Kocuria marina sp. nov., a novel actinobacterium isolated from marine sediment

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An actinobacterial strain was isolated from marine sediment taken from the Troitsa Bay of the Gulf of Peter the Great, East Siberian Sea, and subjected to a taxonomic investigation. The isolate, designated KMM 3905^T, was Gram-positive, aerobic, non-motile and coccoid. The strain tolerated up to 15% NaCl in growth media, although its presence was not essential for growth. Chemotaxonomic and phylogenetic properties of the strain were consistent with its classification in the genus *Kocuria*. The strain formed an independent phylogenetic line in the 16S rRNA gene tree encompassed by members of *Kocuria*. Comparison of phenotypic characteristics also supported its assignment to a novel species within *Kocuria*. The name *Kocuria marina* sp. nov. (type strain KMM 3905^T = KCTC 9943^T) is proposed for this halotolerant actinobacterium from the marine environment.

The genus *Kocuria* was first proposed by Stackebrandt *et al.* (1995) to accommodate phylogenetically distinct actinobacteria formerly classified in the genus *Micrococcus*, within which heterogeneity had been suggested in earlier studies (Kloos, 1969; Ogasawara-Fujita & Sakaguchi, 1976). *Kocuria* is clearly separated from *Micrococcus* and other related Gram-positive cocci such as *Arthrobacter* and *Rothia* in phylogenetic analyses using 16S rRNA gene sequences (Stackebrandt *et al.*, 1995).

Six species with validly published names currently comprise the genus, namely *Kocuria kristinae*, *Kocuria palustris*, *Kocuria polaris*, *Kocuria rhizophila*, *Kocuria rosea* and *Kocuria varians* (Stackebrandt *et al.*, 1995; Kovács *et al.*, 1999; Reddy *et al.*, 2003). Members of *Kocuria* are Gram-positive, aerobic, coccoid, non-encapsulated, non-halophilic and non-endospore-forming. Their normal habitats include mammalian skin, soil, the rhizoplane and fresh water (Kloos *et al.*, 1974; Kocur, 1986; Kovács *et al.*, 1999). Little is known regarding their pathogenicity to humans and other mammals, but they are not considered to be a primary pathogen (Kocur, 1986; Kocur *et al.*, 1991).

Members of *Kocuria* are characterized by the presence of menaquinones MK-7(H₂) and MK-8(H₂), lysine-based A3 α -type peptidoglycan, PI-type phospholipids and

saturated branched fatty acids (anteiso-C_{15:0} as the major component) in their cell envelopes (Stackebrandt *et al.*, 1995). The G + C content of the genomic DNA ranges from 66 to 75 mol% (Boháček *et al.*, 1969; Kloos *et al.*, 1974; Kocur *et al.*, 1971; Kovács *et al.*, 1999; Reddy *et al.*, 2003).

There is no record on the isolation of strains belonging to the genus *Kocuria* from the marine environment, although many members exhibit tolerance to high salt concentrations (Stackebrandt *et al.*, 1995; Kovács *et al.*, 1999). During an investigation exploring bacterial diversity in the marine environment in the Gulf of Peter the Great, East Siberian Sea, a coccoid actinobacterial strain was isolated and designated KMM 3905^T. The strain was subjected to a polyphasic investigation, and its taxonomic position is discussed below.

Strain KMM 3905^T was isolated from a marine sediment sample taken from Troitsa Bay. After primary isolation and purification on marine agar 2216 (Difco) at 28 °C, the strain was subcultured on the same medium and stored at –80 °C in marine broth (Difco) supplemented with 20% (v/v) glycerol.

The presence of enzyme activities, degradation of organic compounds, growth on NaCl, production of acid from carbohydrates, susceptibility to antibiotics and other physiological characteristics of the organism were determined as described by Han *et al.* (2003). DNA extraction, PCR and sequencing of 16S rRNA genes were performed according to previously described procedures (Kim *et al.*,

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1998). The 16S rRNA gene sequence obtained was aligned together with those of representative members of *Kocuria* using PHYDIT version 3.2 (<http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred using suitable programs of the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated from the model of Kimura

Table 1. Phenotypic properties that separate KMM 3905^T and six other species of the genus *Kocuria*

Strain/species: 1, KMM 3905^T; 2, *K. kristinae*; 3, *K. palustris*; 4, *K. polaris*; 5, *K. rhizophila*; 6, *K. rosea*; 7, *K. varians*. Data for reference species were taken from Stackebrandt *et al.* (1995), Kovács *et al.* (1999) and Reddy *et al.* (2003). +, Positive; (+), weakly positive; –, negative; V, results variable between strains; ND, not determined.

