Microterricola viridarii gen. nov., sp. nov., a new member of the family *Microbacteriaceae*

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Strain KV-677^T, a Gram-positive, aerobic, motile, rod-shaped bacterium, was isolated from park soil in Tokyo, Japan, and characterized. It grew well at 15–30 °C on nutrient agar and colonies were pale yellow. The cell-wall peptidoglycan contained diaminobutyric acid, glycine, alanine and glutamic acid and the muramic acid acyl type was acetyl. The predominant menaquinone was MK-12. Mycolic acids were not detected. The DNA G + C content was 70 mol%. 16S rRNA gene sequence analysis revealed that strain KV-677^T fell within the cluster of the family *Microbacteriaceae* and formed a separate lineage joining the genera *Salinibacterium*, *Rhodoglobus*, *Subtercola* and *Agreia*, showing 95.5–96.9 % sequence similarities with the type strains of the type species of the above genera. However, strain KV-677^T clearly differed from these and other genera with relatively high sequence similarity in its chemotaxonomic characteristics. Therefore, it is proposed that strain KV-677^T represents a novel species in a new genus, *Microterricola viridarii* gen. nov., sp. nov., in the family *Microbacteriaceae*. The type strain of *Microterricola viridarii* is KV-677^T (=NRRL B-24538^T =NBRC 102123^T).

A novel strain, KV-677^T, was isolated from park soil in Tokyo. The strain contained diaminobutyric acid (DAB) as the diamino acid in the cell wall and other chemotaxonomic characteristics and phylogenetic analysis showed that the strain belonged to the family *Microbacteriaceae* (Park *et al.*, 1993; Stackebrandt *et al.*, 1997). Currently, the family *Microbacteriaceae* consists of 21 genera, including *Agreia* (Evtushenko *et al.*, 2001), *Agrococcus* (Groth *et al.*, 1996), *Agromyces* (Gledhill & Casida, 1969; Zgurskaya *et al.*, 1992), *Clavibacter* (Davis *et al.*, 1984), *Cryobacterium* (Suzuki *et al.*, 1997), *Curtobacterium* (Yamada & Komagata, 1972), *Frigoribacterium* (Kämpfer *et al.*, 2000), *Gulosibacter* (Manaia *et al.*, 2004), *Leifsonia* (Evtushenko *et al.*, 2000), *Leucobacter* (Takeuchi *et al.*, 1996), *Microbacterium* (Takeuchi & Hatano, 1998), *Microcella* (Tiago *et al.*, 2005), *Mycetocola* (Tsukamoto *et al.*, 2001), *Okibacterium* (Evtushenko *et al.*, 2002), *Plantibacter* (Behrendt *et al.*, 2002), *Pseudoclavibacter* (Manaia *et al.*, 2004), *Rathayibacter* (Zgurskaya *et al.*, 1993), *Rhodoglobus* (Sheridan *et al.*, 2003), *Salinibacterium* (Han *et al.*, 2003),

Subtercola (Männistö *et al.*, 2000) and *Yonghaparkia* (Yoon *et al.*, 2006); *Zimmermannella* (Lin *et al.*, 2004) is a homotypic synonym of *Pseudoclavibacter*. At the time of writing, *Labeledella* (Lee, 2007) was also added. In this study, a novel species in a new genus of the family *Microbacteriaceae* is described.

Strain KV-677^T was isolated using GPM agar (1 % D-glucose, 0.5 % peptone, 0.5 % meat extract, 0.3 % NaCl, 1.2 % agar, pH 7) containing 0.002 % Benlate (Dupont) and 0.0025 % nalidixic acid (Sigma) from soil collected at Arisugawa Park in the Tokyo metropolitan area, Japan, after incubation for 1 week at 27 °C. Morphological characteristics of the strain were observed by scanning electron microscopy (SEM) (JSM-5600; JEOL) and transmission electron microscopy (TEM) (JEM-1200EXII; JEOL). For SEM, cells grown in TSB (Difco) for 3 days at 27 °C were rinsed with 0.05 M Tris/HCl buffer (pH 7.6) and filtered onto a membrane. The sample was fixed using 4 % osmium tetroxide vapour following lyophilization with liquid nitrogen. For TEM, cells grown on nutrient agar for 2 days at 27 °C were stained with 1.5 % (w/v) uranyl acetate after cell motility was observed with a light microscope. Gram-staining was performed using a Gram's stain reagent kit (Nacalai Tesque). Cultural and physiological characteristics were observed after incubation for 2 days at 27 °C. The NaCl tolerance and pH range for growth were determined on YD agar (1.0 % yeast extract,

Abbreviations: DAB, diaminobutyric acid; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KV-677^T is AB282862.

Scanning electron micrographs of cells of KV-677^T cultured on nutrient agar at 27 °C for 8 h and for 10 days are available as supplementary material with the online version of this paper.

1.0 % glucose, 1.2 % agar, pH 7.0) and the temperature range for growth was examined on nutrient agar (Difco) between 5 and 50 °C using a gradient incubator. Resistance to antibiotics was tested on nutrient agar using antibiotic discs (KB discs; Eiken Chemical). Carbon source utilization was determined using API 50CH test strips (bioMérieux) and general biochemical characteristics were determined using API ZYM test strips (bioMérieux) according to the manufacturer's instructions.

