Reclassification of strain CCM 132, previously classified as *Kocuria varians*, as *Kocuria carniphila* sp. nov.

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A Gram-positive actinobacterium, previously classified as *Kocuria varians*, was subjected to a polyphasic taxonomic study. The bacterium showed the peptidoglycan type Lys–Ala₃ (variation A3 α), MK-7(H₂) was the major menaquinone and anteiso-C_{15:0} and anteiso-C_{17:0} were the major fatty acids. On the basis of the phylogenetic and phenotypic characteristics of the actinobacterium, a novel species, *Kocuria carniphila* sp. nov. (type strain, CCM 132^T = DSM 16004^T), is proposed.

Strain CCM 132 was deposited in the CCM as '*Micrococcus lactis*' in 1959. This strain, isolated from meat (Kocur & Martinec, 1962), was extensively studied in the 1960s and 1970s (Boháček *et al.*, 1967; Jeffries *et al.*, 1969; Kocur *et al.*, 1971) and was classified as *Micrococcus varians*. The genus *Kocuria* was established by Stackebrandt *et al.* (1995) by taxonomic dissection of the genus *Micrococcus* and consequently CCM 132 was reclassified as *Kocuria varians*. Because the phenotypic features of strain CCM 132 differed from those of the *K. varians* species description, strain CCM 132 was subjected to a detailed taxonomic study.

Strain CCM 132 was cultivated aerobically on tryptone soya medium (Oxoid) at 28 °C. Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of PCR products were carried out as described previously (Rainey *et al.*, 1996). Purified PCR products were sequenced using the CEQ DTCS Quick Start kit (Beckman Coulter), as directed in the manufacturer's protocol. The CEQ 8000 Genetic Analysis System was used for electrophoresis of the sequence reaction products.

The ae2 editor (Maidak *et al.*, 1999) was used to align the 16S rRNA gene sequence of CCM 132 against those

of representatives of the main bacterial lineages available from the public databases. A phylogenetic tree was constructed, the topology of which was evaluated by bootstrap resampling (Felsenstein, 1993) with 1000 replicates. An almost-complete 16S rRNA gene sequence, of 1515 nucleotides, was determined for strain CCM 132 ranging from position 15 (5') to 1536 (3') (*Escherichia coli* numbering; Brosius *et al.*, 1978). Phylogenetic analyses based on a dataset consisting of 1406 unambiguous nucleotides between positions 42 and 1458 showed that strain CCM 132 represented a distinct lineage within the radiation of the genus *Kocuria*, in a tight phylogenetic group together with *Kocuria rhizophila* and *K. varians* (bootstrap resampling value, 99 %). The highest binary 16S rRNA similarity values were found with *K. rhizophila* DSM 11926^T (97.4 %) and *K. varians* DSM 20033^T (96.9 %). The phylogenetic tree showing the placement of CCM 132 in the genus *Kocuria* is available as supplementary material in IJSEM Online.

DNA for DNA–DNA hybridization was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970), with the modifications described by Huss *et al.* (1983) and Escara & Hutton (1980), using a model 2600 spectrophotometer equipped with a model 2527-R thermoprogrammer and plotter (Gilford Instrument Laboratories). Renaturation rates were computed with the TRANSFER.BAS program of Jahnke (1992). The DNA–DNA reassociation value between strain CCM 132 and its closest phylogenetic neighbour, *K. rhizophila*

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A phylogenetic tree showing the placement of strain CCM 132^T in the genus *Kocuria* is available as supplementary material in IJSEM Online.

DSM 11926^T, was 36.2%, clearly below the 70% value considered to be the threshold for the delineation of genomic species (Wayne *et al.*, 1987).

A new species of the genus *Kocuria*, *Kocuria marina*, has been described recently (Kim *et al.*, 2004). A comparison of the 16S rRNA gene sequences of the type strain of *K. marina* and strain CCM 132 revealed 97.9% similarity between these two taxa. The type strains were subjected to DNA–DNA hybridization. The DNA–DNA reassociation value between strain CCM 132 and *K. marina* DSM 16420^T was 28.0% (repetition, 26.4%), indicating that the strains belong to distinct genomic species. Moreover, CCM 132 differs from *K. marina* DSM 16420^T (Kim *et al.*, 2004) by negative hydrolysis of gelatin and urea and by positive production of acid from lactose and glucose.