Property	1	2	3	4	5	6	7
Oxidase	–	–	–	+	–	–	–
Alkaline phosphatase	–	–	–	–	+	–	–
Urease	+	–	+	–	–	–	+
Nitrate reduction	+	–	+	+	–	+	–
H ₂ S production	–	–	(+)	ND	(+)	–	–
Hydrolysis of:							
Gelatin	+	(+)	+	–	–	+	–
Starch	–	–	–	–	–	+	–
Tween 80	–	+	+	ND	+	–	+
Urea	+	–	+	–	–	–	+
Growth at/in:							
5 °C	+	–	–	+	–	–	–
37 °C	+	+	–	–	+	+	+
5 % NaCl	+	+	(+)	–	+	+	+
10 % NaCl	+	+	–	–	+	–	–
15 % NaCl	+	–	–	–	(+)	–	–
Utilization as sole carbon source:							
Adonitol	–	+	+	+	+	–	+
L-Arabinose	–	+	–	+	+	+	+
meso-Inositol	–	+	–	+	–	–	–
Mannitol	–	+	–	–	–	+	–
Mannose	+	+	–	ND	+	+	+
Melibiose	–						
Sorbitol	–	+	–	ND	–	+	+
Acid production from:							
L-Arabinose	–	–	–	ND	–	+	–
Galactose	–	–	+	ND	–	–	–
Glucose	–	+	+	+	+	+	V
Glycerol	–	+	–	ND	–	–	–
Lactose	–	–	–	ND	+	–	+
Maltose	–	+	–	ND	–	–	–
Mannitol	–	–	–	+	–	+	–
Sorbitol	–	+	–	ND	–	–	–
Sucrose	–	+	+	ND	+	–	–
Xylose	–	–	–	+	–	+	–
DNA G+C content (mol%)	60.0	67.0	69.6	72.5	69.4	66–75	66–72

(1980) and phylogenetic trees were constructed following the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) algorithms. *Micrococcus luteus* (M38242) was used as an outgroup to infer the root position (data not shown). Bootstrap analysis was performed with 1000 resampled datasets, using the SEQBOOT and CONSENSE programs of PHYLIP. Extraction of menaquinones and analysis by HPLC were carried out using a standard procedure (Minnikin *et al.*, 1984). To determine fatty acid methyl esters, strains were cultivated on marine agar (Difco) at 25 °C for 24–48 h. Extraction and analysis of cellular fatty acids were performed according to the procedures for the SHERLOCK Microbial Identification System (MIDI Inc.).

Strain KMM 3905^T formed aerobic, catalase-positive, coccoid, Gram-positive cells. The strain was able to grow in the presence of up to 15 % NaCl, although its presence was not required for growth (Table 1). The G+C content of the genomic DNA was 60 mol%. The cell envelope contained a major amount of 12-methyl-tetradecanoic acid (anteiso-C_{15:0}), and also smaller amounts of 14-methyl hexadecanoic acid (anteiso-C_{17:0}) and 14-methyl pentadecanoic acid (iso-C_{16:0}; Table 2). The dominance of anteiso-C_{15:0} is consistent for *Kocuria*, although the test was carried out in different media than reported previously (Table 2). The morphological, phenotypic and

Table 2. Fatty acid composition of KMM 3905^T and six other species of the genus *Kocuria*

Strain/species: 1, KMM 3905^T; 2, *K. kristinae*; 3, *K. palustris*; 4, *K. polaris*; 5, *K. rhizophila*; 6, *K. rosea*; 7, *K. varians*. Data for reference species were taken from Stackebrandt *et al.* (1995), Kovács *et al.* (1999) and Reddy *et al.* (2003).

Fatty acid	1	2	3	4	5	6	7
iso-C _{14:0}	1.9	2.6	2.2		1.1	1.8	1.4
C _{14:0}	2.3	1.0	3.1	1.1	2.4	1.5	2.9
iso-C _{15:0}	2.5	1.6	1.0	3.2	13.8	7.6	1.3
anteiso-C _{15:0}	74.0	70.1	59.2	70.6	48.4	70.9	60.3
C _{15:0}							1.3
C _{15:1}		12.1	1.0				
iso-C _{16:0}	7.0	1.7	3.3		5.9	1.6	11.1
C _{16:0}	2.9		2.1	2.4	2.6	1.6	9.6
C _{16:1}				2.2		5.9	
iso-C _{17:0}		9.3		2.9	1.2		
anteiso-C _{17:0}	8.4		9.7	5.6	21.1	4.3	12.1
anteiso-C _{17:1}			3.0	4.8		3.0	
iso-C _{18:1}			4.6				
iso-C _{18:0}				3.0			
C _{18:0}				1.2			
C _{18:1}							
C _{18:2}			1.4				

chemotaxonomic descriptions of strain KMM 3905^T are also consistent with its assignment to the genus *Kocuria* (Stackebrandt *et al.*, 1995; Kovács *et al.*, 1999).

