NOTE

***Rhodococcus jostii* sp. nov., isolated from a medieval grave**

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The taxonomic position of a bacterial strain isolated from the femur of the remains of Jošt Lucemburský, margrave in Moravia, Brno (Czech Republic), was investigated by phenotypic, chemotaxonomic and molecular taxonomic methods. The chemotaxonomic characteristics, including the cell-wall amino acid and sugar compositions, the quinone system and the fatty acid profile, were in good agreement with those of the genus *Rhodococcus*. The G+C content of the DNA was 67.4 mol%. Comparative 16S rRNA gene sequencing demonstrated that the unknown strain represents a distinct line of descent within the genus *Rhodococcus*. The nearest relatives of the bacterium were *Rhodococcus opacus* and *Rhodococcus percolatus*. The unknown bacterium was readily distinguished from these species by using phenotypic methods. On the basis of phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium be classified as *Rhodococcus jostii* sp. nov. The type strain is strain IFO 16295^T (= CCM 4760^T).

Keywords: *Rhodococcus jostii* sp. nov., taxonomy, chemotaxonomy, 16S rDNA sequence

The grave of Margrave Jošt Lucemburský, an important Czech ruler who reigned over Moravia at the turn of the 14th century, was opened for the second time in 1999 (having been opened for the first time in the 17th century) for the purpose of archaeological and anthropological research by the Brno City Museum. The grave is located under the floor of St Thomas' church in Brno, and this relatively stable environment (in terms of temperature, humidity and exposure to light) had been closed for several centuries and was expected to yield a rich microbial population.

In the course of microbiological study of the grave and skeletal remains (Marvanová *et al.*, 1999; Takeuchi & Hatano, 2001), three colonies of similar appearance, with pink-pigmented 'nocardioform' morphological features, were isolated from a femur in the remains. Strain IFO 16295^T (used in this study) is representative of these isolates. The organism is a Gram-positive, catalase-positive, strictly aerobic, asporogenous, irregularly rod-shaped bacterium with all of the characteristic chemotaxonomic markers of the genus *Rhodococcus* (Goodfellow, 1986), including wall chemotype IV (*meso*-diaminopimelic acid, arabinose and galactose; Lechevalier & Lechevalier, 1970), an MK-8(H₂)-

type menaquinone and mycolic acids. In this article, we report the characterization of this strain. On the basis of phenotypic and phylogenetic evidence, a novel species of the genus *Rhodococcus*, *Rhodococcus jostii* sp. nov., is described.

Before the grave was opened, a small opening had been made in a side wall. After removal of the marble tombstone, the grave was opened. The skeletal remains were swabbed and micro-organisms were isolated on five isolation media (Marvanová *et al.*, 1999). Strain IFO 16295^T was cultured at 30 °C on peptone/yeast extract medium (PY medium), which contained the following (l⁻¹ distilled water): 10 g Polypepton (Wako Pure Chemicals), 2 g yeast extract and 1 g MgSO₄·7H₂O (pH 7.2). For investigation of morphological characteristics, cultures grown on PY medium to early growth phase (i.e. approx. 16 h) and to stationary phase at 30 °C were observed with a light microscope and a scanning electron microscope (model JSM-5400; JEOL) (Mikata & Nakase, 1997). Motility was determined by using the hanging-drop method. The growth temperature was determined on PY medium at 10, 15, 25, 30, 37, 40 and 45 °C. The strain was characterized biochemically by using the API CORYNE system according to the manufacturer's instructions (API bioMérieux). Other physiological characteristics were determined as follows: hydrolysis

The GenBank/EMBL/DBJ accession number for the 16S rDNA sequence for *Rhodococcus jostii* IFO 16295^T is AB046357.

Table 1. Phenotypic and chemotaxonomic characteristics of *Rhodococcus jostii* IFO 16295^T

+, Positive reaction; + w, weakly positive reaction; —, negative reaction. Differential characteristics are listed in Table 2.

