Rhodococcus tukisamuensis sp. nov., isolated from soil

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A novel strictly aerobic, heterotrophic, mesophilic bacterium, strain Mb8^T, was isolated from soil in Sapporo City, Hokkaido, Japan. The G+C content of strain Mb8^T was 66·0 mol%. It had mycolic acids with 44–52 carbon atoms and C16 : 0 and C18 : 1 (9) as the major fatty acids. The major isoprenoid quinone was MK-8(H_2). The cell wall contained *meso*-diaminopimelic acid, arabinose and galactose. 16S rDNA, chemotaxonomic and morphological data indicated that this strain clearly belonged to the genus *Rhodococcus*. Based on phenotypic properties and DNA–DNA hybridization data, strain Mb8^T (=JCM 11308^T=NCIMB 13903^T) has been assigned to the genus *Rhodococcus* as the type strain of *Rhodococcus tukisamuensis* sp. nov.

There have been many reports of micro-organisms that produce exopolysaccharides (EPS). In our attempts to find novel polysaccharide-producing bacteria from wastewater obtained from several factories, *Microbacterium kitamiense* was isolated from the wastewater of a beet sugar factory in Kitami, Hokkaido, Japan (Matsuyama *et al.*, 1999). Many bacterial enzymes that degrade EPS have been purified for use in analysing EPS structure. Bacteria that degrade EPS produced by *M. kitamiense* have been isolated in our studies. One strain, Mb8^T, was considered to be a *Rhodococcus*-like strain. The genus *Rhodococcus* comprises strains that are metabolically versatile (Finnerty, 1992). Recently, species of the genus *Rhodococcus* have been assigned to four 16S rDNA subclades: *Rhodococcus equi*, *Rhodococcus rhodnii*, *Rhodococcus rhodochrous* and *Rhodococcus erythropolis* (McMinn *et al.*, 2000). In this study, the physiological and biochemical features, chemotaxonomic characteristics and phylogeny of strain Mb8^T were examined. DNA–DNA relatedness data showed that the strain should be classified as a novel species of the genus *Rhodococcus*.

Strain Mb8^T was isolated by selective enrichment from soil in Sapporo City, Japan. The soil sample was inoculated in 50 ml minimal salts medium containing (l^{-1}) 0·1 g KNO_3, 10 ml trace element solution (Patel, 1984) and 3 g EPS produced by *M. kitamiense*. This medium was incubated at 25 °C on a horizontal shaker at 150 r.p.m. After being shaken for several days, a portion of the suspension was transferred into 50 ml fresh medium and the medium was re-incubated. After four successive transfers, the suspension was plated onto solid medium containing 0·3 % (w/v) EPS to isolate pure cultures. Of the strains found, Mb8^T, which showed a good growth rate, was selected for further study. To investigate its morphological and physiological characteristics, strain Mb8^T was cultivated aerobically at 25 °C in tryptic soy agar (TSA). Cell morphology was examined by light microscopy and SEM. For SEM, cells from the early growth phase (1 day incubation) and stationary phase (5 days incubation) were prepared by the method described by Kormendy (1975) and the specimens were examined with a model JSM 5200 SEM.

Strain Mb8^T was also examined for a range of phenotypic properties using standard procedures (Gordon *et al.*, 1974; Komagata, 1985). In addition, decomposition of aesculin and arbutin were examined according to Kurup & Fink (1975). NaCl tolerance and the temperature range for growth of strain Mb8^T and representatives of *Rhodococcus* species were determined on yeast extract-malt extract agar (ISP medium no. 2) for 5 days at 30 °C, except for *Rhodococcus marinonascens* JCM 6241^T, which was incubated on yeast extract-malt extract agar containing 3 % (w/v) NaCl for 10 days at 17 °C.

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Abbreviation: EPS, exopolysaccharide.

The DDBJ/GenBank/EMBL accession number for the 16S rRNA sequence of strain Mb8^T is AB067734.

The isomer type of the diamino acid in the cell wall was analysed according to Schleifer & Kandler (1972) at NCIMB, Japan. Cell-wall sugars were analysed according to Yokota & Takashima (2001). Analysis of mycolic acids was performed as described by Tomiyasu (1982). Cells cultivated overnight in tryptic soy broth at 25 °C were used to determine quinone composition using the method of Komagata & Suzuki (1987). Cells cultivated overnight in tryptic soy broth at 25 °C were used for cellular fatty acid analysis. Cellular fatty acid composition was determined using the method of Suzuki & Komagata (1983). Chromosomal DNA was prepared from bacterial cells using the method of Marmur (1961). The G + C content of DNA was determined according to Tamaoka & Komagata (1984). Levels of DNA relatedness were determined using the method of Ezaki *et al.* (1989). A probe for DNA–DNA hybridization was prepared from strain Mb8^T. Sequencing of 16S rRNA genes was carried out by the method described previously (Shida *et al.*, 1997). Multiple alignments of the sequence were performed and the nucleotide substitution rate (K_{nuc} value) was calculated. A phylogenetic tree was constructed by the neighbour-joining method (Kimura, 1980; Saitou & Nei, 1987) using the program CLUSTAL W (Thompson *et al.*, 1994). Similarity values for sequences were calculated using the program GENETYX (Software Development).

