Rhodococcus jialingiae sp. nov., an actinobacterium isolated from sludge of a carbendazim wastewater treatment facility

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A novel carbendazim-degrading actinobacterium, designated djl-6-2^T, was isolated from the sludge of a carbendazim wastewater treatment facility in Jiangsu province, China. The morphological and chemotaxonomic properties of the isolate were typical of members of the genus *Rhodococcus*. Strain djl-6-2^T formed a coherent cluster with *Rhodococcus qingshengii* djl-6^T, *Rhodococcus baikonurensis* DSM 44587^T, *Rhodococcus erythropolis* DSM 43066^T and *Rhodococcus globerulus* DSM 43954^T in 16S rRNA gene sequence analysis. The results of DNA–DNA hybridization with the above strains (27.7, 19.3, 18.6 and 10.6 % relatedness, respectively), in combination with differences in biochemical and physiological properties, suggest that strain djl-6-2^T should be classified within a novel species of the genus *Rhodococcus*, for which the name *Rhodococcus jialingiae* sp. nov. is proposed, with djl-6-2^T (=DSM 45257^T =CCTCC AB 208292^T) as the type strain.

The genus *Rhodococcus* was described by Zopf (1891), and the description of the genus has subsequently been emended as the classification of the rhodococci has been greatly improved by the application of a polyphasic approach (Goodfellow *et al.*, 1998, 1999). Members of the genus show broad catabolic diversity and enzymic capabilities of environmental and biotechnological importance (Warhurst & Fewson, 1994; Bell *et al.*, 1998). In the present study, an efficient carbendazim-degrading actinobacterium, strain djl-6-2^T, was isolated from sludge of a carbendazim wastewater treatment facility in Jiangsu province, China. Carbendazim, one of the most widely used fungicides, can harm the liver and endocrine system and has mutagenic and teratogenic effects on animals even at low concentrations (Mazellier *et al.*, 2003). Some strains of *Rhodococcus* capable of degrading carbendazim have been reported, but only a few of these can degrade

carbendazim completely (Holtman & Kobayashi, 1997; Zhang *et al.*, 2005a; Xu *et al.*, 2006a, b, 2007).

Strain djl-6-2^T grew well on Luria–Bertani (LB) medium or minimal medium with carbendazim as sole carbon and energy source under aerobic conditions at 30 °C (Xu *et al.*, 2006b) and was maintained as glycerol stocks (20 %, v/v) at –70 °C. For studying cell morphology, the strain was incubated on LB medium at 30 °C. Morphology was observed by light microscopy (BH-2; Olympus) and transmission electron microscopy (H-7650; Hitachi). The Gram reaction was determined according to the procedure of Buck (1982). Acid-fast staining was performed as described by Ebersole (1992). Strain djl-6-2^T was Gram-positive, non-spore-forming, non-motile and non-acid-fast, showed elementary branching, had a rod-coccus growth cycle and formed opaque, slightly pink, moist, smooth and domed colonies with regular edges on LB agar.

Physiological tests, such as growth at different temperatures, pH and NaCl concentrations, were examined by growing the strain on LB medium using the methods of Gordon (1966, 1967). Carbon-source utilization by djl-6-2^T and related type strains was investigated by using API 50CH strips (bioMérieux), while some substrates were

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain djl-6-2^T is DQ185597.

A comparison of fatty acid profiles of strain djl-6-2^T and related strains and an extended neighbour-joining tree are available as supplementary material with the online version of this paper.

tested as described by Shirling & Gottlieb (1966). Urease production was determined as described by Cowan & Steel (1965), nitrate reduction was determined as described by Lányi (1987), hydrolysis of starch and gelatin was determined as described by Smibert & Krieg (1994) and the Voges–Proskauer and methyl red tests and the production of H₂S and indole were tested as described by Goodfellow (1986). Catalase activity was determined by bubble formation after the addition of a drop of 3 % H₂O₂. Oxidase activity was determined from the oxidation of tetramethyl-*p*-phenylenediamine. Detailed physiological and biochemical results for strain djl-6-2^T are given in Table 1 and in the species description.

