

***Rhodococcus koreensis* sp. nov., a 2,4-dinitrophenol-degrading bacterium**

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A 2,4-dinitrophenol-degrading bacterial strain, DNP505^T, which was isolated from an industrial wastewater, was taxonomically studied by a polyphasic approach using phenotypic, chemotaxonomic and genetic methods. Strain DNP505^T has a cell wall of chemotype IV containing meso-diaminopimelic acid, arabinose and galactose. The predominant menaquinone is MK-8(H₂). Mycolic acids contain 43–53 carbon atoms. Strain DNP505^T has a cellular fatty acid profile containing straight-chain saturated, unsaturated and 10-methyl-branched fatty acids and has C_{16:0} as the major fatty acid. The DNA G+C content is 66 mol%. Strain DNP505^T formed a coherent cluster with *Rhodococcus* species in a phylogenetic inference based on 16S rDNA sequences. Interestingly, strain DNP505^T was found to have two types of 16S rDNA sequence, which showed 10 bp sequence differences (99.3% nucleotide similarity). Its differences in some phenotypic characteristics and its genetic distinctiveness indicate that strain DNP505^T is separate from *Rhodococcus* species described previously. It is therefore proposed that strain DNP505^T should be placed in the genus *Rhodococcus* as a new species, *Rhodococcus koreensis*. The type strain of the new species is strain DNP505^T (= KCTC 0569BP^T = JCM 10743^T).

Keywords: *Rhodococcus koreensis* sp. nov., 2,4-dinitrophenol degradation, polyphasic taxonomy

INTRODUCTION

Nitroaromatic compounds are frequently used as building blocks for dyes, plastics, explosives, herbicides and pesticides, and are also important as solvents (Lenke *et al.*, 1992; Marvin-Sikkema & de Bont, 1994). However, despite their industrial importance, nitroaromatic compounds are in most cases highly toxic to living organisms, including microorganisms. They are abundantly present in nature and, in particular, are found as contaminants in waste waters, rivers and herbicide- or pesticide-treated soils (Lenke *et al.*, 1992; Marvin-Sikkema & de Bont, 1994). In spite of the toxicity of nitroaromatic compounds, several microorganisms that are able to convert or degrade nitroaromatic compounds have been found to exist in nature (Marvin-Sikkema & de Bont, 1994). Such microbes have been used for degrading many nitroaromatic compounds or cleaning of nitro-

aromatic-contaminated sites (Bruhn *et al.*, 1987; Cho *et al.*, 1998; Zeyer & Kearney, 1984).

2,4-Dinitrophenol is a well known toxic aromatic compound. It causes toxicity by uncoupling oxidative phosphorylation in the mitochondria of cells (Ilvicky & Casida, 1969; Simon, 1953). It causes 'dinitrophenol poisoning' to man exposed to contaminated environments (Leftwich *et al.*, 1982). The classic syndrome of 'dinitrophenol poisoning' includes lassitude, malaise, headache, increased perspiration, thirst, profound weight loss and respiratory failure (Leftwich *et al.*, 1982). Some bacterial strains have been reported to degrade 2,4-dinitrophenol (Hess *et al.*, 1990; Lenke *et al.*, 1992). Such useful strains utilizing 2,4-dinitrophenol as sole carbon and nitrogen sources have been isolated in our laboratory. Among them, one strain (DNP505) was found to have high ability to utilize or degrade 2,4-dinitrophenol and was considered to be a member of the genus *Rhodococcus*. The aim of this study was to determine the exact phylogenetic position of strain DNP505. We describe its morphological and

The GenBank accession numbers for the two types of 16S rDNA sequence of strain DNP505^T are AF124342 and AF124343, respectively.

physiological characteristics, its phylogeny based on 16S rDNA sequences and its DNA relatedness with some *Rhodococcus* species and other mycolic-acid-containing taxa. On the basis of this data, we propose a new species of the genus *Rhodococcus*, *Rhodococcus koreensis*, for strain DNP505^T.

METHODS

Bacterial strains. Strain DNP505^T was isolated by selective enrichment from an industrial wastewater in Cheong-Ju, Korea. The wastewater samples were inoculated in 50 ml minimal salts medium (Yoon *et al.*, 1997) containing (per litre) 1 g K₂HPO₄, 0.5 g NaH₂PO₄·2H₂O, 0.25 g KCl, 0.25 g MgSO₄·7H₂O, 1 ml trace element solution (Lee *et al.*, 1991) supplemented with 100 mg 2,4-dinitrophenol l⁻¹ and 15 g agar (if needed). This medium was incubated at 30 °C on a horizontal shaker at 150 r.p.m. When the yellow colour of the medium had disappeared, 5 ml of the suspension was transferred into 50 ml fresh medium. To isolate pure cultures, the suspension was plated on solid media containing 2,4-dinitrophenol. Single colonies were tested to investigate their ability to utilize 2,4-dinitrophenol as sole carbon and energy sources for growth. One strain (DNP505) was selected for further studies. *Rhodococcus* species used as reference organisms were obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) and KCTC (Korean Collection for Type Cultures).

