Kocuria koreensis sp. nov., isolated from fermented seafood

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A Gram-positive, aerobic, non-motile and coccoid actinobacterium, designated P31^T, was isolated from a traditional, fermented seafood. The strain was catalase-positive and oxidase-negative. Cells grew in the presence of 0–15.0 % (w/v) NaCl, and at pH 5–10 and 15–37 °C. Major cellular fatty acids were anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$. Strain P31^T contained MK-7 as the predominant menaquinone. The DNA G+C content of the genomic DNA of strain P31^T was 65.2 mol%. A phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain P31^T was most closely related to *Kocuria kristinae* DSM 20032^T, with 96.9 % similarity, and these two strains clustered together in constructed phylogenetic trees. The DNA–DNA hybridization value between strain P31^T and *K. kristinae* DSM 20032^T was 21.1 %. On the basis of the phenotypic, chemotaxonomic and phylogenetic data, it is suggested that strain P31^T represents a novel species of the genus *Kocuria*, for which the name *Kocuria koreensis* sp. nov. is proposed. The type strain is P31^T (=KCTC 19595^T=JCM 15915^T).

The genus *Kocuria* was first proposed by Stackebrandt *et al.* (1995) on the basis of a detailed phylogenetic and chemotaxonomic analysis of the genus *Micrococcus* (Stackebrandt *et al.*, 1995). Fifteen species, namely *Kocuria aegyptia*, *K. carniphila*, *K. erythromyxa*, *K. flava*, *K. gwangalliensis*, *K. halotolerans*, *K. himachalensis*, *K. kristinae*, *K. marina*, *K. palustris*, *K. polaris*, *K. rhizophila*, *K. rosea*, *K. turfanensis* and *K. varians*, currently comprise the genus *Kocuria* (Stackebrandt *et al.*, 1995; Rainey *et al.*, 1997; Kovacs *et al.*, 1999; Reddy *et al.*, 2003; Kim *et al.*, 2004; Tvrzová *et al.*, 2005; Mayilraj *et al.*, 2006; Li *et al.*, 2006; Zhou *et al.*, 2008; Seo *et al.*, 2009; Tang *et al.*, 2009). The aim of this study was to characterize a strain of the genus *Kocuria*, which was isolated from a traditional, fermented seafood in Korea.

A novel bacterium, designated P31^T, was isolated from jeotgal in Korea by using the standard dilution-plating method at 30 °C on marine agar 2216 (MA; BBL) medium. The isolate was subcultured several times to obtain a pure culture. To determine the optimum culture conditions of strain P31^T, growth under various conditions was tested. Temperatures for growth were tested on MA at 4, 10, 15, 20, 25, 30, 37 and 40 °C. The optimum pH range for growth was determined in marine broth (MB; BBL) adjusted to pH 3–10, at intervals of 1.0 pH unit, with HCl or NaOH. NaCl tolerance was tested in MB prepared without NaCl to which NaCl was added to the final concentrations 0.5, 1, 2, 3, 5, 10, 15, 20, 25 and 30 %, w/v (Gordon *et al.*, 1974; Reichert *et al.*, 1998; Liu *et al.*, 2006). The optimum growth conditions were used for the routine cultivation of strain P31^T for physiological and morphological tests. The Gram-staining reaction was performed using a Gram-stain kit (BBL). Motility was determined by the use of semi-solid agar (Tittsler & Sandholzer, 1936). Morphological characteristics were observed using a light microscope (E600; Nikon) and by transmission electron microscopy. API ZYM and API 20 NE test strips (bioMérieux) and GP2 MicroPlates (Biolog) were used to determine enzyme activities and substrate utilization. Catalase activity was determined by observing bubble production in a 3 % (v/v) hydrogen peroxide solution and oxidase activity was determined using an oxidase reagent (bioMérieux).

Strain P31^T was able to grow at 15–37 °C (optimum, 30–37 °C), at pH 5–10 (optimum, pH 5–6) and in the presence of 0–15 % NaCl (optimum, 0–4 %), but not under anaerobic conditions. Colonies of the strain grown on MA medium were pale cream to pale orange, circular,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain P31^T is FJ607312.

A supplementary table showing the cellular fatty acid contents of strain P31^T and closely related species is available with the online version of this paper.

smooth and opaque. Cells were Gram-positive, non-motile and coccoid with a diameter of 1.0–1.5 μm. The cultural, morphological, physiological and biochemical characteristics of strain P31^T and three closely related species of the genus *Kocuria* are summarized in Table 1.

The extraction and purification of chromosomal DNA were performed as described by Sambrook *et al.* (1989).

Table 1. Characteristics that differentiate strain P31^T from closely related type strains of the genus *Kocuria*

Strains: 1, P31^T (*Kocuria koreensis* sp. nov.); 2, *K. kristinae* DSM 20032^T; 3, *K. rhizophila* DSM 11926^T; 4, *K. varians* DSM 20033^T. Data for reference type strains were taken from Stackebrandt *et al.* (1995) and Tvrzová *et al.* (2005). +, Positive; −, negative; W, weakly positive; NR, no data available.