The muramic acid acyl type was determined by the method of Uchida & Aida (1977). Purified cell wall was obtained using the method of Kawamoto *et al.* (1981) and 1 mg purified cell wall preparation was hydrolysed at 100 °C with 1 ml 6 M HCl for 15 h. The hydrolysate was dissolved in 100 µl water and used for amino acid analysis. Amino acid composition was determined by HPLC using the Pico-Tag method (Waters). The presence of mycolic acids was examined by TLC according to Tomiyasu (1982) and phospholipids were extracted and identified following the method of Minnikin *et al.* (1977). Menaquinones were extracted and purified by the method of Collins *et al.* (1977) and then analysed by HPLC (model 802-SC; Jasco) equipped with a CAPCELL PAK C18 column (Shiseido) (Tamaoka *et al.*, 1983). Analysis of fatty acids in cells grown on TSA (Becton Dickinson) at 28 °C for 52 h was performed according to the procedures of the Sherlock microbial identification system (Microbial ID).

DNA was isolated as described by Saito & Miura (1963). DNA base composition was estimated by the HPLC method of Tamaoka & Komagata (1984).

DNA for analysis of the 16S rRNA gene sequence was prepared by the method of Yu *et al.* (2002). The 16S rRNA gene was amplified by PCR using previously described methods (Takahashi *et al.*, 2002) and was sequenced directly on an ABI model 3130 automatic DNA sequencer using a BigDye terminator cycle sequencing kit (Applied Biosystems). The CLUSTAL W software (Thompson *et al.*, 1994) was used for multiple alignment with selected sequences and for calculating evolutionary distances (Kimura, 1980) and the phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987). Data were resampled with 1000 bootstrap replications (Felsenstein, 1985). The PHYLIP software (Felsenstein, 1981) was used to create a phylogenetic tree by the maximum-likelihood method. Sequence similarity values were determined by visual comparison and manual calculation.

Strain KV-677^T grew well on nutrient agar and colonies were pale yellow. The strain was Gram-positive. Cells were irregular rods (0.4–0.5 × 0.6–1.2 µm) in TSB (Fig. 1a) and were longer in early cultures on nutrient agar (see Supplementary Fig. S1 in IJSEM Online). Cell motility was observed and TEM observation showed that cells had long flagella (Fig. 1b). Strain KV-677^T grew at 10–38 °C, with optimum growth at 15–30 °C. Growth was observed at initial pH 6–11. The strain did not grow in the presence

Fig. 1. Electron micrographs of strain KV-677^T. (a) SEM of cells cultured in TSB for 3 days at 27 °C. (b) TEM of cells cultured on nutrient agar for 2 days at 27 °C.

of 4 % NaCl and was resistant to nalidixic acid (30 µg), aztreonam (30 µg) and oxacillin (1 µg).

The cell-wall peptidoglycan contained DAB, glycine, alanine and glutamic acid. The muramic acid acyl type was acetyl. The predominant menaquinone was MK-12 (69 %); MK-11 (21 %) and MK-13 (10 %) were minor menaquinones (Table 1). For unknown reasons, the amount of menaquinones in this strain was found to be significantly smaller than in members of the family *Microbacteriaceae* analysed previously. Mycolic acids were not detected. The dominant cellular fatty acid components were 12-methyltetradecanoic (anteiso-15:0, 63 %), 14-methylhexadecanoic (anteiso-17:0, 21 %), 14-methylpentadecanoic (iso-16:0, 9 %), 13-methyltetradecanoic (iso-15:0, 3 %) and n-hexadecanoic (16:0, 3 %) acids. The DNA G + C content was 70 mol%. An almost complete 16S rRNA gene sequence (1442 bp) was determined for strain KV-677^T. The phylogenetic tree revealed that strain KV-677^T forms a separate lineage within the clade of the family *Microbacteriaceae*, joining the genera *Salinibacterium*, *Rhodoglobus*, *Subtercola* and *Agreia* (Fig. 2). Sequence similarities between strain KV-677^T and the type strains of the type species of the above genera were 96.5, 95.5, 96.0 and 96.9 %, respectively. Strain KV-677^T clearly differed

Table 1. Characteristics of genera in the family Microbacteriaceae

Data for reference genera are from the descriptions of genera from the following references: Behrendt *et al.* (2002), Davis *et al.* (1984), Evtushenko *et al.* (2000, 2001, 2002), Han *et al.* (2003), Groth *et al.* (1996), Kämpfer *et al.* (2000), Manaia *et al.* (2004), Männistö *et al.* (2000), Sasaki *et al.* (1998), Schumann *et al.* (2003), Suzuki *et al.* (1997, 1999), Takeuchi *et al.* (1996), Takeuchi & Hatano (1998), Tiago *et al.* (2005), Tsukamoto *et al.* (2001), Wieser *et al.* (1999), Yoon *et al.* (2006) and Zgurskaya *et al.* (1993). ND, No data available.