Culture conditions. For investigation of morphological and physiological characteristics, strain DNP505^T was cultivated on trypticase soy agar (TSA; BBL) at 28 °C. Cell mass for analyses of cell wall, mycolic acids and menaquinones was obtained from trypticase soy broth (BBL) cultures. Cell mass for DNA extraction was produced in trypticase soy broth supplemented with glucose (0.75%, w/v). Strain DNP505^T was cultivated at 28 °C on a horizontal shaker at 150 r.p.m. The broth cultures were checked for purity before they were harvested by centrifugation. For fatty acid methyl ester (FAME) analysis, cell mass of strain DNP505^T and *Rhodococcus* species was obtained from agar plates after growing for 6 d on TSA.

Morphological and physiological characterization. The morphology of cells was examined by light microscopy, and scanning- and transmission electron microscopy. For scanning electron microscopy, cells from early-growth phase and stationary phase were prepared by the method described by Bozzola & Russell (1991) and the specimens were examined with a model 535M scanning electron microscope (Philips). For transmission electron microscopy, cells from the stationary phase were negatively stained with 1% (w/v) phosphotungstic acid, and after air-drying the grids were examined by using a model CM-20 transmission electron microscope (Philips). Motility was determined with an optical microscope using the hanging-drop technique (Skerman, 1967). Catalase activity was determined by bubble formation in a 3% hydrogen peroxide solution. Oxidase activity was determined by oxidation of 1% *p*-aminodimethylaniline oxalate. Hydrolysis of casein and of starch, and production of urease were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin and of arbutin were determined according to the method of Kurup & Fink (1975). Hydrolysis of elastin was determined according to the method of Williams *et al.* (1983). Tests for utilization of various substrates as sole carbon and energy sources were performed as described by Shirling & Gottlieb (1966). Most of the substrates were tested at a concentration

of 1% (w/v); the exception was glycerol (0.1%, w/v). The utilization results were checked over a period of 4 weeks.

Isolation of DNA. Chromosomal DNA was isolated and purified according to the method described previously (Yoon *et al.*, 1996), with the exception that ribonuclease T1 was used together with ribonuclease A.

Chemotaxonomic characterization. The isomer of diamino-pimelic acid in the peptidoglycan was determined by the method described by Komagata & Suzuki (1987). The sugar composition of the cell wall was determined by the method described by Saddler *et al.* (1991). The acyl type of the peptidoglycan was determined by the method of Uchida & Aida (1977). Menaquinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. Mycolic acids were extracted and purified as their methyl esters according to the procedures described by Minnikin (1988). The numbers of carbon atoms in the mycolic acids were calculated from the mass spectra of the methyl esters taken from an Autospec M mass spectrometer (Micromass) operated in electron impact mode. Fatty acids were extracted and analysed according to the instructions of the Microbial Identification System (MIDI).

Determination of G+C content. The G+C content was determined by the method of Tamaoka & Komagata (1984). DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

DNA-DNA hybridization. *Rhodococcus erythropolis* KCTC 1062^T, *Rhodococcus fascians* DSM 20669^T, *Rhodococcus globerulus* DSM 43954^T, *Rhodococcus marinonascens* DSM 43752^T, *Rhodococcus opacus* DSM 43205^T and *Rhodococcus percolatus* DSM 44240^T were used as reference strains for DNA-DNA hybridization. DNA-DNA hybridization was performed fluorometrically in microdilution wells according to the procedures described by Ezaki *et al.* (1989).

16S rDNA sequencing. The 16S rDNA of strain DNP505^T was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). The PCR amplification of the 16S rDNA of strain DNP505^T was also performed at different annealing temperatures and various concentrations of DNP mix and primers. Cloning of 16S rDNA was carried out by using the method described previously (Kim *et al.*, 1995). Sequencing of 16S rDNA and the cloned 16S rDNA was performed as described previously (Yoon *et al.*, 1998).

Phylogenetic analysis. The 16S rDNA sequence of strain DNP505^T was aligned with 16S rRNA gene sequences of *Rhodococcus* species and some other related actinomycete taxa by using CLUSTAL W software (Thompson *et al.*, 1994). 16S rDNA similarity values were calculated from the alignment. Gaps at the 5' and 3' ends of the alignment were omitted from further analyses. Evolutionary distance matrices were calculated by using the algorithm of Jukes & Cantor (1969) with the DNADIST program within the PHYLIP package (Felsenstein, 1993). A phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) as implemented within the NEIGHBOR program of the same package. The stability of relationships was assessed by a bootstrap analysis of 1000 data sets by using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package.