Strain Mb8^T was an aerobic, non-motile, non-acid-fast, non-spore-forming, Gram-positive bacterium. Cells were rod-shaped and formed filaments or showed elementary branching in the early growth phase. Most cells in the stationary phase were cocci (Fig. 1). Colonies were cream-coloured, opaque and with slightly irregular edges on TSA. Strain Mb8^T showed catalase activity, but no oxidase activity. The pH range for growth was pH 5·5–8·5 and the temperature range for growth was 15–45 °C.

The cell wall of strain Mb8^T contained *meso*-diaminopimelic acid as the diamino acid and arabinose and galactose in the whole-cell hydrolysate (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970). The major isoprenoid quinone was dihydrogenated menaquinone with eight isoprenoid units [MK-8(H_2)]. Strain Mb8^T contained large amounts of mycolic acids with 44–52 carbon atoms. Major cellular fatty acids were C16 : 0 (33·7 % total cellular fatty acids) and C18 : 1 (9) (18·6 % total cellular fatty acids). The G + C content of strain Mb8^T was 66·0 mol%. It was evident from the polyphasic taxonomic study that strain Mb8^T had properties typical of members of the genus *Rhodococcus* (Goodfellow *et al.*, 1998).

16S rDNA data supported the results of morphological and chemotaxonomic analysis. The 16S rDNA sequence of strain Mb8^T, which was determined directly after PCR amplification, was 1331 nt. Strain Mb8^T clearly belonged to the *R. erythropolis* subclade (Fig. 2). The organism was most closely related to the type strain of *Rhodococcus maanshanensis*; the two strains shared 98·0 % 16S rDNA similarity, which corresponds to 27 nt differences over 1331 positions.

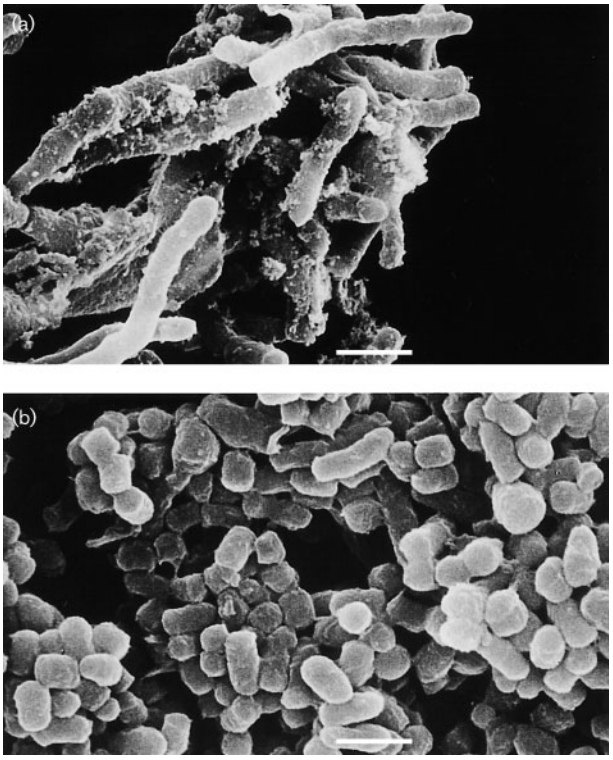

Fig. 1. SEM of strain Mb8^T. (a) Early growth phase; (b) stationary phase. Bars, 1 μm.

DNA–DNA relatedness was examined between strain Mb8^T and members of the *R. erythropolis* 16S rDNA subclade. Strain Mb8^T exhibited levels of DNA–DNA relatedness of 5, 7, 21, 41, 11, 35, 34, 34 and 45 % to *Rhodococcus wratislaviensis* JCM 9689^T, *Rhodococcus percolatus* JCM 10087^T, *Rhodococcus fascians* JCM 10002^T, *Rhodococcus opacus* JCM 9703^T, *R. marinonascens* JCM 6241^T, *Rhodococcus koreensis* JCM 10743^T, *R. erythropolis* JCM 3201^T, *Rhodococcus globerulus* JCM 7472^T and *R. maanshanensis* JCM 11374^T, respectively. All of these values are well below the 70 % cut-off point recommended for assignment of organisms to the same genomic species (Wayne *et al.*, 1987). Strain Mb8^T could also be distinguished from the type strains that form a coherent cluster in the *R. erythropolis* subclade by phenotypic properties (Table 1). However, the phenotypic properties of *R. marinonascens* were not examined because of its growth temperature range and NaCl requirement.

