Rhodococcus qingshengii sp. nov., a carbendazim-degrading bacterium

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A Gram-positive, aerobic, non-motile, mesophilic strain, djl-6T, able to degrade carbendazim, was isolated from a carbendazim-contaminated soil sample from Jiangsu province, China. The taxonomic position of this isolate was analysed by using a polyphasic approach. Chemotaxonomic analysis including peptidoglycan type, diagnostic sugar composition, fatty acid profile, menaquinones, polar lipids and mycolic acids showed that the characteristics of strain djl-6T were in good agreement with those of the genus Rhodococcus. DNA–DNA hybridization showed that it had low genomic relatedness with Rhodococcus baikonurensis DSM 44587T (31.8 %), Rhodococcus erythropolis DSM 43066T (23.8 %) and Rhodococcus globerulus DSM 43954T (17.7 %), the three type strains to which strain djl-6T was most closely related based on 16S rRNA gene sequence analysis (99.78, 99.25 and 98.91 % similarity, respectively). Based on the phenotypic properties and DNA–DNA hybridization data, strain djl-6T (=CGMCC 1.6580T =KCTC 19205T) is proposed as the type strain of a novel Rhodococcus species, Rhodococcus qingshengii sp. nov.

Members of the genus Rhodococcus are common in nature, possess a wide spectrum of catabolic activities and are able to survive under extremely harsh conditions, which makes them potentially useful in environmental and industrial biotechnology (Shao et al., 1995). With the development of polyphasic procedures, the taxonomic positions of Rhodococcus species have undergone extensive changes over recent decades. The genus Rhodococcus was first described by Zopf (1891), reintroduced by Tsukamura (1974) and redefined by Goodfellow & Alderson (1977). At the time of writing, there are 40 species of the genus Rhodococcus with validly published names (Euzéby, 2007).

Strain djl-6T, isolated in our laboratory as described below, is capable of degrading carbendazim efficiently (Xu et al., 2006). Carbendazim is one of the most widely used benzimidazole fungicides and is also a conversion product of benomyl and thiophanate-methyl. This fungicide can harm the liver and endocrine system and has mutagenic and teratogenic effects on animals, even at low concentrations (Mazellier et al., 2003). Microbial metabolism is the main mechanism of eliminating or minimizing contamination by carbendazim (WHO, 1993). To date, only a few pure cultures of bacteria with the ability to degrade carbendazim have been documented (Holtman & Kobayashi, 1997; Zhang et al., 2005a; Xu et al., 2006).

Strain djl-6T was isolated by selective enrichment from a carbendazim-contaminated soil sample from Jiangsu province, China, and grew well on Luria–Bertani (LB) medium or minimal medium with carbendazim as sole carbon and energy source at 30 °C (Xu et al., 2006). Rhodococcus baikonurensis DSM 44587T, Rhodococcus erythropolis DSM 43066T, and Rhodococcus globerulus DSM 43954T, used as reference organisms, were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany), and were cultivated in tryptic soy broth at 30 °C (Zhang et al., 2002).

For investigation of morphological and physiological characteristics, strain djl-6T was cultivated on LB agar at 30 °C. The morphology of cells was examined by using light microscopy (BH-2; Olympus) and transmission electron microscopy (H-7650; Hitachi). Cells were stained according to the classical Gram procedure (Buck, 1982). Physiological tests such as determination of growth at different temperatures and pH were done by growing the strain on LB medium by the methods of Gordon (1966, 1967). Tests for utilization of various substrates as sole carbon and energy sources were performed as described by Shirling & Gottlieb (1966); substrates were tested at a concentration of 0.1 % (w/v). Oxidase activity was determined by oxidation of 1 % 2-amino-3,4-methylenediamine

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain djl-6T is DQ090961.
oxalate. Catalase activity was determined by bubble formation in a 3% hydrogen peroxide solution. Urease production and methyl red and Voges–Proskauer reactions were determined as described by Lanyi (1987). Acid-fast staining was performed as described by Ebersole (1992).

Amino acid and sugar analysis of cell walls was conducted according to the procedures described by Hasegawa et al. (1983). Polar lipids were extracted, examined by two-dimensional TLC and identified using previously described procedures (Minnikin et al., 1984). Menaquinones were isolated according to Minnikin et al. (1984) and separated by HPLC (Kroppenstedt, 1982). Mycolic acids were analysed by using the method of Minnikin et al. (1975). Fatty acids were extracted and analysed according to the instructions of the Microbial Identification System (MIDI).

Chromosomal DNA was extracted and purified according to the method of Loeffelholz & Scholl (1989). Amplification and phylogenetic analysis of the 16S rRNA gene were carried out according to the method of Xu et al. (2006). The G+C content of DNA was determined by thermal denaturation (Marmur & Doty, 1962) with *Escherichia coli* K-12 used as a standard. DNA–DNA hybridization between strain djl-6<sup>T</sup> and reference strains was determined on the basis of the initial DNA–DNA liquid reassociation rate method, as described by De Ley et al. (1970). Renaturation was achieved at 77 °C. The procedure was performed on a Lambda 35 UV/Vis spectrometer equipped with a temperature program controller (Perkin Elmer).

