Transfer of *Nocardioides fastidiosa* Collins and Stackebrandt 1989 to the Genus *Aeromicrobium* as *Aeromicrobium fastidiosum* comb. nov.

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The 16S rRNA sequences of aerobic LL-diaminopimelic acid-containing coryneform bacteria were compared, and the sequence of *Nocardioides fastidiosa* exhibited a high level of similarity to the sequence of *Aeromicrobium erythreum* but not to the sequences of the other species of the genus *Nocardioides*. Furthermore, analyses of menaquinone systems and cellular fatty acids revealed that *N. fastidiosa* was similar to *A. erythreum* but differed from the other *Nocardioides* species. On the basis of chemotaxonomic data and DNA-DNA hybridization and comparative 16S rRNA results, we propose that *N. fastidiosa* should be transferred to the genus *Aeromicrobium* as *Aeromicrobium fastidiosum* comb. nov. (type strain, IFO 14897).

Miller et al. (9) described a nonmycelial, nonsporulating actinomycete that produces the macrolide antibiotic erythromycin and named it *Aeromicrobium erythreum*. This organism has LL-diaminopimelic acid (LL-DAP) as the diamino acid in its cell wall. In addition to the genus *Aeromicrobium*, workers have described two other genera of aerobic, LL-DAP-containing coryneform and nocardioform bacteria, the genera *Nocardioides* and *Terrabacter*. In the genus *Nocardioides*, there are currently five species: *Nocardioides albus* (12), *Nocardioides simplex* (10), *Nocardioides luteus* (13), *Nocardioides jensenii* (1), and *Nocardioides fastidiosa* (4). *Terrabacter tumescens* (1) is the only species in the genus *Terrabacter*.

In the course of our taxonomic study of LL-DAP-containing actinomycetes, *N. fastidiosa* was found to be more closely related to *Aeromicrobium erythreum* than to the other *Nocardioides* species on the basis of the results of phylogenetic and chemotaxonomic analyses. *N. fastidiosa* and *Aeromicrobium erythreum* have the following characteristics in common: the major menaquinone is MK-9(H₄); the major fatty acid is 10-methyloctadecanoic acid (10Me C_{18:0}); phosphatidylethanolamine is present; and the organisms require biotin and thiamine for growth. In this paper, we reclassify *N. fastidiosa*, and we propose that *N. fastidiosa* should be transferred to the genus *Aeromicrobium* as *Aeromicrobium fastidiosum* comb. nov.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *N. fastidiosa* IFO 14897^T (T = type strain) and *Aeromicrobium erythreum* IFO 15406^T were obtained from the culture collection of the Institute for Fermentation, Osaka. For chemotaxonomic studies these strains were grown in shaken cultures in nutrient broth (Difco Laboratories, Detroit, Mich.) at 28°C; and cells were harvested in the stationary phase, washed twice with water, and then freeze-dried.

Chemical analyses. Preparation of peptidoglycan and analyses of cellular fatty acids, polar lipids, and isoprenoid quinones were carried out by previously described methods (16).

DNA homology experiment. DNA-DNA homology was stud-

ied by the photobiotin-microplate hybridization method of Ezaki et al. (5).

Comparison of 16S rRNA sequences and nucleotide sequence accession numbers. All sequence data were obtained from the DDBJ, EMBL, and GenBank nucleotide sequence databases, where the nucleotide sequences are available under the following accession numbers: M37200 (*Aeromicrobium erythreum*), M23411 (*Arthrobacter globiformis*), M38242 (*Micrococcus luteus*), X53211 (*N. albus*), X53189 (*N. fastidiosa*), X53214 (*N. jensenii*), X53212 (*N. luteus*), X72377 (*Sporichthya polymorpha*), X61478 (*Streptomyces griseus*), and X53215 (*T. tumescens*). The nucleotide sequence of *N. simplex* was obtained from the study of Collins et al. (1). DNA sequences were aligned by using the ODEN system (7). Evolutionary distances were represented by using K_{nuc} values as described by Kimura (8). A phylogenetic tree was constructed by the neighbor-joining method (14) from the K_{nuc} values derived from the sequences.

RESULTS AND DISCUSSION

Phylogenetic analysis. The 16S rRNA sequence of *N. fastidiosa* exhibited a high level of relatedness (98.2%) with the 16S rRNA sequence of *Aeromicrobium erythreum*, based on a comparison of 1,260 nucleotides, and this value was higher

FIG. 1. Unrooted phylogenetic tree showing the positions of *N. fastidiosa* and some other actinomycete taxa. The tree is based on the results of a comparison of 1,260 bases.