Characteristic	1	2	3	4
Growth with:				
10 % (w/v) NaCl	+	+	+	−
15 % (w/v) NaCl	+	−	W	−
Enzyme activity				
Oxidase	−	+	−	−
Alkaline phosphatase	+	−	+	−
L-Arginine dihydrolase	+	+	−	−
β-Galactosidase	−	+	−	+
β-Glucuronidase	−	NR	−	−
Urease	+	+	−	+
Hydrolysis of:				
Aesculin	−	+	−	−
Gelatin	−	−	+	+
Utilization of:				
Adenosine	−	NR	−	+
L-Arabinose	−	−	+	+
Dextrin	−	NR	+	+
L-Fucose	−	NR	+	+
Methyl α-D-galactoside	−	NR	−	−
D-Galacturonic acid	−	NR	−	−
N-Acetyl-D-glucosamine	−	NR	+	−
3-Methyl glucose	−	NR	−	W
D-Glucose-6-phosphate	−	NR	−	+
N-Acetyl-L-glutamic acid	−	NR	−	+
Glycerol	+	+	−	−
Glycogen	−	NR	+	+
β-Hydroxybutyric acid	−	NR	−	+
myo-Inositol	+	NR	−	−
D-Malic acid	−	NR	+	+
Maltose	−	+	−	NR
D-Mannitol	+	−	−	−
N-Acetyl-β-D-mannosamine	−	NR	−	−
Melibiose	−	−	−	+
D-Sorbitol	+	NR	−	+
Tween 40	+	NR	+	+
Tween 80	+	−	+	+
Turanose	−	NR	+	+
Uridine	−	NR	+	+
Xylitol	+	NR	+	+

DNA–DNA hybridization was determined using photobiotin-labelled DNA probes as described by Roh *et al.* (2008). The 16S rRNA gene sequence was amplified by PCR using PCR Pre-Mix (Solgent) and the amplified PCR product was purified using a purification kit (Solgent). Sequencing and phylogenetic analysis were performed as described previously (Roh *et al.*, 2008). The 16S rRNA gene sequences of strain P31^T and related taxa were aligned using the multiple-sequence alignment program CLUSTAL X (Thompson *et al.*, 1997). The MEGA4 program (Tamura *et al.*, 2007) was used to determine phylogenetic relationships between representative strains of the genus *Kocuria*. The construction of phylogenetic trees was performed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) methods. Distance matrices were calculated with the method described by Kimura (1980). To investigate the stability of the constructed trees, bootstrap analysis was performed with 1000 replicates.

16S rRNA gene sequence analysis indicated that strain P31^T belongs to the genus *Kocuria*, with highest sequence similarities to sequences from *K. kristinae* DSM 20032^T and *K. polaris* CMS 76or^T (96.9 and 96.4 %, respectively). Strain P31^T and *K. kristinae* DSM 20032^T formed a clade in the phylogenetic tree (Fig. 1). The low DNA–DNA relatedness value between strain P31^T and *K. kristinae* DSM 20032^T (21.1 ± 5.97 %; mean ± SD, n=3) confirmed that strain P31^T represents a distinct species.

The genomic G+C content was determined by using the thermal denaturation method with SYBR Green and a real-time PCR thermocycler, as described by Gonzalez & Saiz-Jimenez (2002). The cellular fatty acid compositions of strain P31^T and the three most closely related type strains, *K. kristinae* DSM 20032^T, *K. rhizophila* DSM 11926^T and *K. varians* DSM 20033^T, were determined for cultures grown under the same conditions, MA for 2 days at 30 °C. The analysis of the cellular fatty acids was performed by using the standard protocols provided by the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990) with a 6890 gas chromatograph (Hewlett Packard). Menaquinones were analysed by TLC as described by Hiraishi *et al.* (1996).

The G+C content of the genomic DNA of strain P31^T was 65.2 mol%. This was consistent with values for the other members of the genus *Kocuria*, which are in the range 60–75 mol% (Stackebrandt *et al.*, 1995; Rainey *et al.*, 1997; Kovacs *et al.*, 1999; Reddy *et al.*, 2003; Kim *et al.*, 2004; Tvrzová *et al.*, 2005; Mayilraj *et al.*, 2006; Li *et al.*, 2006; Zhou *et al.*, 2008). The cellular fatty acid contents of strain P31^T and three *Kocuria* species are shown in Supplementary Table S1 (available in IJSEM Online). Total cellular fatty acids of the genus *Kocuria* consist of saturated and unsaturated fatty acids (Kim *et al.*, 2004). The predominant cellular fatty acids of strain P31^T were anteiso-$C_{15:0}$ (42.9 %), anteiso-$C_{17:0}$ (35.9 %) and iso-$C_{16:0}$ (14.9 %). This trend was found in all tested strains