Nucleotide sequence accession numbers. GenBank and EMBL accession numbers for reference 16S rDNA sequences used in this analysis are as follows: X80626 (*Rhodococcus coprophilus* DSM 43347^T), X79289 (*Rhodococcus erythropolis* DSM 43066^T), X80614 (*Rhodococcus*

equi DSM 20307^T), X79186 (*Rhodococcus fascians* DSM 20669^T), X80619 (*Rhodococcus globerulus* DSM 43954^T), X80617 (*Rhodococcus marinonascens* DSM 43752^T), X80630 (*Rhodococcus opacus* DSM 43205^T), X92114 [*Rhodococcus percolatus* strain MBS1^T (Briglia *et al.*, 1996)], X80621 (*Rhodococcus rhodii* DSM 43336^T), X79288 (*Rhodococcus*

rhodochrous DSM 43241^T), X80625 (*Rhodococcus ruber* DSM 43338^T), X81934 (*Rhodococcus zopfii* ATCC 51349^T), X84248 (*Corynebacterium diphtheriae* NCTC 11397^T), X79290 (*Dietzia maris* DSM 43672^T), X79287 (*Gordonia bronchialis* DSM 43247^T), X80632 (*Gordonia rubripertincta* DSM 43197^T), X52917 [*Mycobacterium tuberculosis* (Rogall *et al.*, 1990)], X80606 (*Nocardia asteroides* DSM 43757^T), X80608 (*Nocardia brasiliensis* DSM 43758^T), X80611 (*Nocardia otitidiscaviarum* DSM 43242^T), Z35435 (*Skermania piniformis* IFO 15059^T) and X80628 (*Tsukamurella paurometabola* DSM 20162^T).

RESULTS

Morphological and physiological characteristics

Strain DNP505^T is an aerobic, non-motile and Gram-positive bacterium. A substrate mycelium that penetrates into agar media is observed which often fragments into rod to coccus elements. Cells are rods, form filaments or show elementary branching at early-

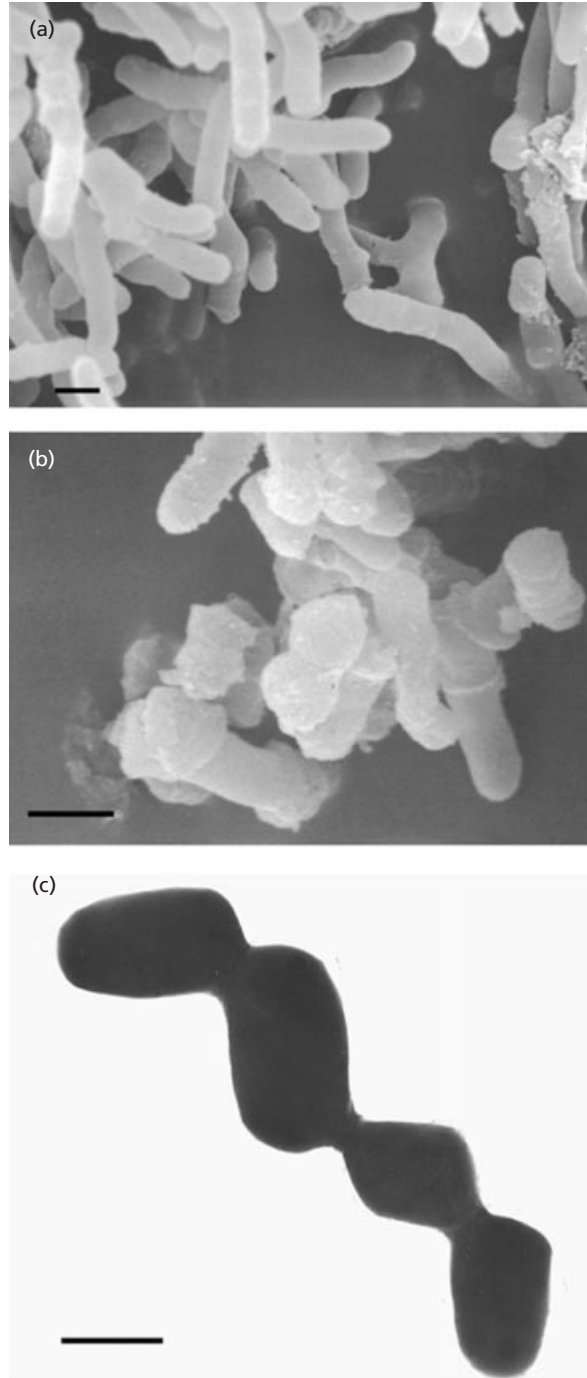


Fig. 1. Scanning- and transmission electron microscopy of strain DNP505^T grown on TSA. (a, b) Scanning electron micrographs of strain DNP505^T at early-growth phase (a) and at exponential-growth phase (b). (c) Transmission electron micrograph of strain DNP505^T at exponential-growth phase. Bars, 1 µm.

