Transfer of *Arthrobacter picolinophilus* Tate and Ensign 1974 to *Rhodococcus erythropolis*

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The type strain of *Arthrobacter picolinophilus*, strain DSM 20665 (= ATCC 27854), was found to be so closely related to strains of *Rhodococcus erythropolis* that we propose that this misclassified taxon should be removed from the genus *Arthrobacter* and reclassified as a strain of *R. erythropolis*. Our conclusion is based on the results of a comparative analysis of the chemotaxonomic properties of the organism, including the amino acid composition of the peptidoglycan, the mycolic acid composition, and the fatty acid composition, the very similar physiological properties and 16S ribosomal DNA sequences, and the results of DNA-DNA reassociation experiments performed by the renaturation rate method.

Arthrobacter picolinophilus was described on the basis of a strain which resembled *Arthrobacter* species morphologically and in which branching was not observed (19). The ability to utilize picolinate as a sole carbon and energy source in a mineral salts medium when the organism was provided with L-methionine and thiamine was the most salient feature. This species was included on the Approved Lists of Bacterial Names (17). Shortly after the name became valid, a mycolic acid with a chain length of 32 to 34 carbon atoms was reported to be present in this organism, which indicated that this species should not be considered a genuine member of the genus *Arthrobacter* but is a member of the genus *Rhodococcus* (2, 6). Thus, *A. picolinophilus* was considered a species incertae sedis, but formal transfer to the genus *Rhodococcus* has not formally proposed previously (6).

Assignment of *A. picolinophilus* DSM 20665^T (T = type strain) to the species *Rhodococcus erythropolis* is strongly supported by the presence of identical amino acids in the peptidoglycan and identical isoprenoid quinones and by the presence of mycolic acids in the two taxa. The peptide subunits of the peptidoglycan are directly cross-linked; the diagnostic amino acid of the peptidoglycan is meso-diaminopimelic acid (Al_γ peptidoglycan type according to Schleifer and Kandler [16]), and the glycan moiety of the cell walls contains N-glycolyl residues (N-glycolyl muramic acid) (20).

The whole-cell sugar pattern is a type A pattern (12); the whole-cell sugars include an arabinogalactan and no xylose. The prominent menaquinone is MK-8(H₂), determined as described previously (11). The G+C content of the DNA of *A. picolinophilus* is 65.2 mol% (19), which is within the ranges reported for *Rhodococcus* species (60 to 69 mol% [2]) and *Arthrobacter* species (59 to 66 mol% [6]).

In the course of a phylogenetic analysis of the genus *Arthrobacter* (9, 10), a comparative 16S ribosomal DNA analysis (15) indicated that *A. picolinophilus* DSM 20665^T did not cluster with members of the genus *Arthrobacter*, but was highly related to *Rhodococcus* species. The highest 16S ribosomal DNA similarity values found were the values between *A. picolinophilus* and two strains of *R. erythropolis*, type strain DSM

43066 (99.4% similarity) and strain DSM 43188 (formerly classified as the type strain of *Nocardia calcarea* [14] (99.7% similarity). As even a very high binary 16S ribosomal DNA similarity value does not eliminate the possibility that there are two species (18), DNA similarity studies were performed with *A. picolinophilus* and *R. erythropolis* DSM 43066^T and DSM 43188 by using the renaturation rate method (1, 3, 4); while the similarity value found for the two *R. erythropolis* strains was 99.0%, the values obtained for the *Arthrobacter* strain and the two *Rhodococcus* strains were 72 and 75%, respectively. These values are higher than the DNA similarity threshold value recommended for species delineation (70%) (21).

Transfer of *A. picolinophilus* to *R. erythropolis* is also justified by the very similar mycolic acid and fatty acid compositions and very similar physiological reactions of these organisms. An analysis of the mycolic acids (8) (Table 1) revealed that strain DSM 20665^T could be identified as an *R. erythropolis* strain with a high score (similarity index, 0.778) by using the Microbial Identification System (MIDI, Newark, Del.) modified for mycolic acids (8). Similarly, an analysis of the fatty acids of these strains (11, 13) (Table 2) gave a high score for strain DSM 20665^T and *R. erythropolis* (similarity index, 0.835) with the Microbial Identification System when the modified database of Klatte et al. was used (8). A numerical analysis of the mycolic acid and fatty acid compositions of *Rhodococcus* species and a determination of the levels of similarity based on Euclidian distances revealed that species of this genus can be separated from each other by distances ranging from 24 to 30 for mycolic acids and from 12 to 15 for fatty acids (7). In both kinds of analyses *A. picolinophilus* and strains of *R. erythropolis* formed a single coherent cluster within the branching pattern of *Rhodococcus* species (7).