Amino acids and sugars of whole-cell hydrolysates were determined by using TLC as described by Stanek & Roberts (1974). Menaquinones were extracted using the methods of Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). The presence of mycolic acids was analysed by TLC (Minnikin *et al.*, 1980). Analysis of the cellular fatty acid composition followed the instructions of the Microbial Identification System (MIDI) (Sasser, 1990).

Phospholipids were extracted and identified using published procedures (Collins & Jones, 1980; Minnikin *et al.*, 1979).

Extraction of genomic DNA and amplification of the 16S rRNA gene were done as described by Xu *et al.* (2006b). The 16S rRNA gene sequence of strain djl-6-2^T and type strains within the genus *Rhodococcus* were selected for the calculation of pairwise sequence similarity using a global alignment algorithm, which was implemented at the EzTaxon server 2.1 (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Phylogenetic analysis was performed using MEGA version 2.1 (Kumar *et al.*, 2001). Evolutionary distances and the *K*_{nuc} value (Kimura, 1983) were calculated and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. DNA–DNA hybridization was performed by the method of De Ley *et al.* (1970). The G + C content of the genomic DNA was determined by thermal denaturation (Marmur & Doty, 1962) with DNA from *Escherichia coli* JM109 as a standard.

Table 1. Phenotypic characteristics that differentiate strain djl-6-2^T from closely related type strains

Strains: 1, djl-6-2^T; 2, *R. qingshengii* djl-6^T; 3, *R. baikonurensis* DSM 44587^T; 4, *R. erythropolis* DSM 43066^T; 5, *R. globerulus* DSM 43954^T. Some data for reference type strains were taken from previous studies (Yoon *et al.*, 2000; Li *et al.*, 2004; Zhang *et al.*, 2002, 2005b; Xu *et al.*, 2007); data for strain djl-6-2^T are from the present study. +, Positive; w, weakly positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5
Colony colour	Slightly pink	Orange	Slightly pink	Slightly pink	Orange
Maximum NaCl concentration (% w/v)	7	5	6	5	3
pH for growth	4.0–9.0	5.0–8.0	5.0–9.0	5.0–8.0	5.0–8.0
Utilization as sole carbon source of:					
Arabitol	+	–	–	+	+
L-Asparagine	–	–	+	w	+
Benomyl	+	+	–	–	–
Carbendazim	+	+	–	–	–
Catechol	+	+	–	–	–
D-Fructose	+	–	+	+	+
Inulin	–	–	–	–	+
myo-Inositol	+	–	–	+	–
L-Lactic acid	–	–	+	+	+
Maltose	–	–	–	+	+
D-Mannitol	+	–	–	+	+
D-Mannose	–	+	w	–	+
Methyl pyruvate	–	–	+	+	+
D-Sorbitol	–	–	+	+	+
Sucrose	–	w	–	+	+
Trehalose	–	–	–	+	+
Xylitol	–	–	–	–	+
D-Xylose	–	–	–	–	+
Phospholipids*	DPG, PE, PG, PI, PIM	PG, PI, PME	CL, PE, PI, PIM	ND	ND
DNA G + C content (mol%)	60.1	59.1	55.5	ND	ND

*CL, Cardiolipin; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PME, phosphatidylmethylethanolamine.

Strain djl-6-2^T contained *meso*-diaminopimelic acid as the diamino acid in the cell wall. Whole-cell sugars were ribose, arabinose and galactose. The predominant menaquinone was MK-8(H₂). The phospholipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol mannoside, phosphatidylethanolamine and phosphatidylinositol. The major fatty acids were C_{16:0} (36.8%), C_{18:1ω9c} (27.3%), C_{14:0} (10.0%), C_{18:0} (4.9%), 10-methyl C_{18:0} (tuberculostearic acid) (3.7%), C_{17:1ω8c} (1.4%) and C_{15:0} (1.3%) (only components that represent ≥1% of the total are reported). Mycolic acids were present. The G+C content of strain djl-6-2^T was 60.1 mol%. The results of the chemotaxonomic and morphological tests were consistent with its classification in the genus *Rhodococcus*.