Table 1. Physiological characteristics of some *Rhodococcus* species and strain DNP505^T

All results are from this study. +, Positive reaction; -, negative reaction; w, weakly positive reaction. All strains were positive for catalase, hydrolysis of Tween 80 and urea; and utilization of D-ribose, D-fructose, D-glucose, D-mannose, sucrose, D-trehalose, D-mannitol, D-sorbitol and glycerol as sole carbon and energy sources. All strains were negative for oxidase; hydrolysis of casein, elastin and starch; and utilization of D-cellobiose, adonitol, dulcitol and sodium benzoate as sole carbon and energy sources. Species: 1, *R. erythropolis* KCTC 1062^T; 2, *R. fascians* DSM 20669^T; 3, *R. globerulus* DSM 43954^T; 4, *R. opacus* DSM 43205^T; 5, *R. percolatus* DSM 44240^T; 6, strain DNP505^T.

Character	1	2	3	4	5	6
Hydrolysis of:						
Arbutin	+	-	+	-	-	-
Aesculin	+	-	+	-	-	-
Utilization of as sole carbon and energy sources:						
L-Arabinose	-	+	-	-	-	w
D-Xylose	-	+	+	w	+	+
D-Galactose	-	w	-	+	+	+
L-Rhamnose	-	-	-	-	-	+
Lactose	-	-	-	+	+	+
Maltose	-	-	-	+	+	+
Melezitose	-	-	-	+	+	+
D-Melibiose	-	-	-	+	+	+
D-Raffinose	-	-	-	+	+	+
D-Turanose	-	-	-	+	+	+
Arabitol	-	+	+	+	+	+
myo-Inositol	+	-	-	+	+	+
Xylitol	-	+	+	+	+	+
Inulin	+	w	+	+	+	+
Sodium acetate	+	-	+	+	+	+
Trisodium citrate	+	-	+	+	+	+
Disodium succinate	+	-	+	+	+	+
2,4-Dinitrophenol	-	-	-	-	+	+

Table 2. Cellular fatty acid profiles of some *Rhodococcus* species and strain DNP505^T

Species: 1, *R. coprophilus* DSM 43347^T; 2, *R. equi* KCTC 9082^T; 3, *R. erythropolis* KCTC 1062^T; 4, *R. fascians* DSM 20669^T; 5, *R. globerulus* DSM 43954^T; 6, *R. opacus* DSM 43205^T; 7, *R. percolatus* DSM 44240^T; 8, *R. rhodii* DSM 43336^T; 9, *R. rhodochrous* KCTC 9086^T; 10, *R. ruber* DSM 43338^T; 11, *R. zopfii* DSM 44108^T; 12, strain DNP505^T.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12
Saturated												
C _{13:0}						0.6						
C _{14:0}	1.8	2.9	7.3	4.6	2.3	3.0	3.4	3.1	1.7	1.5	1.7	2.8
C _{15:0}	2.7	1.3	5.0	5.2	1.6	15.6	10.7	3.3	1.9	2.9	1.0	11.6
C _{16:0}	23.1	26.1	22.0	26.4	27.4	24.6	28.6	34.3	22.7	27.4	32.7	28.4
C _{17:0}	1.8	17.3		1.7	2.7	7.5	6.6	1.4	2.8	4.3		11.4
C _{18:0}		2.4		1.4	4.0	1.3	1.9	1.0	1.9	2.4	8.8	2.7
C _{19:0}		3.4	10.9		13.0				2.7		0.6	0.5
C _{20:0}			5.1		2.6				5.2		2.1	
Unsaturated												
C _{15:1 cis} 10						0.8	0.5					
C _{15:1 B*}						0.7	0.6					0.5
C _{16:1 cis} 9	5.6	1.5	2.8	0.9		6.9	9.0		4.1	1.2	6.6	7.0
C _{16:1 B*}					2.1				1.0			
C _{17:1 cis} 9	1.3		3.0	3.1	1.0	17.8	14.2	2.3	1.6	4.3	1.2	16.7
C _{17:1 cis} 10		4.4										
C _{18:1 cis} 9	2.4	3.2	9.7	26.2	15.5	9.5	11.3	13.7	6.0	20.7	16.5	10.6
C _{20:4 cis} 5, 8, 11, 14									2.4			
C _{20:1 cis} 11									0.6		1.7	
Cyclo												
C _{17:0}	1.8											
10-Methyl												
C _{16:0}	12.5	2.7	2.1			0.5	0.7		5.2	0.6	2.9	
C _{17:0}	4.5	1.6	2.4	1.3	0.9	2.8	2.3	2.1	2.2	1.8		1.5
C _{18:0}	24.1	18.2	11.0	11.5	14.3	1.8	2.4	23.7	22.8	15.6	13.1	1.3
Summed features†												
Summed feature 4	16.1	8.2	16.6	14.1	10	5.2	4.7	15.2	14.3	15.9	9.4	2.6
Summed feature 6				0.9								
Summed feature 7		1.3									1.3	
Summed feature 8			2.1	2.9	1.2	1.1	1.2		0.9	1.3		1.1
Summed feature 9		3.7										
Unknown fatty acid	2.4	2.0			1.5							