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TABLE 1. K_{nuc} values and levels of homology for a 1,260-nucleotide region of the 16S rRNAs of *Aeromicrobium erythreum*, *N. fastidiosus*, and related taxa

Species	K_{nuc} value or % homology with ^a :											
	<i>Aeromicrobium erythreum</i>	<i>N. fastidiosus</i>	<i>N. jensenii</i>	<i>N. albus</i>	<i>N. luteus</i>	<i>N. simplex</i>	<i>T. tumescens</i>	<i>Streptomyces griseus</i>	<i>Streptomyces lividans</i>	<i>Sporichthya polymorpha</i>	<i>M. luteus</i>	<i>Arthrobacter globiformis</i>
<i>Aeromicrobium erythreum</i>		98.2	95.7	93.7	93.8	94.1	91.6	91.1	91.3	91.4	91.5	92
<i>N. fastidiosus</i>	0.0185		95.6	94.0	94.0	93.9	91.0	90.3	90.8	90.6	90.8	91
<i>N. jensenii</i>	0.0443	0.0451		95.3	95.3	95.2	92.1	91.2	92.0	91.7	91.1	91.3
<i>N. albus</i>	0.0665	0.0621	0.0484		98.7	95.9	91.3	89.6	90.8	90.4	90.2	90.3
<i>N. luteus</i>	0.0648	0.0621	0.0484	0.0136		95.9	91.7	89.8	90.6	90.8	90.2	90.6
<i>N. simplex</i>	0.0614	0.0639	0.0494	0.0425	0.0426		91.5	89.4	90.4	90.3	91.0	91.6
<i>T. tumescens</i>	0.0895	0.0959	0.0833	0.0922	0.0877	0.0904		91.6	91.5	93.1	93.7	94
<i>Streptomyces griseus</i>	0.0952	0.1042	0.0943	0.1124	0.1104	0.1143	0.0901		96.8	92.8	91.5	92
<i>Streptomyces lividans</i>	0.0933	0.0986	0.0851	0.0986	0.1003	0.1033	0.0907	0.0325		92.7	92.1	92.2
<i>Sporichthya polymorpha</i>	0.0914	0.1005	0.0887	0.1031	0.0986	0.1040	0.0731	0.0764	0.0773		91.7	92.8
<i>M. luteus</i>	0.0906	0.0987	0.0950	0.1058	0.1048	0.0957	0.0657	0.0909	0.0834	0.0882		96.4
<i>Arthrobacter globiformis</i>	0.0854	0.0960	0.0934	0.1042	0.1013	0.0895	0.0622	0.0854	0.0826	0.0765	0.0366	

^a The values on the upper right are percentages of homology, and the values on the lower left are K_{nuc} values.

than the value obtained with any of the other four *Nocardioides* species (93.9 to 95.6%) (Table 1). Among the *Nocardioides* species, the level of sequence relatedness between *N. albus* and *N. luteus*, which are mycelium-forming species, was 98.7%, and *N. jensenii* exhibited intermediate levels of similarity (95.2 to 95.3%) with all of the other species in this genus except *N. fastidiosus* (Table 1). The branching pattern in Fig. 1 clearly demonstrates that *N. fastidiosus* is phylogenetically a member of the genus *Aeromicrobium* rather than a member of the genus *Nocardioides*.

Cellular fatty acids. The fatty acid patterns of *N. fastidiosus* and *Aeromicrobium erythreum* were similar to each other but differed from those of the other *Nocardioides* species. Both species contained significant amounts of 10Me C_{18:0}, octadecenoic acid (C_{18:1}), and hexadecanoic acid (C_{16:1}). 2-Hydroxy fatty acid was also detected in these species. The cellular fatty acid profiles of these two species are quite different from the cellular fatty acid profiles of the other *Nocardioides* species (Table 2), whose distinguishing fatty acid constituents are iso-C_{16:0}, C_{16:0}, C_{18:1}, and 10Me C_{18:0}.

Isoprenoid quinones. The major menaquinone in *N. fastidiosus* was found to be MK-9(H₄), while the major menaquinone in the other *Nocardioides* species and *T. tumescens* (1) is MK-8(H₄). Miller et al. (9) reported that menaquinone was

absent or present at a very low concentration in *Aeromicrobium erythreum*. However, we detected an ordinary amount of menaquinone in *Aeromicrobium erythreum* in this study, and the major menaquinone was MK-9(H₄) (Table 3).

