translucent, mucoid and orange in colour. Psychrophilic. Grows between 5 and 30 °C, with optimum growth at 20 °C. Grows in ABM adjusted to pH 7–12 and tolerates up to 2.9 % NaCl. Catalase- and lipase-positive. Negative for L-lysine decarboxylase, L-arginine decarboxylase, β-galactosidase, L-arginine dihydrolase and levan formation. Negative results in the indole test, the methyl red test and the Voges–Proskauer test. Utilizes L-rhamnose, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol, D-mannose, D-xylose, acetate, sorbitol, L-glutamine, L-glycine, L-serine and L-threonine as sole carbon sources, but can not utilize *meso*-erythritol, melezitose, D-ribose, D-sorbose, citrate, dextran, lactic acid, dulcitol, sucrose, succinic acid, thioglycollate, D-trehalose, L-cysteine, L-histidine, L-isoleucine, L-lysine, L-proline, L-methionine, L-tyrosine, L-tryptophan, L-valine or β-hydroxybutyric acid as sole carbon sources. Does not produce gas from D-glucose, D-fructose, L-arabinose, D-xylose, L-rhamnose, D-galactose, D-mannose, D-mannitol, lactose or sucrose. Acid is produced in the presence of D-fructose, D-galactose,

lactose and sucrose. Oxidizes D-glucose, D-maltose, D-ribose, D-trehalose and D-xylose, but not L-arabinose or D-galactose. Ferments D-fructose, but not L-arabinose, D-galactose, D-glucose, D-maltose, D-ribose, D-trehalose or D-xylose. Other characteristics of the species can be found in Table 1. Sensitive to ampicillin, cefoperazone, co-trimoxazole, amikacin, cefazoline, ciprofloxacin, penicillin, vancomycin, tetracycline, lomefloxacin, erythromycin, roxithromycin, tobramycin, cephalexin, lincomycin, chloramphenicol, streptomycin, kanamycin, norfloxacin, amoxycillin and cefuroxime, but resistant to nitrofurantoin, nalidixic acid and colistin. Cell-wall peptidoglycan is L-Lys-Ala₃ (type A3 α). D-Galactose, D-glucose and D-ribose are the major cell-wall sugars. Menaquinones and fatty acid composition of the species are shown in Tables 1 and 2, respectively. Polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and an unidentified phospholipid. Cells contain six water-insoluble pigments that are soluble in methanol; pigment production is not dependent on any specific growth conditions or on the composition of the medium. DNA G+C content is 72.5 mol%. Isolated from a cyanobacterial mat sample from McMurdo Dry Valley, Antarctica (77°33'3"S, 160°38'E). The type strain is CMS 76or^T (=MTCC 3702^T =DSM 14382^T).

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