* The double-bond position indicated by a capital letter is unknown.

† Summed features represent groups of two or three fatty acids which could not be separated by GLC with the MIDI system. Summed features 4, 6, 7, 8 and 9 contained one or more of the following fatty acids: summed feature 4, 15:0 iso 2-OH and/or 16:1 *trans* 9; summed feature 6, 18:0 anteiso and/or 18:2 *cis* 9, 12; summed feature 7, 18:1 *cis* 11, 18:1 *trans* 6 and/or 18:1 *trans* 9; summed feature 8, unknown 18:756 and/or 19:1 *cis* 10; summed feature 9, unknown 18:846, unknown 18:858 and/or 19:0 cyclo.

growth phase and fragment into short rods or cocci during exponential-growth phase (Fig. 1). Most cells in stationary phase are cocci. Colonies are cream-coloured, opaque and convex with slightly irregular edges on TSA. Strain DNP505^T showed catalase and urease activities but no oxidase activity. It grew optimally over a broad pH range of 6.0–8.0, and growth was slow or inhibited at pH values below 5.0 and above 10.0. It grew optimally at temperatures of

25–30 °C. Some other physiological properties of strain DNP505^T are shown in Table 1, together with those of some *Rhodococcus* species.

Chemotaxonomic characteristics and DNA base composition

The cell wall of strain DNP505^T contained *meso*-diaminopimelic acid as the diamino acid, and ar-