Characteristic	Reaction	Characteristic	Reaction
Motility	—	Growth on sole carbon sources (% w/v): (cont.)	
Catalase	+	Ethanol (0.5)	—
Urease	—	DL-Tartrate (0.5)	+ w
DNase	—	N-Acetylglucosamine (0.5)	+
β -Galactosidase	+	Sodium succinate (0.5)	+
Lecithinase (egg yolk)	—	Sodium fumarate (0.5)	+
Pyrazinamidase	—	m-Hydroxybutyrate (0.5)	+
Pyrrolidonyl arylamidase	—	Proline (0.25)	—
Alkaline phosphatase	—	L-Serine (0.05)	—
β -Glucuronidase	—	L-Leucine (0.25)	—
α -Glucosidase	—	Valine (0.05)	+ w
β -Glucosidase	—	Quinate (0.25)	+
N-Acetyl- β -glucosaminidase	—	Mycolic acids	+
Nitrate reduction	—	Predominant menaquinone	MK-8(H ₂)
Hydrolysis of:		Acyl type of the peptidoglycan	Glycolyl
Casein	—	Cellular fatty acid composition (%)	
Aesculin	—	14:0	4
Gelatin	—	15:0	8
Starch	—	16:1	13
Tween 80	—	16:0	29
Growth at:		17:1	13
30 °C	+	17:0	9
37 °C	+ w	18:1	16
Acid production from:		18:0	4
Glucose	+	10-Me-18 (tuberculostearic acid)	4
Fructose	+	Cell wall amino acids (molar ratio to diamino acid):	
Lactose	+	meso-Diaminopimelic acid	1:0
Maltose	—	Alanine	2:1
Mannitol	+	Glutamic acid	1:2
Xylose	+	Cell-wall sugar composition:	
Growth on sole carbon sources (% w/v):		Arabinose	+
D-Galactose (0.5)	—	Galactose	+
D-Ribose (0.5)	—	DNA G + C content (mol %)	67.4

of Tween 80 and of gelatin were determined as described by Páková & Kocur (1984); the activity of β -galactosidase was determined according to Lowe (1962); DNase activity was determined on DNase agar base Oxoid CM321 (BBL); and assimilation of organic acids was tested in a medium containing (l⁻¹ distilled water) 5 g organic acid (sodium salt), 1 g NH₄NO₃, 1 g KH₂PO₄, 0.5 g MgSO₄·7H₂O and 20 mg KCl (pH 7.2) (Iizuka & Komagata, 1965).

The cell wall of strain IFO 16295^T was prepared by the method of Schleifer & Kandler (1972) and cell-wall amino acid and sugar compositions were identified by HPLC as described by Takeuchi *et al.* (1999). Glycolate tests were performed by the method described by Uchida *et al.* (1999). Fatty acids were extracted from dried cells, purified and then analysed by GC-MS as described previously (Takeuchi *et al.*, 1999). Menaquinones were extracted from dry cells with chloroform/methanol (2:1, v/v), purified by TLC

(Kieselgel 60F₂₅₄ plates; Merck) and then analysed by HPLC by using a Shimadzu model LC-5A instrument as described previously (Takeuchi *et al.*, 1999). Mycolic acids were analysed by using the method of Minnikin *et al.* (1975). The G + C content of the DNA of strain IFO 16295^T was determined by the method of Mesbah *et al.* (1989) after treatment with P1 nuclease and alkaline phosphatase, and was analysed by HPLC (Tamaoka & Komagata, 1984). The DNA–DNA hybridization test was carried out fluorometrically in microdilution wells by using biotinylated DNA (Ezaki *et al.*, 1989). The phylogenetic analysis was performed by using comparative 16S rRNA gene sequence analyses. A 16S rDNA fragment corresponding to positions 10–1510 in the *Escherichia coli* numbering system (Brosius *et al.*, 1978) was amplified by PCR with primers 5'-AGTTTGATCCTGGCTC-OH-3' (identical to positions 10–25) and 5'-GGCTACCTTGTTACGA-OH-3' (complementary to positions 1510–1495) (Takeuchi *et al.*, 1994), sequenced directly

Fig. 1. Unrooted phylogenetic tree displaying the relationship between *Rhodococcus jostii* IFO 16295^T and related organisms. The numbers indicate the numbers of bootstrap samplings, derived from 1000 samplings of internal branches (Felsenstein, 1985).

with a Thermo Sequenase cycle sequencing kit with 7-deaza-dGTP (Amersham Pharmacia Biotech) and analysed with a LI-COR 4200 (two-dye system) DNA sequencer according to the manufacturers' instructions. The 16S rDNA sequence was aligned with published sequences available from DDBJ, EMBL and GenBank. The CLUSTAL W version 1.6 software (Thompson *et al.*, 1994) was used to generate evolutionary distances (K_{nuc} values; Kimura, 1980) and similarity values. A phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) and the topology of the tree was evaluated by using the bootstrap resampling method (Felsenstein, 1985) with 1000 replicates.