An analysis of physiological properties in which we tested the ability of DSM 20665^T and 13 strains of *R. erythropolis* to metabolize 35 compounds by the system of Kämpfer et al. (5) as modified by Klatte et al. (9) resulted in a Willcox probability value of 100%. The following tests were found to be positive for all strains of *R. erythropolis* and strain DSM 20665^T: utilization of gluconate, myo-inositol, citrate, succinate, L-alanine, L-valine, putrescine, quinate, para-nitrophenol-β-D-xyloside, para-nitrophenol-phosphoryl-choline, and 2-deoxythymidine-5-para-nitrophenol-phosphate. Between 7 and 12 of the 13 strains of *R. erythropolis* exhibited the same reactions as strain DSM 20665^T in tests to determine the use of D-galactose, D-ribose, saccharose, caprate, D-arabitol, 2-hydroxyvalerate, 4-aminobutyrate, L-leucine, acetamide, 4-hydroxybenzoate,

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TABLE 1. Qualitative and quantitative distribution of mycolic acids in *R. erythropolis* DSM 43066^T and *A. picolinophilus* DSM 20665^T

Mycolic acid	% of total mycolic acids in:	
	DSM 43066 ^T	DSM 20665 ^T
30:0 ^a		3
31:0		
32:0	4	5
33:0	3	3
34:0	12	12
35:0	4	6
36 unsaturated ^{a,b}	2	2
36 unsaturated ^b	7	6
36:0	10	11
37 unsaturated	4	5
37:0	3	4
38 unsaturated	12	11
38:0	3	4
39:0	6	5
40:0	11	11
41:0	4	4
42 unsaturated	2	
42:0	5	4
43:0	1	1
44 unsaturated	1	1
44:0	2	1

^a Number of carbon atoms:number of double bonds.^b Unsaturated mycolic acid in which the position and degree of unsaturation are not known.

and phenylacetate, and these strains were not able to use D-glucosamine, L-rhamnose, D-furanose, pimelate, L-aspartate, and 3-hydroxybenzoate. Strain DSM 20665^T did not metabolize N-acetyl-D-glucosamine and 2-oxoglutarate, while the ma-

TABLE 2. Qualitative and quantitative distribution of fatty acids in strains of *R. erythropolis* and *A. picolinophilus* DSM 20665^T

Fatty acid	% of total fatty acids in:	
	<i>R. erythropolis</i> strains ^a	DSM 20665 ^T
Unknown ^b	0.53	0.55
14:0	6.11	5.85
15:1 <i>cis</i> -9	1.07	0.63
15:0	4.71	3.87
16:1 <i>cis</i> -7	1.95	2.86
16:1 <i>cis</i> -9	14.53	14.87
16:0	24.53	25.03
17:1 <i>cis</i> -9	3.45	3.30
17:1 <i>cis</i> -11	2.85	2.11
17:0	1.61	1.28
17:0 10-methyl	3.56	2.65
18:0 <i>cis</i> -9	9.69	14.24
18:0	0.43	0.67
18:0 10-methyl	17.47	16.39
Unknown ^c	0.82	0.85

^a Mean values for the fatty acids of type strain DSM 43066 and 11 reference strains (DSM 43060, DSM 43135, DSM 43188, DSM 43200, DSM 43296, DSM 43297, DSM 43440, DSM 43441, DSM 43955, DSM 43957, and DSM 43987).^b Fatty acid of unknown structure having an equivalent chain length of 13.961.^c Fatty acid of unknown structure having an equivalent chain length of 19.743.

jority of *R. erythropolis* strains were positive for these characteristics (10 and 9 strains, respectively); four strains of *R. erythropolis* and strain DSM 20665^T utilized benzoate.

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