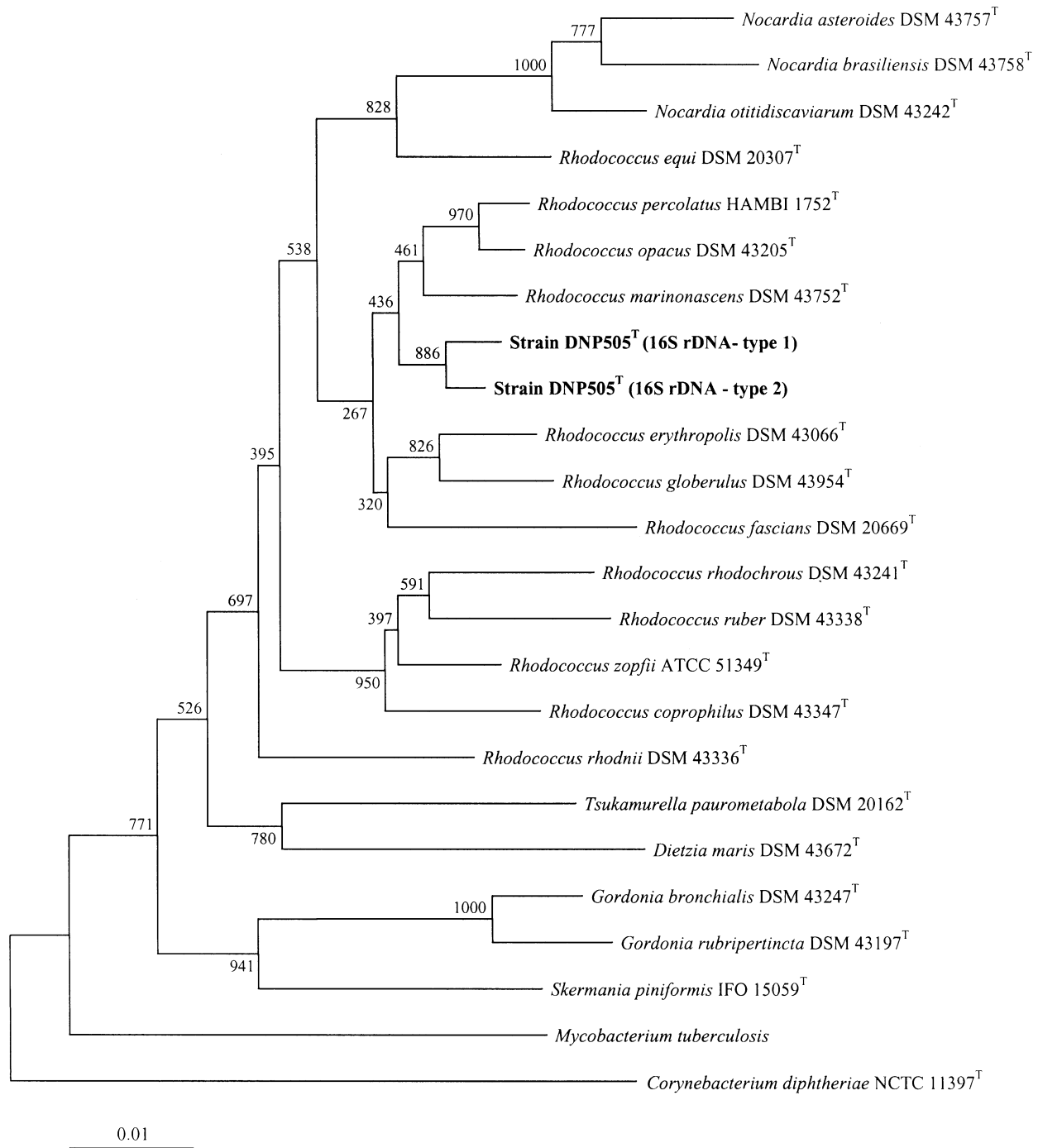


Fig. 2. Phylogenetic tree based on 16S rDNA sequences showing the positions of strain DNP505^T, *Rhodococcus* species and representatives of other mycolic-acid-containing actinomycete taxa. Scale-bar represents 0.01 substitution per nucleotide position. Bootstrap values are shown at the branch points.

abinose and galactose as major cell wall sugars, indicating wall chemotype IV (Lechevalier & Lechevalier, 1970). The acyl type in the muramic acid of the peptidoglycan was glycolyl. The major isoprenoid quinone was dihydrogenated menaquinone with eight isoprenoid units [MK-8(H₂)]. Strain DNP505^T contained major amounts of mycolic acids

with 43–53 carbon atoms. It had a cellular fatty acid profile containing straight-chain saturated, unsaturated and 10-methyl-branched fatty acids, and had C_{16:0} as the major fatty acid and relatively high proportions of C_{15:0}, C_{17:0}, C_{17:1} (*cis* 9) and C_{18:1} (*cis* 9) (Table 2). Branched saturated and hydroxy fatty acids were not detected (Table 2).

The genomic DNA G + C content of strain DNP505^T was 66 mol%, a value within the range known in the genus *Rhodococcus* (Takeuchi & Hatano, 1998).

16S rDNA sequence analysis

An almost complete nucleotide sequence of the 16S rDNA was determined by directly sequencing single strands of 16S rDNAs obtained by treatment with λ exonuclease following PCR amplification. Interestingly, strain DNP505^T was found to have two types of 16S rDNA sequence, which showed 10 bp sequence differences (99.3% nucleotide similarity). These differences were found in the region corresponding to that between positions 508 and 631 of the 16S rDNA of *Escherichia coli*. This observation has three possible explanations: contamination with other organisms, PCR amplification error and heterogeneity in the 16S rDNA sequences within strain DNP505^T. The possibility of contamination was eliminated because an identical result was obtained from new cultures that had been checked and confirmed to be uncontaminated. The possibility of PCR amplification error was also eliminated because identical results were obtained when repeated PCR, PCR under different conditions and PCR with an enzyme having 3' to 5' exonuclease proofreading activity were performed. Accordingly, it was concluded that strain DNP505^T shows heterogeneity in 16S rDNA sequences between rRNA gene clusters. When the region showing sequence differences from several (10) clones containing 16S rDNA was sequenced, two types of sequence were found. However, the number and heterogeneity of rRNA gene clusters of strain DNP505^T were not investigated in *rrn* loci distinguished by the use of restriction endonucleases. The two types of 16S rDNA sequence were simply designated type 1 and type 2.