Phylogenetic analysis based on 16S rRNA gene sequences showed that strain djl-6-2^T was closely related to *Rhodococcus qingshengii* djl-6^T (99.80% similarity), *Rhodococcus baikonurensis* GTC 1041^T (99.78%), *Rhodococcus erythropolis* DSM 43066^T (99.12%) and *Rhodococcus globerulus* DSM 43954^T (98.75%), but showed less than 97% similarity to other members of the genus *Rhodococcus* (Fig. 1). However, the levels of DNA–DNA relatedness of strain djl-6-2^T to *R. qingshengii* djl-6^T, *R. baikonurensis* DSM 44587^T, *R. erythropolis* DSM 43066^T and *R. globerulus* DSM 43954^T were 27.7, 19.3, 18.6 and 10.6%, respectively. These data are consistent with the conclusion drawn by Yassin (2005) and Ghosh *et al.* (2006) that representatives of *Rhodococcus* species with 16S rRNA gene sequence similarity greater than 98% share whole genomic relatedness values well below the 70% threshold value for the delineation of genomic species (Stackebrandt & Goebel, 1994).

Fig. 1. Neighbour-joining tree showing the phylogenetic relationship between strain djl-6-2^T and its closest relatives, based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at branch points. Bar, 0.005 substitutions per site. Accession numbers are given in parentheses. An extended version of this tree is available as Supplementary Fig. S1.

Based on the phenotypic (Table 1 and Supplementary Table S1, available in IJSEM Online) and phylogenetic (Fig. 1 and Supplementary Fig. S1) evidence presented, strain djl-6-2^T should be assigned to the genus *Rhodococcus* as the type strain of a novel species, for which the name *Rhodococcus jialingiae* sp. nov. is proposed.

Description of *Rhodococcus jialingiae* sp. nov.

Rhodococcus jialingiae (jia.lin'gi.ae. N.L. fem. gen. n. *jialingiae* of Jia-Ling, to honour Jia-Ling Wang, a respected Chinese microbiologist, for her enormous contributions to the development of environmental microbiology in China).

Gram-stain-positive, aerobic, non-motile, non-spore-forming, non-acid-fast bacterium, showing elementary branching and a rod-coccus life cycle. Colonies on LB plates are opaque, slightly pink, moist, smooth and convex with regular edges. Growth occurs at 4–35 °C and pH 4.0–9.0. NaCl is tolerated at up to 7%. Negative for oxidase, production of H₂S and indole, gelatin liquefaction, starch hydrolysis, nitrate reduction and the methyl red test, while positive for catalase, the Voges–Proskauer test and urease production. Utilizes D-arabitol, benomyl, carbendazim, catechol, aesculin ferric citrate, D-fructose, D-mannitol and myo-inositol as sole carbon sources, but inulin, D-mannose, D-sorbitol, sucrose, trehalose and D-xylose are not utilized. The diagnostic amino acid in the peptidoglycan is *meso*-diaminopimelic acid, and ribose, arabinose and galactose are dominant in whole-cell hydrolysates. Phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol mannoside, phosphatidylethanolamine and phosphatidylinositol. The predominant menaquinone is MK-8(H₂). Mycolic acids are present. The major fatty acids (≥10%) are C_{16:0}, C_{18:1ω9c} and C_{14:0}. The DNA G+C content of the type strain is 60.1 mol%.

The type strain, djl-6-2^T (=DSM 45257^T =CCTCC AB 208292^T), was isolated from sludge of a carbendazim wastewater treatment facility in Jiangsu province, China.

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