Strain IFO 16295^T is an aerobic, non-motile, Gram-positive bacterium. The substrate mycelium, which often fragmented into rods or coccoid elements, penetrates into agar media. The cells take the form of irregular rods and/or filaments (0.4–0.6 × 8–12 µm) in the early growth phase and fragment into short rods (0.2–0.5 × 1.7–3 µm) or cocci during the exponential growth phase. The colonies are light pink, opaque and convex with slightly irregular edges on PY agar. The strain grows optimally at 15–30 °C, grows slightly at 10 °C and does not grow at 37, 40 or 45 °C. Some other phenotypic and chemotaxonomic properties are shown in Table 1. The cell wall of strain IFO 16295^T contains *meso*-diaminopimelic acid as the diamino acid and arabinose and galactose as the major cell wall sugars, indicating wall chemotype IV (Lechevalier & Lechevalier, 1970). The chemotaxonomic features show that

strain IFO 16295^T should be allocated to the genus *Rhodococcus*. To investigate the phylogenetic relationships between strain IFO 16295^T and *Rhodococcus* species, the 16S rRNA gene sequence was compared with those of representative members of the genus *Rhodococcus*. A phylogenetic tree (Fig. 1) showed that strain IFO 16295^T represents a distinct lineage within the genus *Rhodococcus*. The species most closely related to strain IFO 16295^T are *Rhodococcus opacus* (Klatte *et al.*, 1994) and *Rhodococcus percolatus* (Briglia *et al.*, 1996) (having 98.6 and 98.1 % sequence similarity, respectively). However, strain IFO 16295^T can be separated from these two species by the following characteristics (Table 1): decomposition of urea; assimilation of inositol, sorbitol, sucrose, glycerol and trehalose; and the absence of growth at 40 °C. The level of DNA–DNA relatedness between strain IFO 16295^T and *R. opacus* IFO 16217^T was 20–26 %, while that between strain IFO 16295^T and *R. percolatus* IFO 16218^T was 18–19 % (data not shown).

Thirteen species have hitherto been accommodated within the genus *Rhodococcus*. Table 2 shows some of the diagnostic and differentiating characteristics of strain IFO 16295^T and 13 previously described species of the genus *Rhodococcus*. It is evident that strain IFO 16295^T can be separated from other species in the genus *Rhodococcus* by virtue of its morphological and physiological characteristics. On the basis of these data, we propose a novel species of the genus *Rhodococcus*, *Rhodococcus jostii* sp. nov., for strain IFO 16295^T.

Table 2. Physiological characteristics that differentiate the species of the genus *Rhodococcus*

