Reclassification of *Nocardioides simplex* ATCC 13260, ATCC 19565, and ATCC 19566 as *Rhodococcus erythropolis*

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Our phylogenetic analysis based on 16S ribosomal DNA (rDNA) sequences and chemotaxonomic analyses showed that *Nocardioides simplex* ATCC 13260, ATCC 19565, and ATCC 19566 are more closely related to the genus *Rhodococcus*, especially *Rhodococcus erythropolis*, than to the genus *Nocardioides*. *N. simplex* ATCC 13260 and *N. simplex* ATCC 19565 and ATCC 19566 exhibited levels of 16S rDNA similarity of 99.4 and 100%, respectively, to *R. erythropolis* DSM 43066^T. Strains ATCC 13260, ATCC 19565, and ATCC 19566 had meso-diaminopimelic acid in their peptidoglycan and MK-8(H₂) as their predominant menaquinone. These three strains produced cellular fatty acid patterns similar to those of *R. erythropolis* strains rather than those of *Nocardioides* species. Therefore, *N. simplex* ATCC 13260, ATCC 19565, and ATCC 19566 should be reclassified as strains of *R. erythropolis* Gray and Thornton 1928.

Arthrobacter simplex was assigned to the genus *Nocardioides* as *Nocardioides simplex* by O'Donnell et al. in 1982 (9). Suzuki and Komagata proposed that the genus *Pimelobacter* should accommodate *Arthrobacter* species containing LL-diaminopimelic acid in their peptidoglycan (14). *Arthrobacter globiformis*, the type species of the genus *Arthrobacter*, contains lysine as the dibasic amino acid in its peptidoglycan (12). A strain of *Arthrobacter simplex* and two strains of "*Brevibacterium lipolyticum*" were assigned to *Pimelobacter simplex*, and the name *Pimelobacter jensenii* was proposed for an atypical *Arthrobacter simplex* strain (14). However, *P. simplex* and *P. jensenii* were found to be indistinguishable from members of the genus *Nocardioides* with respect to certain chemotaxonomic traits (3, 9). These two taxa were shown to be related to the genus *Nocardioides* by phylogenetic inferences based on 16S rRNA sequences (1). Therefore, *P. simplex* and *P. jensenii* have been described as members of the genus *Nocardioides* (1).

N. simplex is distinguishable from other *Nocardioides* species by its motility and does not form an extended substrate and aerial mycelium (14). Some strains of *N. simplex*, including industrially significant patented strains, have been described. However, some strains of *N. simplex*, *N. simplex* ATCC 13260, ATCC 19565, and ATCC 19566, differ from the type strain of *N. simplex* in colonial morphology. Therefore, we compared these strains with the type strain of *N. simplex* by using chemotaxonomic markers and 16S ribosomal DNA (rDNA) sequences.

N. simplex ATCC 13260, ATCC 19565, and ATCC 19566 were purchased from American Type Culture Collection in 1996 and were grown in shake flasks containing tryptone soy broth supplemented with glucose (0.75%, wt/vol) at 26°C. The broth cultures were checked for purity before they were harvested by centrifugation. Chromosomal DNA was isolated and purified by a method described previously (16). The 16S rDNA sequences were determined by directly se-

quencing nonphosphorylated strands of PCR-amplified 16S rDNAs whose 5'-phosphorylated strands were selectively digested by λ exonuclease. Two previously described primers (13), primers 9F (5'-GAGTTTGATCCTGGCTCAG-3'; positions 9 to 27 [*Escherichia coli* 16S rRNA numbering]) and 1542R (5'-AGAAAGGAGGTGATCCAGCC-3'; positions 1542 to 1525 [*E. coli* 16S rRNA numbering]), were used for amplification of the 16S rRNA gene. The 5' ends of the two primers were phosphorylated by using T4 polynucleotide kinase (New England Biolabs, Inc., Beverly, Mass.) according to the instructions included in a Strandase ssDNA preparation kit (Novagen, Inc., Madison, Wis.). The 16S rRNA gene was amplified as described previously (17) by using phosphorylated primer 9F plus nonphosphorylated primer 1542R and phosphorylated primer 1542R plus nonphosphorylated primer 9F. The PCR products were precipitated with 1 μ l of 1% (wt/vol) glycogen, 9 μ l of 3 M sodium acetate (pH 5.2), and 70 μ l of isopropanol and were resuspended in 10 μ l of distilled water.