The two types of 16S rDNA sequence determined were 1473 bp long, and corresponded to the region between positions 28 and 1524 of the 16S rDNA of *E. coli*. The 16S rDNA sequences of strain DNP505^T were compared with those of *Rhodococcus* species and representatives of other mycolic-acid-containing taxa. The phylogenetic tree (Fig. 2) shows that strain DNP505^T occupies a distinct lineage within the cluster enclosed by the genus *Rhodococcus* and forms a coherent cluster with *R. opacus*, *R. percolatus*, *R. marinonascens*, *R. fascians*, *R. globerulus* and *R. erythropolis*. Strain DNP505^T exhibited levels of 16S rDNA similarity of 95.6–98.8% with type strains of validly described *Rhodococcus* species. It was interesting that one type (type 1) of 16S rDNA sequence of strain DNP505^T exhibited highest similarity to *R. opacus* at 98.8% whereas the other type (type 2) exhibited highest similarity to *R. percolatus* at 98.6%.

DNA–DNA relatedness test

DNA–DNA relatedness tests were performed between strain DNP505^T and the type strains of some *Rhodococcus* species with which it formed a coherent cluster

according to phylogenetic analysis based on 16S rDNA sequences. Strain DNP505^T exhibited levels of DNA–DNA relatedness of 29, 30, 19, 32, 27 and 35% to *R. opacus*, *R. percolatus*, *R. marinonascens*, *R. fascians*, *R. globerulus* and *R. erythropolis*, respectively.

DISCUSSION

The genus *Rhodococcus* is an industrially important taxon which includes many strains used for amino acid production, transformation of steroids, etc. The genus *Rhodococcus* is also a very significant taxon from the point of view of bioremediation. Many *Rhodococcus* strains have been described as degrading toxic aromatic compounds such as chlorinated phenols (Briglia *et al.*, 1996), dinitrophenol (Lenke *et al.*, 1992) and naphthalene (Grund *et al.*, 1992). However, mycolic-acid-containing taxa, including the genus *Rhodococcus*, had been taxonomically confused because of similar morphological and chemotaxonomic properties (Goodfellow, 1992). Recently, reliable classification of mycolic-acid-containing taxa has been achieved by integrating 16S rDNA sequences and chemotaxonomic properties (Chun *et al.*, 1997; Rainey *et al.*, 1995a, b; Takeuchi & Hatano, 1998). Many species belonging to this group have been transferred to other genera or reclassified as new genera by integration of phylogenetic analyses based on 16S rDNA sequences and chemotaxonomic analyses (Chun *et al.*, 1997; Klatte *et al.*, 1994; Rainey *et al.*, 1995b). Mycolic-acid-containing genera are currently classified into six families within the suborder *Corynebacterineae* and the genus *Rhodococcus* is included in the family *Nocardiaceae* together with the genus *Nocardia* (Stackebrandt *et al.*, 1997). The genus *Rhodococcus* can be taxonomically distinguished from other mycolic-acid-containing actinomycete genera by a combination of chemotaxonomic and phylogenetic data.

Chemotaxonomic properties and phylogenetic inference based on 16S rDNA sequences indicate clearly that strain DNP505^T belongs to the genus *Rhodococcus*. The predominant menaquinone type of strain DNP505^T [MK-8(H₂)] distinguishes this organism from other mycolic-acid-containing genera, except the genus *Dietzia* containing MK-8(H₂) and the genus *Corynebacterium* containing MK-8(H₂) or MK-9(H₂) (Takeuchi & Hatano, 1998). The genera *Gordonia* and *Mycobacterium* have MK-9(H₂), the genera *Nocardia* and *Skermania* have MK-8(H₄, ω -cycl) and the genus *Tsukamurella* has MK-9, as predominant menaquinones (Takeuchi & Hatano, 1998). The acyl type (glycolyl) found in the peptidoglycan of strain DNP505^T distinguishes this organism from the genera *Dietzia* and *Corynebacterium*, whose acyl type is acetyl. The length of carbon chains of the mycolic acids of strain DNP505^T is different from those of the genera *Gordonia*, *Mycobacterium*, *Skermania* and *Tsukamurella* (Takeuchi & Hatano, 1998). The phylogenetic inference based on 16S rDNA sequences is consistent

with results obtained from chemotaxonomic analyses. Strain DNP505^T exhibited its highest homology to *Rhodococcus* species and formed a coherent cluster with *R. opacus*, *R. percolatus*, *R. marinonascens*, *R. fascians*, *R. globerulus* and *R. erythropolis* (Fig. 2).