Data for all strains except *R. jostii* IFO 16295^T, *R. opacus* IFO 16127^T and *R. percolatus* IFO 16218^T are from Goodfellow (1986), Stoecker *et al.* (1994) and Yoon *et al.* (2000). Strains: 1, *R. jostii* IFO 16295^T; 2, *R. opacus* IFO 16217^T; 3, *R. percolatus* IFO 16218^T; 4, *Rhodococcus coprophilus*; 5, *Rhodococcus equi*; 6, *Rhodococcus erythropolis*; 7, *Rhodococcus fascians*; 8, *Rhodococcus globerulus*; 9, *Rhodococcus koreensis*; 10, *Rhodococcus marinonascens*; 11, *Rhodococcus rhodnii*; 12, *Rhodococcus rhodochrous*; 13, *Rhodococcus ruber*; 14, *Rhodococcus zopfii*. +, At least 90% of strains are positive; −, at least 90% of strains are negative; v, 11–89% of strains are positive; +w, weakly positive reaction; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Morphogenetic sequence*	H-RC	H-RC	H-RC	H-RC	H-RC	EB-RC	H-RC	EB-RC	EB-RC	H-RC	EB-RC	EB-RC	RC	H-RC
Decomposition of:														
Urea	−	+	+	v	+	+	+	+	+	−	+	v	+	ND
Tyrosine	+	ND	ND	−	−	v	+	−	ND	v	+	+	+	ND
Growth on sole carbon sources (% w/v):														
L-Rhamnose (0.5)	−	−	−	−	−	−	−	−	+	−	−	−	−	−
Maltose (0.5)	+	+	+	+	−	+	−	+	+	−	−	+	−	+
Mannitol (0.5)	+	+	+	−	−	+	+	ND	+	−	+	+	+	−
Inositol (0.5)	−	+	+	−	−	v	−	−	+	+	−	−	−	−
Sorbitol (0.5)	−	+	+	−	−	+	+	ND	+	v	+	+	+	−
Sucrose (0.5)	−	−	+	v	+	+	+	+	+	−	+	+	+	−
Ethanol (0.5)	−	−	−	+	+	+	+	+	ND	−	+	+	+	+
Glycerol (0.5)	−	−	+	−	−	+	+	+	+	v	ND	+	+	+
Trehalose (0.5)	−	−	+	v	+	+	+	+	+	−	+	+	+	−
Sodium malate (0.5)	−	−	−	−	+	+	+	+	+	v	+	ND	+	ND
Sodium gluconate (0.5)	+w	+	+	−	−	+	+	−	ND	v	+	ND	v	ND
Sodium citrate (0.5)	−	+w	−	−	−	+	+	+	+	−	v	+	+	−
Sodium lactate (0.5)	−	−	−	v	+	+	+	−	ND	−	v	ND	v	ND
p-Hydroxybenzoate (0.5)	+	+	+	−	+	v	+	v	−	−	v	+	+	ND
Growth at:														
10 °C	+w	+	+	+	+	+	+	+	ND	+	−	+	ND	ND
40 °C	−	+	+	+	+	+	+	+	ND	−	−	+	+	ND

* RC, Rod-coccus cycle; H-RC, hypha-rod-coccus growth cycle; EB-RC, elementary branching-rod-coccus growth cycle.

Description of *Rhodococcus jostii* sp. nov.

Rhodococcus jostii (jos'ti.i. N.L. adj. *jostii* of Jošt, in honour of Margrave Jošt Lucemburský, one of the most important Czech rulers, from whose skeletal remains strain CCM 4760^T was isolated).

Cells are aerobic, non-motile and Gram-positive. The substrate mycelium, which is often fragmented into rods or coccoid elements, penetrates into agar media. The cells are irregular rods, which form filaments (0.4–0.6 × 8–12 µm) in the early growth phase and form mostly cocci or short rods (0.2–0.5 × 1.7–3 µm) during the exponential growth phase. The colonies are light pink, opaque and convex with slightly irregular edges on PY agar. Grows optimally at 15–30 °C, but does not grow at 37 °C. Catalase- and β-galactosidase-positive and urease-, DNase- and lecithinase-negative. Tyrosine is hydrolysed. Aesculin, gelatin, Tween 80, casein and starch are not hydrolysed. Maltose, mannitol, *N*-acetylglucosamine, sodium succinate, sodium fumarate, *m*-hydroxybutyrate, *p*-hydroxybenzoate and quinate are utilized as sole carbon and energy sources. D-Galactose, L-rhamnose, D-ribose, inositol, sorbitol, sucrose, glycerol, trehalose, sodium malate, sodium citrate, sodium lactate, proline, L-serine and L-leucine are not utilized. Acid is produced from glucose, mannitol, fructose, xylose and lactose. The cell wall contains meso-diaminopimelic acid, arabinose and galactose (wall chemotype IV). The predominant menaquinone is MK-8(H₂). The major fatty acid is C_{16:0}, and 10-methyl-branched fatty acids are present. Mycolic acids are present. The G+C content of the DNA is 67.4 mol%. Isolated from a femur in the remains in the grave of Jošt Lucemburský. The type strain is strain IFO 16295^T (= CCM 4760^T).

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