The strand containing the phosphorylated primer from the PCR product was selectively digested with λ exonuclease according to the instructions of the Strandase ssDNA preparation kit. The single-stranded DNA templates produced were directly used for sequencing. Sequencing was performed as described previously (7) by using α -³⁵S-labelled dATP and a DNA sequencing kit (U.S. Biochemicals, Cleveland, Ohio) and by using primers derived from conserved regions of the 16S rRNA gene. The 16S rDNA sequences of *N. simplex* ATCC 13260, ATCC 19565, and ATCC 19566 were aligned with the 16S rRNA sequences of representatives of the genera *Nocardioides* and *Rhodococcus* and some other actinomycete taxa by using CLUSTAL W software (15). Gaps at the 5' and 3' ends of the alignment were omitted from further analysis. Evolutionary distances calculated by using the Kimura-2 parameter correction with CLUSTAL W software were used to construct a phylogenetic tree by the neighbor-joining method (11).

The diamino acid of the peptidoglycan was determined by a method described previously (8). Menaquinones were analyzed as described previously (6) by using reverse-phase high-performance liquid chromatography. *Nocardioides albus* KCTC 9186^T, *Nocardioides jensenii* KCTC 9134^T, *Nocardioides luteus* KCTC 9575^T, *N. simplex* KCTC 9106^T, ATCC 13260, ATCC 19565, and ATCC 19566, and *Rhodococcus erythropolis*

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FIG. 1. Unrooted phylogenetic tree showing the positions of *N. simplex* ATCC 13260, ATCC 19565, and ATCC 19566, of representatives of the genera *Nocardioideis* and *Rhodococcus*, and of some other actinomycete taxa.

KCTC 1062^T, KCTC 1061, and KCTC 9077 were also grown for 4 days on nutrient agar (Difco) for a fatty acid methyl ester analysis. Fatty acids were extracted and analyzed by using the instructions of the Microbial Identification System (Microbial ID, Inc., Newark, Del.).

N. simplex ATCC 13260, ATCC 19565, and ATCC 19566 exhibited low levels of 16S rDNA similarity with *Nocardioideis* strains, including the type strain of *N. simplex*, and a closer relationship with *Rhodococcus* species. *N. simplex* ATCC 19565 and ATCC 19566 had the same 16S rDNA sequence and exhibited levels of 16S rDNA similarity of 90.1% with the type strain of *N. simplex* and 89.1 to 90.3% with other *Nocardioideis* species. *N. simplex* ATCC 19565 and ATCC 19566 had 16S rDNA sequences that were identical to the *R. erythropolis* DSM 43066^T 16S rDNA sequence in the region corresponding to the region between positions 28 and 1524 in the 16S rRNA of *E. coli*. *N. simplex* ATCC 13260 exhibited a level of 16S rDNA similarity of 99.3% with *N. simplex* ATCC 19565 and ATCC 19566. *N. simplex* ATCC 13260 also exhibited levels of 16S rDNA similarity of 90% with the type strain of *N. simplex*, 89 to 90.4% with other *Nocardioideis* species, and 99.4% with *R. erythropolis* DSM 43066^T. Therefore, *N. simplex* ATCC 13260, ATCC 19565, and ATCC 19566 are thought to be strains of *R. erythropolis* Gray and Thornton 1928 (5) rather than strains of *N. simplex* (Fig. 1).

Chemotaxonomic traits of *N. simplex* ATCC 13260, ATCC 19565, and ATCC 19566 also provide supporting evidence that these three strains are members of the genus *Rhodococcus* rather than the genus *Nocardioideis*. *N. simplex* ATCC 13260, ATCC 19565, and ATCC 19566 have *meso*-diaminopimelic acid as the dibasic amino acid in their cell wall peptidoglycan. *Rhodococcus* strains have been known to have *meso*-diaminopimelic acid in their cell walls (12). However, the genus *Nocardioideis* is one of the few taxa that contain *LL*-diaminopimelic

acid in the cell wall (1, 10). *N. simplex* ATCC 13260, ATCC 19565, and ATCC 19566 have a dihydrogenated menaquinone with eight isoprene units [MK-8(H₂)] as their predominant quinone. The predominant menaquinones found in the genus *Rhodococcus* and the genus *Nocardioideis* are MK-8(H₂) (2, 4) and MK-8(H₄) (9), respectively. In the cellular fatty acid methyl ester analysis, *N. simplex* ATCC 13260, ATCC 19565, and ATCC 19566 produced patterns similar to those of *R. erythropolis* strains but different from those of *Nocardioideis* species (Table 1). *N. simplex* ATCC 13260, ATCC 19565, and ATCC 19566 and strains of *R. erythropolis* were found to contain a significant amount of C_{16:0} and 10-methyl-branched C_{18:0} as major fatty acids, whereas *Nocardioideis* species, including the type strain of *N. simplex* contained iso-C_{16:0} as the major fatty acid.

Our phylogenetic analysis based on 16S rDNA sequences and chemotaxonomic analyses showed that *N. simplex* ATCC 13260, ATCC 19565, and ATCC 19566 are more closely related to the genus *Rhodococcus*, especially *R. erythropolis*, than to the type strain of *N. simplex*. Therefore, we propose that *N. simplex* ATCC 13260, ATCC 19565, and ATCC 19566 should be reclassified as strains of *R. erythropolis* Gray and Thornton 1928.