Strain DNP505^T was found to be a very interesting organism, showing heterogeneity in its 16S rDNA sequences. This is the first report of a strain showing 16S rDNA sequence heterogeneity within the actinomycetes. The existence of heterogeneous 16S rDNA sequences within a single organism has been reported in only a few bacteria. *Camphylobacter helveticus* (Linton *et al.*, 1994), *Clostridium paradoxum* (Rainey *et al.*, 1996), *Paenibacillus polymyxa* (Nübel *et al.*, 1996) and the archaeon *Haloarcula marismortui* (Mylvaganam & Dennis, 1992) are known to contain multiple 16S rRNA genes with intervening sequences (IVSs) or sequence heterogeneity between 16S rRNA genes within one organism. Many bacteria are known to contain multiple *rrn* loci in their genomes (Baylis & Bibb, 1988; Kim *et al.*, 1993) and most investigations have noted a high degree of sequence identity of *rrn* genes within one organism. Therefore, heterogeneity of 16S rRNA gene sequences between *rrn* loci within one organism might be questioned. However, such a possibility might have been overlooked because the method of choice for most 16S rRNA gene sequencing involves the use of 16S rDNA amplified by PCR using total genomic DNA without distinction between rRNA operons within an organism (Weisburg *et al.*, 1991). The 16S rRNA heterogeneity shown by strain DNP505^T supports the possibility that inter-operon variability within a single strain may exist in more cases than known previously. The existence of heterogeneous 16S rRNA gene sequences may also be considered from the point of view of phylogenetic analysis. Among the two types of 16S rDNA sequence of strain DNP505^T, one type exhibited highest similarity to the 16S rDNA of *R. percolatus* whereas the other type exhibited highest similarity to the 16S rDNA of *R. opacus*. It is therefore evident that strain DNP505^T possesses a phylogenetically unique property which is not shown by other *Rhodococcus* species.

Physiological characteristics of strain DNP505^T were compared with some *Rhodococcus* species (type strains of *R. opacus*, *R. percolatus*, *R. fascians*, *R. globerulus* and *R. erythropolis*) that were phylogenetically related based on 16S rDNA sequence analysis. Strain DNP505^T was similar to *R. opacus* and *R. percolatus* in physiological characteristics as shown in cellular fatty acid profiles and phylogenetic inference based on 16S rDNA sequences. However, the ability to utilize rhamnose as carbon source was found only in strain DNP505^T and not in other species, including *R. opacus* and *R. percolatus*. Also, weak utilization of arabinose was shown by strain DNP505^T but not by *R. opacus* and *R. percolatus*. The ability to degrade 2,4-dinitrophenol was shown by both strain DNP505^T and *R. percolatus*, but strain DNP505^T did not show the ability to degrade 2,4,6-trichlorophenol which is charac-

teristic of *R. percolatus*. DNA–DNA relatedness provided important data for determining the taxonomic position of strain DNP505^T within the genus *Rhodococcus*. Although not all *Rhodococcus* species were used in DNA–DNA relatedness tests, those used in this study were considered to be sufficient because they and strain DNP505^T formed a coherent phylogenetic cluster (Fig. 2). Levels of DNA–DNA relatedness between strain DNP505^T and the type strains of some *Rhodococcus* species exhibited values that genetically distinguish strain DNP505^T from other species (Wayne *et al.*, 1987). Its differences in some phenotypic characteristics and its genetic distinctiveness indicate that strain DNP505^T is separate from *Rhodococcus* species described previously.

On the basis of data described above, strain DNP505^T should be placed in the genus *Rhodococcus* as a new species, for which we propose the name *Rhodococcus koreensis* sp. nov.

Description of *Rhodococcus koreensis* sp. nov.

Rhodococcus koreensis (ko.re.en'sis. M.L. adj. *koreensis* referring to Korea, the country where strain DNP505^T was isolated and taxonomically studied).

Cells are Gram-positive and non-motile. They are rods, form filaments or show elementary branching at early-growth phase and are mostly cocci in stationary phase. Colonies are cream-coloured, opaque and convex, with slightly irregular edges on TSA. Forms a substrate mycelium that fragments into rod to coccus elements. Grows optimally at pH 7.0–7.8 and at 25–30 °C. Oxidase-negative and catalase- and urease-positive. Tween 80 is hydrolysed. Aesculin, arbutin, casein, elastin and starch are not hydrolysed. L-Arabinose, D-ribose, D-xylose, D-fructose, D-galactose, D-glucose, D-mannose, L-rhamnose, lactose, maltose, melezitose, D-melibiose, sucrose, D-trehalose, D-raffinose, turanose, *myo*-inositol, D-mannitol, D-sorbitol, arabitol, xylitol, inulin, glycerol, sodium acetate, trisodium citrate and disodium succinate are utilized as sole carbon and energy sources. D-Cellobiose, adonitol, dulcitol and sodium benzoate are not utilized. Degrades 2,4-dinitrophenol. 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}. Mycolic acids contain 43–53 carbon atoms. The G + C content of the DNA is 66 mol % (as determined by HPLC). Isolated from an industrial wastewater in Cheong-ju, Korea. The type strain is strain DNP505^T, which has been deposited in the Korean Collection for Type Cultures as KCTC 0569BP^T and in the Japan Collection of Microorganisms as JCM 10743^T.

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