Polar lipids. In *Aeromicrobium erythreum* and *N. fastidiosus*, we detected phosphatidylethanolamine and phosphatidylglycerol, but not phosphatidylcholine. Phosphatidylethanolamine, which was detected in these two species, is not present in the other *Nocardioides* species (3).

DNA-DNA hybridization. In the DNA-DNA hybridization study we found low DNA-DNA similarity values for *N. fastidiosus* IFO 14897^T and *Aeromicrobium erythreum* IFO 15406^T (12 to 14%) (data not shown), which indicates that *N. fastidiosus* IFO 14897^T and *Aeromicrobium erythreum* IFO 15406^T are members of different species.

Analysis of 16S rRNA sequences is a very useful tool for determining the taxonomic status of G+C-rich gram-positive bacteria at both the genus and species levels. Miller et al. (9) reported that the level of sequence homology between *Aeromicrobium erythreum* and *N. jensenii* was 94.6% and that the levels of sequence homology between *Aeromicrobium erythreum* and other *Nocardioides* species were less than 94%. These values are somewhat lower than the value of 95% which is sometimes considered the limit for species belonging to the same genus

TABLE 2. Cellular fatty acid compositions of *N. fastidiosus* IFO 14897^T, *Aeromicrobium erythreum* IFO 15406^T, and related bacteria

Strain or species	Fatty acid composition (%)																		Other acids	2-OH acids ^a
	anteiso acids		iso acids		Saturated acids					Unsaturated acids				10-Methyl acids						
	C ₁₅	C ₁₇	C ₁₆	C ₁₈	C ₁₄	C ₁₅	C ₁₆	C ₁₇	C ₁₈	C ₁₉	C ₁₆	C ₁₇	C ₁₈	C ₁₉	C ₁₆	C ₁₇	C ₁₈			
<i>Aeromicrobium erythreum</i> IFO 15406 ^T		3	1	1		1	17	3	8	1	1	2	17	1		4	39	+		
<i>N. fastidiosa</i> IFO 14897 ^T		4		1	1	1	10	3	7	1	3	2	14	1		4	42	+		
<i>N. albus</i> ^b	3		34				3	4	2			9	5		5	10	26	ND		
<i>N. luteus</i> ^b	3		47				3	7				8	3		6	10	13	ND		
<i>N. jensenii</i> ^b	6	4	22					2	2		3		12		4	5	19	17	+	
<i>N. simplex</i> ^b	2	2	10				5		5		2	3	41		3	4	18	2	+	
<i>T. tumescens</i> ^b	20		5		5	8	3	2			5	11	16		4	3	2	7	—	

^a +, present; -, absent; ND, not determined.

^b Data from reference 9.

TABLE 3. Menaquinone compositions of *Aeromicrobium*, *Nocardioides*, and *Terrabacter* species

Species	Menaquinone composition ^a						
	MK-7 (H ₄)	MK-8	MK-8 (H ₂)	MK-8 (H ₄)	MK-8 (H ₆)	MK-9 (H ₄)	MK-9 (H ₆)
<i>Aeromicrobium erythreum</i>	+			+		+++	
<i>N. fastidiosa</i>				+		+++	+
<i>N. albus</i> ^b	+	-	+	+++	+	tr	
<i>N. jensenii</i> ^b				+++			
<i>N. luteus</i> ^b	+	-	+	+++	+	tr	
<i>N. simplex</i> ^b	+	+	-	+++	+	tr	
<i>T. tumescens</i> ^b	+			+++	+	tr	

^a +++, main component (>20% of total); +, significant component (>5% of total); -, absent; tr, trace.

^b Data from references 2 and 3.

(9). However, *N. fastidiosa* was not included in the study of Miller et al.

In our study, *Aeromicrobium erythreum* exhibited 95.7% sequence homology with *N. jensenii*. Collins and Stackebrandt (4) reported that *N. fastidiosa* exhibited 94.1% sequence homology with *N. jensenii* and 92.4 to 92.8% sequence homology with *N. luteus*, *N. albus*, and *N. simplex*. However, *N. fastidiosa* exhibited 95.6% sequence homology with *N. jensenii* and 93.9 to 94.0% sequence homology with *N. luteus*, *N. albus*, and *N. simplex* in our study. The slight differences in the values described above were probably due to differences in the numbers of nucleotides compared. In the studies of Miller et al. (9) and Collins and Stackebrandt (4), the level of sequence homology between *Aeromicrobium erythreum* and *N. fastidiosa* was not determined. The high level of homology (98.2%) between *Aeromicrobium erythreum* and *N. fastidiosa* found in our study suggests that these two species should be included in the same genus.