It is evident from the genotypic and phenotypic data that strain Mb8^T merits recognition as a representative of a novel species of the genus *Rhodococcus*. It is, therefore, proposed that the organism be classified in the genus *Rhodococcus* as *Rhodococcus tukisamuensis* sp. nov.

Description of *Rhodococcus tukisamuensis* sp. nov.

Rhodococcus tukisamuensis (tu.ki.sa.mu.en′sis. N.L. masc. adj. *tukisamuensis* referring to Tukisamu, a town in

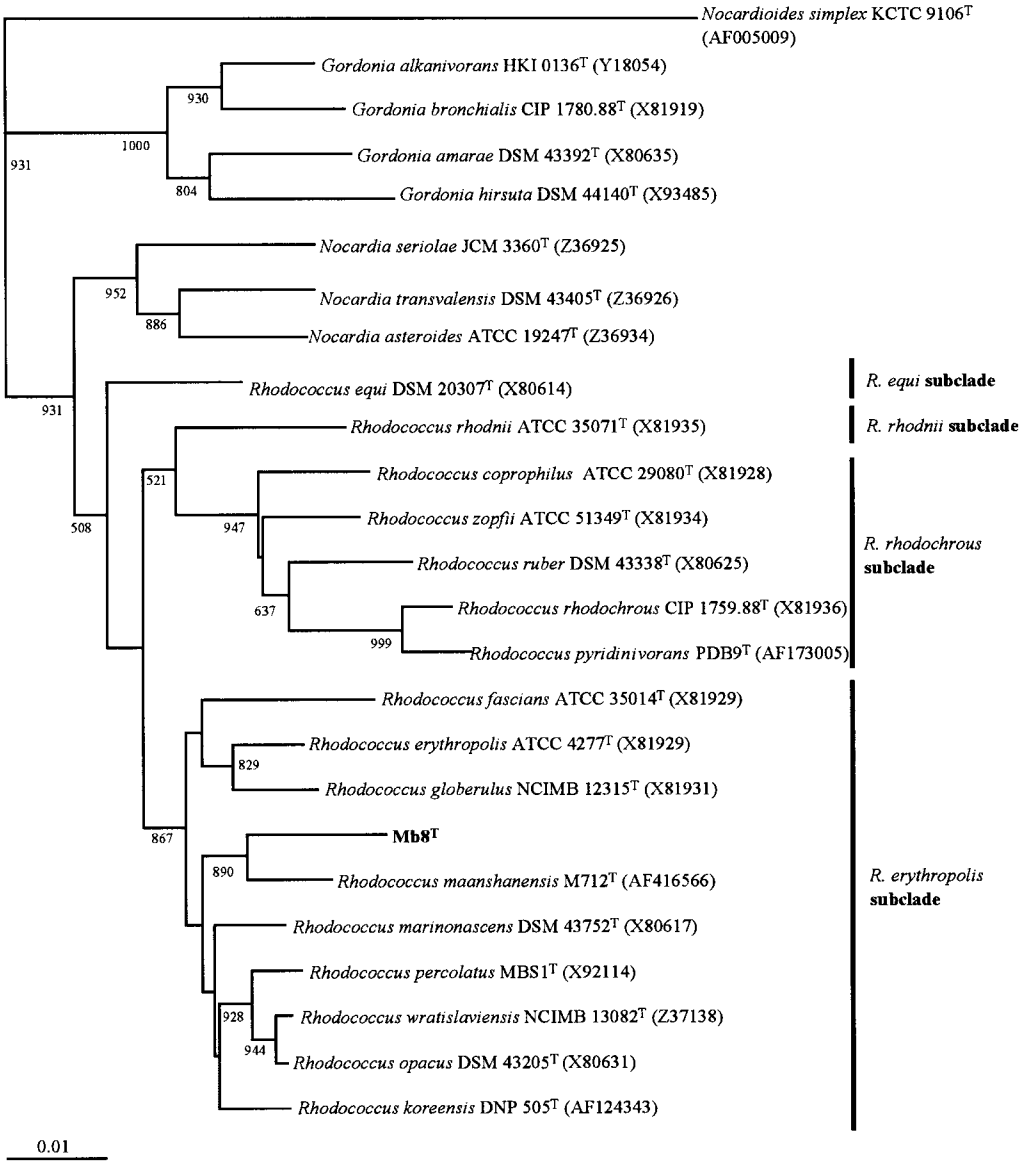

Fig. 2. Phylogenetic tree derived from 16S rDNA sequence data of strain Mb8^T and other species. Bar, 1 substitution per 100 nt.