In the phylogenetic analysis using 16S rRNA gene sequences, KMM 3905^T formed an independent line within the *Kocuria* tree (Fig. 1). KMM 3905^T had 16S rRNA gene sequence similarities ranging from 96.2 to 98.2 % with other species of *Kocuria*. KMM 3905^T was most closely related to *K. rhizophila* DSM 11926^T and *K. varians* DSM 20033^T, and the topology of the branch for KMM 3905^T and these two species was reproducible in the trees using least-squares and maximum-likelihood methods. *K. rhizophila* and *K. varians* have a close relationship with each other, sharing 98.6 % 16S rRNA gene sequence similarity and are always recovered as a single cluster group in previous analyses. However, the two species are clearly distinguished on the basis of phenotypic and genotypic data such as physiological properties and DNA–DNA relatedness (Kovács *et al.*, 1999; Reddy *et al.*, 2003). The high bootstrap value between KMM 3905^T and the *K. rhizophila*–*K. varians* group also strongly supported the tree topology of the three taxa (Fig. 1), indicating that the branching of KMM 3905^T and the *K. rhizophila*–*K. varians* group is stable. 16S rRNA gene sequence analysis clearly supports strain KMM 3905^T as representing a separate phylogenetic lineage.

Strain KMM 3905^T and other members of *Kocuria* were also separated on the basis of phenotypic characteristics; KMM 3905^T grew at both 4 and 43 °C and also in the

presence of 15 % NaCl (Table 1). Strain KMM 3905^T showed different results in at least 11 tested characters from any other known species of *Kocuria*. The DNA G + C content of KMM 3905^T was 60 mol%, the lowest reported among the members of the genus.

Total membrane fatty acids of members of *Kocuria* consist of major amounts of branched, saturated species (over 75 % of the total), with minor amounts of straight or unsaturated fatty acids. In contrast, saturated species comprise over 90 % of the total in all members (Table 2; Stackebrandt *et al.*, 1995; Kovács *et al.*, 1999; Reddy *et al.*, 2003). KMM 3905^T, even allowing that the data were obtained from marine 2216 agar, has a unique fatty acid profile in that anteiso-C_{15:0} comprises 74 % of the total, though this is similar to the profiles for *K. rhizophila* and *K. varians*. KMM 3905^T, *K. rhizophila* and *K. varians* are also unique in that they do not contain any significant proportion of unsaturated fatty acids, whereas other species of *Kocuria* contain at least 7 % unsaturated components.

From the polyphasic taxonomic evidence reported here, KMM 3905^T clearly merits species status within *Kocuria*, for which we propose the name *Kocuria marina* sp. nov.

Description of *Kocuria marina* sp. nov.

Kocuria marina (ma.ri'na. L. fem. adj. *marina* of the sea).

Gram-positive, aerobic, non-motile, halotolerant coccoid cells. Positive for catalase, β -galactosidase and urease, but negative for arginine dihydrolase, lysine and ornithine decarboxylase, oxidase and alkaline phosphatase. Nitrate is reduced, but hydrogen sulphide is not produced. Production of indole and acetoin (Voges–Proskauer reaction) is negative. Growth occurs in the presence of up to 15 % NaCl, although its presence is not required for growth. Grows at 4–43 °C. Casein, gelatin and Tween 40 are hydrolysed, but agar, alginate, cellulose, DNA, starch, Tween 20 and Tween 80 are not. Glucose, lactose, mannose and sucrose are utilized as sole carbon sources, but not adonitol, L-arabinose, *meso*-inositol, mannitol, sorbitol, citric acid or malonic acid. Acid is produced from L-fucose, but not from acetic acid, *N*-acetylglucosamine, adonitol, L-arabinose, cellobiose, dulcitol, fumaric acid, galactose, glucose, glycerol, *meso*-inositol, lactose, malic acid, maltose, mannitol, melibiose, raffinose, rhamnose, sorbitol, sorbose, sucrose and L-xylose. Growth is inhibited by ampicillin, benzylpenicillin, carbenicillin, gentamicin, lincomycin, neomycin, oleandomycin, streptomycin and tetracycline, but not by kanamycin or polymyxin B. Contains major amounts of branched, saturated fatty acids with 14 to ~17 carbons, among which 12-methyl-tetradecanoic acid (anteiso-C_{15:0}) is the predominant form. The G + C content of the genomic DNA is 60 mol%.

The type and only strain is KMM 3905^T (=KCTC 9943^T), isolated from marine sediment.

Fig. 1. Neighbour-joining tree based on the 16S rRNA gene sequences of representative *Kocuria* species and strain KMM 3905^T. Kimura's two-parameter model was used to calculate phylogenetic distances among species. The topology of the entire tree was conserved in all trees using two different algorithms, namely Fitch–Margoliash and maximum-likelihood methods. Numbers at nodes are levels of bootstrap support from 1000 resampled datasets. Bar, 0.01 nucleotide substitutions per position.

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