Chemotaxonomy is very useful for finding characteristics that distinguish neighboring taxa. *N. fastidiosa* can be distinguished readily from the other *Nocardioides* species on the basis of chemical characteristics (Table 4), and together the following characteristics distinguish *Aeromicrobium erythreum* and *N. fastidiosa* from all other LL-DAP-containing actinomycetes that have been described: presence of 10Me C_{16:0} and iso-C_{16:0} as major cellular fatty acids, presence of MK-9(H₄), and presence of phosphatidylethanolamine as a polar lipid.

Recently, Schumann and Prauser (15) suggested that two LL-DAP-containing coryneforms are closely related. In this study, *Aeromicrobium erythreum* and *N. fastidiosa* were differentiated phenotypically from members of the genus *Nocardioides* and from members of the other LL-DAP-containing genera on the basis of chemotaxonomic characteristics. Therefore, we believe that *N. fastidiosa* should be transferred to the genus *Aeromicrobium*.

On the basis of the phylogenetic and chemotaxonomic data described above, we propose that *N. fastidiosa* should be transferred to the genus *Aeromicrobium* as *Aeromicrobium fastidiosum* (Collins and Stackebrandt) comb. nov.

Description of *Aeromicrobium fastidiosum*. *Aeromicrobium fastidiosum* (fas. tid. i. o' sum. Lat. adj. *fastidiosum*, referring to the nutritionally fastidious nature of the organism when it is first isolated). The following description is based on data from references 4, 6, and 11 and our study. Most cells are short rods; coccoid forms occur. Both rods and cocci are gram positive and not acid fast. Motile. Endospores are not formed. Colonies are whitish. Growth occurs at 5 and 30°C. No growth occurs at

TABLE 4. Taxonomic properties of *Aeromicrobium erythreum*, *N. fastidiosa*, and *Nocardioides* species

Taxon	G+C content (mol%)	Color of colonies	Motility	Cell shape	Cell size (μm)	Endospore formation	Growth temp (°C)	Optimum temp (°C)	Growth requirements			Cell wall diamino acid	Menaquinone	Cellular fatty acid	Presence of phosphatidylethanolamine
									Biotin	Nicotinic acid	Thiamine				
<i>Aeromicrobium erythreum</i>	71-73	Yellow	-	Irregular rods, cocci	0.5 × 0.5-1.2	-	21-40	35	+	+	+	LL-DAP	MK-9(H ₄)	10Me C _{18:0}	+
<i>N. fastidiosa</i>	71-72	Whitish	+	Short rods, cocci		-	5-30	25	+		+	LL-DAP	MK-9(H ₄)	10Me C _{18:0}	+
<i>Nocardioides</i> spp.	66-69	White	+/-	Hypae, rods		-	15-37	28				LL-DAP	MK-8(H ₄)	iso-C _{16:0}	-

40°C. The optimum temperature for growth is ca. 25°C. Aerobic. Acid is produced from glucose. Capable of growth only in nutritionally complex media. Thiamine and biotin are required for growth. A wide range of organic compounds are utilized as sole or principal carbon plus energy sources for growth, including L-arabinose, cellobiose, D-fructose, galactose, D-mannose, D-alanine, L-alanine, L-phenylalanine, tyrosine, L-threonine, acetate, citrate, crotonate, oxalacetate, pantoate, pyruvate, suberate, butanol, glycerol, dodecane, and hexadecane. Maltose and rhamnose are not utilized. Cellulose and hippurate are not lysed. Urease negative.

The cell wall peptidoglycan contains LL-DAP as the diamino acid. Alanine, glutamic acid, and glycine are also present. Mycolic acids are absent. 2-Hydroxylated and nonhydroxylated long-chain fatty acids are present. Nonhydroxylated straight-chain saturated, monounsaturated, and 10-methyl-branched acids are present. The major menaquinone is MK-9(H₄). Phosphatidylethanolamine, diphosphatidylglycerol, and phosphatidylglycerol are present. The G+C content of the DNA is 71 to 72 mol% (as determined by the thermal denaturation method). Isolated from herbage.

The type strain is IFO 14897 (= NCIB 12713).

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