Nucleotide sequence accession numbers. The 16S rRNA gene sequences of *N. simplex* ATCC 13260, ATCC 19565, and ATCC 19566 have been deposited in the GenBank database under accession no. U81990, U82666, and U82667, respectively. The accession numbers for the sequences used as reference sequences are as follows: *N. albus*, X53211; *N. jensenii*, X53214; *N. luteus*, X53212; *Nocardioideis plantarum*, X69973; *Aeromicrobium erythreum*, M37200; *Aeromicrobium fastidiosum*, X53189; *Terrabacter tumescens*, X53215; *Rhodococcus rhodochrous*, X70295; *R. erythropolis*, M79289; *Rhodococcus equi*, X80614; *Streptomyces griseus* subsp. *griseus*, M76388;

TABLE 1. Cellular fatty acid profiles of representatives of the genus *Nocardioide*s, *N. simplex* ATCC 13260, ATCC 19565, and ATCC 19566, and some strains of *R. erythropolis*

Fatty acid or summed feature	% in:									
	<i>N. albus</i> KCTC 9186 ^T	<i>N. jensenii</i> KCTC 9134 ^T	<i>N. luteus</i> KCTC 9575 ^T	<i>N. simplex</i> KCTC 9106 ^T	<i>N. simplex</i> ATCC 13260	<i>N. simplex</i> ATCC 19565	<i>N. simplex</i> ATCC 19566	<i>R. erythropolis</i> KCTC 1062 ^T	<i>R. erythropolis</i> KCTC 1061	<i>R. erythropolis</i> KCTC 9077
Saturated fatty acids										
14:0					5.4	5.6	5.5	5.5	4.8	4.7
15:0	0.7	0.3			3.1	3.2	2.6	1.6	1.9	2.8
16:0	0.7	1.3	0.6	1.1	24.0	25.9	25.7	20.2	23.2	24.7
17:0	1.5	2.7	0.5		1.4			1.4	1.3	1.4
18:0		2.0		1.3	0.7	1.5		0.8	0.9	0.7
19:0					3.8	5.7	5.6	7.1	6.6	5.5
20:0					2.0	2.9	2.6	1.9	2.8	2.4
Unsaturated fatty acids										
15:1 ω 5c					0.4			0.5	0.4	
15:1 ω 6c		0.3								
16:1 ω 9c					0.4					
17:1 ω 5c					3.9	4.9	5.1	6.3	5.3	4.6
17:1 ω 6c	15.6	5.2	15.9	13.1						
17:1 ω 8c	2.8	3.4	1.1	2.3	1.3	2.1		1.2	1.3	1.4
18:1 ω 9c		3.3		7.3	3.2	3.0	2.9	4.4	7.5	6.8
19:1 iso I				1.6						
20:2 ω 6, 9c								0.8	0.9	1.0
Branched fatty acids										
iso-14:0	2.2	1.1	0.7	1.6						
iso-14:0 3-OH			0.6							
iso-15:0	3.1	3.7	3.7	1.5						
iso-16:1 H	1.1	14.0	2.1	5.4						
iso-16:0	53.6	45.5	49.1	34.4						
iso-17:0	1.3	1.7	1.0	2.2						
anteiso-17:0	0.7	0.7	1.4	0.5						
iso-18:0	0.9	2.7		0.9						
10-Methyl-branched fatty acids										
16:0	1.5	2.5	5.0	4.0	1.8	1.6	1.8	3.2	1.6	1.2
17:0	12.2	5.4	15.0	5.4	2.9	3.1	2.7	1.7	1.4	2.1
18:0	2.3	2.4	2.9	9.3	29.0	29.7	33.0	25.4	22.5	26.1
19:0					1.0		1.2	0.9	0.7	0.8
Summed features ^a										
Summed feature 4		1.2		5.1	14.4	9.4	11.3	14.7	14.9	12.2
Summed feature 6				0.7						
Summed feature 7				2.4						
Summed feature 8					1.4	1.5		1.5	1.6	1.6

^a Summed features represent groups of two or three fatty acids which could not be separated by gas-liquid chromatography with the MIDI system. Summed feature 4 contained one or more of the following fatty acids: iso-15:0 2-OH and/or 16:1 ω 7c. Summed feature 6 contained one or more of the following fatty acids: anteiso-18:0 and/or 18:2 ω 6,9c. Summed feature 7 contained one or more of the following fatty acids: 18:1 ω 7c, 18:1 ω 9t, and/or 18:1 ω 12t. Summed feature 8 contained one or more of the following fatty acids: unknown 18.756 and/or 19:1 ω 11c.

Streptomyces coelicolor, X60514; *Arthrobacter globiformis*, M23411; and *Micrococcus luteus*, M38242. The 16S rRNA sequence of the type strain of *N. simplex* was obtained from the study of Collins et al. (1).

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