Genus	Motility	Diamino acid(s)*	Acyl type	Major menaquinone(s)
<i>Microterricola</i>	+	DAB	Acetyl	12
<i>Agreia</i>	+, -	DAB, Orn	Acetyl	10
<i>Agrococcus</i>	-	DAB	Acetyl	11, 12
<i>Agromyces</i>	-	DAB	Acetyl	12
<i>Clavibacter</i>	-	DAB	Acetyl	9
<i>Cryobacterium</i>	-	DAB	Acetyl	10
<i>Curtobacterium</i>	+, -	Orn	Acetyl	9
<i>Frigoribacterium</i>	+	Lys	Acetyl	9
<i>Gulosibacter</i>	-	Orn	ND	9
<i>Leifsonia</i>	+, -	DAB	Acetyl	11, 10
<i>Leucobacter</i>	-	DAB	Acetyl	11
<i>Microbacterium</i>	+, -	Lys, Orn	Glycolyl	11, 12, 13
<i>Microcella</i>	-	Lys, Orn	ND	12, 13
<i>Mycetocola</i>	-	Lys	Acetyl	10
<i>Okibacterium</i>	-	Lys	Glycolyl	10, 11
<i>Planibacter</i>	-	DAB	Acetyl	10, 11
<i>Pseudoclavibacter</i>	-	DAB	Acetyl	9
<i>Rathayibacter</i>	-	DAB	ND	10
<i>Rhodoglobus</i>	+	Orn	ND	12, 11
<i>Salinibacterium</i>	-	Lys, Orn	Acetyl	11
<i>Subtercola</i>	-	DAB	Acetyl	10, 9
<i>Yonghaparkia</i>	-	DAB	ND	12

*DAB, Diaminobutyric acid; Lys, lysine; Orn, ornithine.

from the above four genera, as well as from the other genera with high 16S rRNA gene sequence similarity (96.5–96.8%), *Leifsonia*, *Frigoribacterium* and *Okibacterium*, in the presence of DAB as the sole cell-wall diamino acid and MK-12 as the major menaquinone. Therefore, it is proposed that strain KV-677^T represents a novel species in a new genus within the family Microbacteriaceae, *Microterricola viridarii* gen. nov., sp. nov.

Description of *Microterricola* gen. nov.

Microterricola (Mic.ro.ter'ri.co.la. Gr. adj. *micro* small; L. fem. n. *terricola* dweller in soil; L. fem. n. *Microterricola* a small dweller in soil).

Cells are Gram-positive, catalase-positive, aerobic and motile. Cells are irregular rods. The cell-wall peptidoglycan contains DAB, glycine, alanine and glutamic acid. The acyl type is acetyl. Predominant menaquinone is MK-12 and minor menaquinones are MK-11 and MK-13. Predominant cellular fatty acids are anteiso-branched (anteiso-15:0, anteiso-17:0). Mycolic acids are not detected. Mesophilic. The type species is *Microterricola viridarii*.

Description of *Microterricola viridarii* sp. nov.

Microterricola viridarii (vi.ri.da'ri.i. L. gen. n. *viridarii* of a pleasure-garden, the place where the type strain was isolated).

General morphological, chemotaxonomic and growth characteristics are as given above in the genus description. Colonies are pale yellow in colour. Cell size is about 0.4–0.5 × 0.6–1.2 µm. The temperature range for growth is 10–38 °C and the optimum range is 15–30 °C. Growth occurs at initial pH 6–11. Glycerol, L-arabinose, D-ribose, aesculin, maltose and glycogen are utilized as carbon sources, but erythritol, D-arabinose, D- and L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-fructose,

Fig. 2. Phylogenetic tree, derived from 16S rRNA gene sequences, created using the neighbour-joining method and K_{Nuc} values. Only bootstrap values above 50% (percentages of 1000 replications) are indicated. Solid circles indicate that the corresponding nodes are also recovered in the maximum-likelihood tree. Bar, 0.02 substitutions per nucleotide position.

D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, N-acetylglucosamide, amygdalin, arbutin, salicin, cellobiose, D-lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate are not utilized. No growth occurs in the presence of 4% (w/v) NaCl. Cells are susceptible to tetracycline (30 μ g), polymyxin B (300 U), amikacin (30 μ g), chloramphenicol (30 μ g), erythromycin (15 μ g), norfloxacin (10 μ g), ciprofloxacin (5 μ g), tobramycin (10 μ g), vancomycin (30 μ g), kanamycin (30 μ g), gentamicin (10 μ g), ampicillin (10 μ g), imipenem (10 μ g) and ceftazidime (30 μ g). Leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α -glucosidase are present, but alkaline phosphatase, lipase (C8), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase are absent. The predominant cellular fatty acid components are anteiso-15:0, anteiso-17:0, iso-16:0, iso-15:0 and 16:0. The DNA G+C content of the type strain is 70 mol%.

The type strain is KV-677^T (=NRRL B-24538^T =NBRC 102123^T), isolated from park soil in Tokyo (Japan).

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