Sapporo, Hokkaido, Japan, where the type strain was isolated).

Cells are Gram-positive, non-acid-fast and non-motile. They are rods, form filaments or show elementary branching in the early growth phase and are mostly cocci in the stationary phase. Colonies are cream-coloured, opaque and convex, with slightly irregular edges on TSA. Grows at pH 5·5–8·5 and 15–45 °C. Growth occurs in 1–3 % (w/v) NaCl, but not in 5 % (w/v) NaCl. Nitrate is reduced to nitrite. Oxidase-negative and catalase-positive. The pH in Voges–Proskauer broth is 7·6. Hydrogen sulfide and indole are not produced. Decomposes aesculin, arbutin, Tween 80 and testosterone. Does not decompose adenine, tyrosine, urea, uric acid, casein, elastin, hypoxanthine, starch or xanthine. D-Mannose, D-trehalose, glycerol, D-melibiose, D-raffinose, D-ribose (weak), D-fructose (weak), D-glucose, sucrose, D-cellobiose, adonitol (weak), dulcitol, D-galactose, L-rhamnose, maltose, melezitose, D-turanose, xylitol and inulin are utilized. Citrate, D-mannitol, D-sorbitol, D-arabinose, D-xylose, lactose, arabitol and *myo*-inositol are not utilized. Growth is inhibited by 0·02 % (w/v) NaN_3. No acid is produced from glucose, arabinose, fructose, galactose, maltose, lactose, sucrose, xylose, trehalose, glycerol, mannitol, cellobiose, ribose, salicin, sorbitol, sorbose, mannose, melibiose, rhamnose, raffinose, inositol or adonitol. The DNA G+C content of the type strain, Mb8^T (=JCM 11308^T=NCIMB 13903^T), is 66·0 mol%.

Table 1. Differential characteristics of some *Rhodococcus* species and strain Mb8^T

Strains: 1, *R. percolatus* JCM 10087^T; 2, *R. koreensis* JCM 10743^T; 3, *R. wratislaviensis* JCM 9689^T; 4, *R. opacus* JCM 9703^T; 5, *R. maanshanensis* JCM 11374^T; 6, *R. marinonascens* JCM 6241^T; 7, strain Mb8^T. All results are from this study. +, Positive; −, negative; W, weakly positive; ND, not determined. All strains were positive for decomposition of testosterone, utilization of D-mannose, D-trehalose, glycerol, D-melibiose and D-raffinose as sole carbon and energy sources, utilization of succinate, growth at 10–25 °C and growth with 1–3 % (w/v) NaCl. All strains were negative for decomposition of casein, elastin, hypoxanthine, starch and xanthine.

Character	1	2	3	4	5	6	7
Decomposition of:							
Adenine	−	−	−	+	+	ND	−
Aesculin	−	−	−	−	W	ND	+
Arbutin	−	−	−	−	W	ND	+
Tween 80	W	W	−	W	+	ND	+
Tyrosine	+	+	+	+	−	ND	−
Urea	+	+	+	+	W	−	−
Uric acid	+	+	+	+	−	ND	−
Utilization as sole carbon and energy source:							
D-Ribose	+	W	−	+	+	ND	W
D-Fructose	+	+	W	+	+	ND	W
D-Glucose	+	+	W	+	+	ND	+
Sucrose	+	+	+	+	W	ND	+
D-Mannitol	+	+	+	+	−	ND	−
D-Sorbitol	+	+	W	+	−	ND	−
D-Cellobiose	−	W	−	−	−	ND	+
Adonitol	−	−	+	−	−	ND	W
Dulcitol	−	W	+	−	+	ND	+
D-Arabinose	−	W	+	−	−	ND	−
D-Xylose	+	+	+	+	−	ND	−
D-Galactose	+	+	−	+	+	ND	+
L-Rhamnose	−	+	+	−	+	ND	+
Lactose	+	+	+	+	−	ND	−
Maltose	−	+	+	+	W	ND	+
Melezitose	+	+	−	+	+	ND	+
D-Turanose	+	W	+	+	+	ND	+
Arabitol	+	W	+	+	−	ND	−
myo-Inositol	−	+	+	+	−	ND	−
Xylitol	+	+	−	+	−	ND	+
Inulin	+	+	+	+	W	ND	+
Utilization of citrate	+	+	+	+	−	ND	−
Growth in/at:							
30 °C	+	+	+	+	+	−	+
37 °C	+	−	−	−	−	−	−
0 % NaCl	+	+	+	+	+	−	+
4 % (w/v) NaCl	+	+	+	+	−	W	W
5 % (w/v) NaCl	+	+	+	+	−	−	−

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