The affiliation of strain CCM 132 to the genus *Kocuria* was confirmed by chemotaxonomic features that are characteristic of the genus *Kocuria* (Stackebrandt *et al.*, 1995). The peptidoglycan structure was determined by using hydrolysates of purified cell walls according to Schleifer (1985). The amino acids and peptides were separated by two-dimensional ascending TLC on cellulose plates with the solvent systems of Schleifer & Kandler (1972). The molar ratio of the amino acids was determined by GC as described by MacKenzie (1987). Menaquinones were extracted according to Collins *et al.* (1977) and analysed by using HPLC (Groth *et al.*, 1996). Cells for cellular fatty acid analysis were harvested from 24 h cultures grown at 28 °C on tryptone soya medium. Fatty acids were extracted and analysed following the instructions of the Microbial Identification System operating manual (MIDI, 1999). The peptidoglycan of strain CCM 132 contained lysine, glutamic acid and alanine in a molar ratio of about 0.8:1.0:5.0, respectively. Based on these data and on the occurrence of characteristic peptides in the partial hydrolysate of the peptidoglycan (data not shown), the peptidoglycan type is consistent with the type Lys–Ala₃, variation A3 α (Schleifer & Kandler, 1972), A11.6 according to the DSMZ Catalogue of strains (DSMZ, 2001). The interpeptide bridge of the peptidoglycan of strain CCM 132 containing three alanine residues (type A11.6) was also detected in the type strains of *Kocuria polaris* (Reddy *et al.*, 2003), *Kocuria palustris* and *K. rhizophila* (Kovács *et al.*, 1999), and in *Kocuria rosea* DSM 11630 (Schleifer & Kandler, 1972). However, the type strains of *K. rosea* and *K. varians*, as well as the *K. varians* strains DSM 20319 and DSM 20320, were reported to contain four alanine residues in the interpeptide bridge (type A11.7; Schleifer & Kandler, 1970, 1972; DSMZ, 2001). The menaquinone pattern of strain CCM 132, with MK-7(H₂):MK-8(H₂):MK-6(H₂), peak area ratio 88:3:4, corresponds to that of *K. varians* DSM 20033^T in its high content of MK-7(H₂) (Stackebrandt *et al.*, 1995), but differentiates the isolate from the closely related species *K. rhizophila* DSM 11926^T, which also contains significant amounts of MK-8(H₂); MK-7(H₂):MK-8(H₂):MK-6(H₂):MK-9(H₂), 51:39:4:1 (Kovács *et al.*, 1999). The

cellular fatty acids content of strain CCM 132 was as follows. The major fatty acids were anteiso-C_{15:0} (68.0%) and anteiso-C_{17:0} (12.8%). anteiso-C_{13:0} (0.2%), iso-C_{14:0} (2.3%), C_{14:0} (1.8), iso-C_{15:0} (3.8%), iso-C_{16:0} (3.5%), C_{16:0} (5.0%) and iso-C_{17:0} (0.6%) occurred in minor amounts. The fatty acid profile was in agreement with data reported for other *Kocuria* species (Stackebrandt *et al.*, 1995; Kovács *et al.*, 1999; Reddy *et al.*, 2003). The fatty acid methyl ester profile of strain CCM 132 differed from that of *K. rhizophila* DSM 11926^T by a higher amount of anteiso-C_{15:0} and lower amounts of iso-C_{15:0} and anteiso-C_{17:0} (Kovács *et al.*, 1999).

The G+C content of the DNA of strain CCM 132 was 71 mol% (Boháček *et al.*, 1967), which is in agreement with the description of the genus *Kocuria* (Stackebrandt *et al.*, 1995).

For the comparative phenotypic studies, strain CCM 132 and type strains of *Kocuria* species [*K. rhizophila* DSM 11926^T (= CCM 4950^T), *K. varians* DSM 20033^T (= CCM 884^T), *K. palustris* DSM 11925^T (= CCM 4949^T), *K. polaris* DSM 14382^T, *K. rosea* DSM 20447^T (= CCM 679^T) and *K. kristinae* DSM 20032^T (= CCM 2690^T)] were routinely cultivated on tryptone soya medium (Oxoid) at 28 °C. Standardized methods described previously (Barrow & Feltham, 1993) and API ID 32 STAPH, API STAPH and Biolog GP MicroPlate systems were used for phenotypic characterization of strain CCM 132. The commercial kits were applied according to the manufacturer's instructions. The colonies of strain CCM 132 grown aerobically on nutrient agar were yellow-coloured, circular, convex and opaque. Strain CCM 132 can be easily differentiated from all *Kocuria* species by physiological characteristics (Table 1). Additional phenotypic characteristics are given below in the species description.

Based on the results of phylogenetic analysis and its chemotaxonomic and physiological properties, it is proposed to reclassify strain CCM 132 (= DSM 16004) as a novel *Kocuria* species, *Kocuria carniphila* sp. nov.

Description of *Kocuria carniphila* sp. nov.

Kocuria carniphila (car.ni'phi.la. L. n. caro gen. carnis meat; Gr. fem. adj. philos loving; N.L. fem. adj. carniphila meat-loving).