Strain djl-6<sup>T</sup> was an aerobic, non-motile, Gram-positive bacterium. It displayed a hypha–rod–coccus growth cycle and formed orange colonies on LB agar after 2–3 days of incubation, whereas *R. baikonurensis* DSM 44587<sup>T</sup> formed slightly pink colonies on LB agar. Oxidase, starch hydrolysis, nitrate reduction and methyl red tests were found to be negative. Catalase, urease and Voges–Proskauer tests were positive. The acid–alcohol-fastness test was negative. The strain grew well on LB agar or minimal medium with carbendazim as sole carbon and energy source. Optimum growth was observed at 30 °C and pH 7.5–8.0. Strain djl-6<sup>T</sup> could utilize dextrin, carbendazim, d-mannose, catechol and benomyl as sole carbon and energy sources and could not utilize α-, β- or γ-hydroxybutyric acids, methyl pyruvate, L-asparagine or L-lactic acid as sole carbon and energy sources, distinguishing it from *R. baikonurensis* DSM 44587<sup>T</sup>.

The diagnostic cell-wall amino acid of strain djl-6<sup>T</sup> was meso-diaminopimelic acid. The diagnostic cell-wall sugars were arabinose and galactose (cell-wall chemotype IV; Lechevalier & Lechevalier, 1970). The non-diagnostic sugar glucose was also found in the whole-cell hydrolysate. The major menaquinone was MK-8(H<sub>2</sub>). The G+C content of strain djl-6<sup>T</sup> was 59.1 mol%. The results of the chemotaxonomic and morphological tests were consistent with classification in the genus *Rhodococcus*.

To determine the taxonomic position of strain djl-6<sup>T</sup>, its 16S rRNA gene sequence was compared with those of related species of the genus *Rhodococcus* retrieved from GenBank. The 16S rRNA gene sequence of strain djl-6<sup>T</sup> is closely related to those of members of the genus *Rhodococcus*. The most closely related strains were *R. baikonurensis* GTC 1041<sup>T</sup>, *R. erythropolis* DSM 43066<sup>T</sup> and *R. globulatus* DSM 43954<sup>T</sup> (similarity 99.78, 99.25 and 98.91 %, respectively) (Fig. 1). Strain djl-6<sup>T</sup> exhibited low levels of DNA–DNA relatedness of 31.8, 23.8 and 17.7 %, respectively, to *R. baikonurensis* DSM 44587<sup>T</sup>, *R. erythropolis* DSM 43066<sup>T</sup> and *R. globulatus* DSM 43954<sup>T</sup>. All of the values were well below the 70% cut-off point recommended for assignment of organisms to the same genomic species (Wayne et al., 1987).

Evidently, on the basis of the distinctive genotypic and phenotypic properties summarized in Table 1, strain djl-6<sup>T</sup> should be assigned to the genus *Rhodococcus* as a new species, *Rhodococcus qingshengii* sp. nov. (Fig. 1).
representative of a novel species, for which the name *Rhodococcus qingshengii* sp. nov. is proposed.

**Description of Rhodococcus qingshengii** sp. nov.

*Rhodococcus qingshengii* (qing.shen.gi.i. N.L. gen. n. qingshengii of Qing-Sheng, to honour Qing-Sheng Fan, a respected Chinese microbiologist, for his enormous contributions to the development of microbiology in China).

Cells are Gram-positive and non-motile. They show elementary branching during the early growth phase and are mostly short rods or cocci during the stationary phase. Colonies are orange, opaque and convex with slightly irregular edges on LB agar. Grows optimally at pH 7.5–8.0 and 30°C. Oxidase, starch hydrolysis, reduction of nitrate and methyl red tests are negative. Catalase, urease and Voges–Proskauer tests are positive. Reacts positively for utilization of Tween 40, Tween 80, D-fructose, D-gluconic acid, D-mannose, γ-ketovaleric acid, L-malic acid, L-alaninate, D-ribose, acetic acid, α-D-glucose, D-psicose, L-alanine, dextrin, carbendazim, D-mannose, catechol, benomyl and glycerol as sole carbon sources. The acid–alcohol-fastness test is negative. meso-Diaminopimelic acid, arabinose and galactose are present in whole-organism hydrolysates. The predominant menaquinone is MK-8(H2). Major phospholipids are phosphatidylinositol, phosphatidylmethylethanolamine and diphosphatidylglycerol. The major fatty acids (only values ≥ 3% are reported) are C14:0 (8.12%), iso-C15:0 2-OH (8.49%), C16:0 (25.97%), C18:1ω9c (7.32%), C18:0 (7.01%), 10-methyl C18:0 (tuberculostearic acid) (19.81%), C19:0 (4.31%) and C20:0 (3.36%). Mycolic acids are present. The G+C content of the DNA of the type strain is 59.1 mol%.

The type strain is djl-6T (=CGMCC 1.6580T =KCTC 19205T), isolated from a carbendazim-contaminated soil sample obtained from a vegetable field in Jiangsu province, China.

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