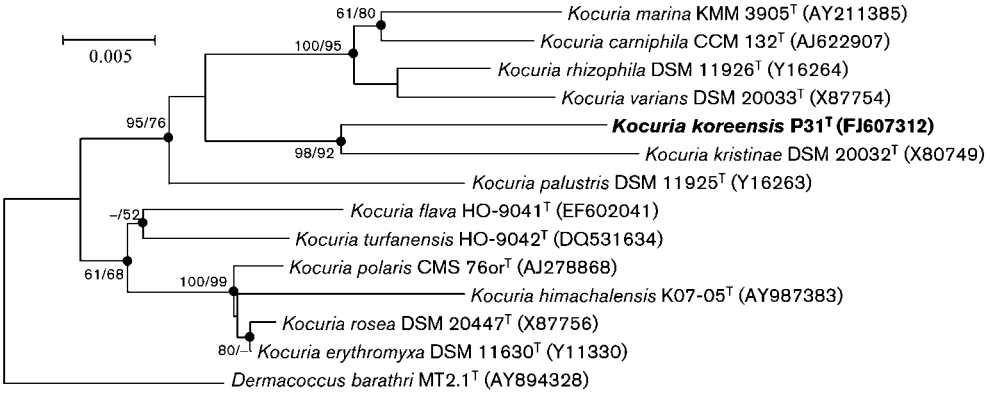

Fig. 1. Neighbour-joining phylogenetic consensus tree showing the relationship between strain P31^T and representative strains of the genus *Kocuria* based on 16S rRNA gene sequences. Filled circles indicate nodes that were also recovered with the maximum-parsimony method. Bootstrap values (>50 %, neighbour joining/maximum parsimony) based on 1000 resampled datasets are shown at branch nodes. *Dermacoccus barathri* MT2.1^T was used as the outgroup. Bar, 0.5 % nucleotide substitutions per site.

shown in Supplementary Table S1. Strain P31^T contained MK-7 as the predominant menaquinone. MK-7 is also a major menaquinone of *K. kristinae* DSM 20032^T, *K. rhizophila* DSM 11926^T and *K. varians* DSM 20033^T (Stackebrandt *et al.*, 1995; Tvrzová *et al.*, 2005).

On the basis of the phenotypic, genotypic and phylogenetic data presented, strain P31^T represents a novel species of the genus *Kocuria*, for which the name *Kocuria koreensis* sp. nov. is proposed.

Description of *Kocuria koreensis* sp. nov.

Kocuria koreensis (ko.re.en′sis. N.L. fem. adj. *koreensis* pertaining to Korea, isolated from Korean fermented seafood, made from comb pen shell).

Cells are Gram-positive, non-motile, aerobic and coccoid with a diameter of 1.0–1.5 μm. Optimal growth occurs at 30–37 °C, at pH 5–6 and with 0–4 % NaCl, over 1–2 days on MA or in MB. Colonies are pale cream or pale orange, circular, smooth and opaque after incubation on MA for 2 days at 30 °C. Catalase-positive and oxidase-negative. Cannot reduce nitrate or produce indole. Cannot hydrolyse aesculin, gelatin or PNPG (*p*-nitrophenyl β-D-galactopyranoside). Positive for alkaline phosphatase, L-arginine dihydrolase, esterase (C4), esterase lipase (C8), β-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and urease. Negative for acid phosphatase, cystine arylamidase, α-chymotrypsin, α-fucosidase, α- or β-galactosidases, α-glucosidase, β-glucuronidase, α-mannosidase, trypsin and valine arylamidase. According to Biolog GP2 MicroPlates, assimilates Tweens 40 and 80, D-fructose, α-D-glucose, *myo*-inositol, D-mannitol, D-mannose, D-psicose, D-ribose, D-sorbitol, xylitol, acetic acid, α-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, L-lactic acid, pyruvic acid methyl ester, pyruvic acid, L-asparagine, glycerol and 2′-deoxyadenosine as sole carbon sources. Contains MK-7 as the predominant menaquinone. Major cellular fatty acids are anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$; minor cellular fatty acids are $C_{16:0}$ and iso-$C_{15:0}$; trace amounts of iso-$C_{14:0}$, $C_{14:0}$, $C_{15:0}$, iso-$C_{17:0}$ and $C_{18:0}$ are found. The DNA G+C content of the type strain is 65.2 mol%.

The type strain, P31^T (=KCTC 19595^T=JCM 15915^T), was isolated from a traditional, fermented seafood in Korea.

Acknowledgements

We thank Dr J. P. Euzéby (École Nationale Vétérinaire, France) for etymological advice. This work was supported by the Environmental Biotechnology National Core Research Center (KOSEF: R15-2003-012-02002-0) and TDPAF (Technology Development Program for Agriculture and Forestry) of Ministry for Agriculture, Forestry and Fisheries.

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