Cells are coccoid, 1–1.5 µm in diameter, occurring in pairs and tetrads, Gram-positive, non-motile, non-acid-fast and non-spore-forming. Colonies are yellow, circular, convex and opaque. Obligately aerobic. Good growth at 28–37 °C. Growth over pH range 7.0–9.1. Very weak growth in the presence of 10% (w/v) NaCl. Catalase-, β -galactosidase- and β -glucuronidase-positive. Negative for oxidase, Voges–Proskauer test, arginine dihydrolase, ornithine decarboxylase, urease, phosphatase and pyrrolidonyl arylamidase. Nitrate is reduced to nitrite. Nitrite is not reduced. Starch, gelatin, Tween 80 and aesculin are not hydrolysed.

Table 1. Differentiating characteristics of *Kocuria carniphila* CCM 132^T and type strains of *Kocuria* species

Strains: 1, *K. carniphila* sp. nov. CCM 132^T; 2, *K. rhizophila* DSM 11926^T; 3, *K. varians* DSM 20033^T; 4, *K. palustris* DSM 11925^T; 5, *K. polaris* DSM 14382^T; 6, *K. rosea* DSM 20447^T; 7, *K. kristinae* DSM 20032^T. +, Positive reaction; –, negative reaction; w, weak reaction.

Characteristic	1	2	3	4	5	6	7
Urease	–	–	+	+	–	–	–
β-Galactosidase	+	–	+	+	+	–	–
β-Glucuronidase	+	–	–	–	–	–	–
Nitrate reduction	+	–	+	+	+	+	–
Hydrolysis of:							
Tween 80	–	+	–	–	+	–	–
Gelatin	–	+	w	–	+	–	w
Acidification of:							
Mannose	–	–*	–	–	w	–	+
Lactose	+	–	+	+	w	–	–
Trehalose	–	–	–	–	w	–	+
Sucrose	w	–*	–	+	+	–	+
Utilization of:							
N-Acetyl-D-mannosamine	+	–	–	–	–	–	–
D-Galacturonic acid	+	–	–	–	–	–	–
Methyl α-D-galactoside	+	–	–	–	–	–	–
3-Methyl glucose	+	–	w	–	–	–	+
β-Hydroxybutyric acid	+	–	+	–	+	–	–
L-Malic acid	–	+	+	–	+	+	+
N-Acetylglutamic acid	+	–	+	–	–	–	–
Uridine	–	+	+	+	–	w	–
Glucose 6-phosphate	+	–	+	–	–	w	–

*Reaction different from data published previously (Stackebrandt *et al.*, 1995; Kovács *et al.*, 1999; Reddy *et al.*, 2003).

Acidification of glucose, fructose and lactose is positive. Weak acid production from maltose and sucrose. Acid production is negative from mannose, raffinose, trehalose, mannitol, xylitol, melibiose, xylose, turanose, ribose, cellobiose, methyl α-D-glucopyranoside, N-acetylglucosamine and arabinose. The following compounds are utilized: dextrin, Tween 40, Tween 80, N-acetyl-D-glucosamine, N-acetyl-D-mannosamine, amygdalin, D-fructose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, D-gluconic acid, α-D-glucose, myo-inositol, maltotriose, D-mannitol, D-mannose, D-melibiose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl glucose, D-psicose, D-ribose, sucrose, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylacetic acid, α-ketoglutaric acid, β-ketovaleric acid, L-lactic acid, mono-methyl succinate, propionic acid, pyruvic acid, succinic acid, N-acetyl-glutamic acid, L-glutamic acid, L-pyrogutamic acid, glycerol, thymidine 5'-monophosphate and glucose 6-phosphate. α-Cyclodextrin, β-cyclodextrin, D-arabitol, arbutin, D-galactose, lactulose, D-melezitose, methyl α-D-glucoside, methyl β-D-glucoside, methyl

α-D-mannoside, palatinose, D-raffinose, salicin, sedoheptulosan, D-tagatose, xylitol, lactamide, D-lactic acid methyl ester, D-malic acid, L-malic acid, alaninamide, D-alanine, L-alanine, L-alanyl glycine, glycyl-L-glutamic acid, putrescine, inosine, uridine, adenosine 5'-monophosphate and uridine 5'-monophosphate can not be utilized. The cell wall diamino acid is lysine. The peptidoglycan type is Lys-Ala₃ (variation A3α). The major menaquinone is MK-7(H₂). The cellular fatty acid pattern is dominated by anteiso-C_{15:0}. The G+C content is 71 mol%.

The type strain is CCM 132^T (=DSM 16004^T), which was isolated from meat.

Note added in proof

A new species of the genus *Kocuria*, *K. marina*, was described as this report was being prepared for press (Kim *et al.*, 2004). Consequently, details of a comparison of the type strain of that species, DSM 16420^T, with *K. carniphila* CCM 132^T have been added to the text.

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