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NOTE

Reclassification of a Carboxydobacterium, *Acinetobacter* sp. Strain JC1 DSM3803, as *Mycobacterium* sp. Strain JC1 DSM 3803

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A carboxydotrophic bacterium, isolated from a soil sample in Seoul, was classified initially as *Acinetobacter* sp. strain JC1 DSM 3803. Chemotaxanomic properties, analysis of the 16s rDNA sequence, fatty acid content, and molecular phylogenetic analysis based on *rpoB* gene, however, suggested that this bacterium belongs to the genus, *Mycobacterium*. On the basis of this evidence, it is proposed that *Acinetobacter* sp. strain JC1 DSM 3803 be reclassified as *Mycobacterium* sp. strain JC1 DSM 3803.

Key words: carbon monoxide, carboxydobacteria, *Mycobacterium*

Carboxydobacteria are a group of bacteria which are capable of growing aerobically on carbon monoxide as a sole source of carbon and energy (Kim and Hegeman, 1983; Meyer and Schlegel, 1983). As the carboxydobacteria are believed to be physiologically related to Gram-negative hydrogen bacteria, earlier studies on this carbon monoxide-utilizing bacteria have been focused mostly on Gram-negative bacteria. Various species of different genera of Gram-negative bacteria, *Pseudomonas*, *Alcaligenes*, *Azotobacter*, and *Azomonas*, are regarded as members of carboxydobacteria (Kim and Hegeman, 1983; Meyer *et al.*, 1986). But the fact that they are scattered among genera of Gram-negative bacteria makes it difficult to define carboxydobacteria as a taxanomic group, and a few new genera have been introduced to accommodate these physiologically unique bacteria (Willems *et al.*, 1989; Meyer *et al.*, 1993). Recently, a large number of carboxydotrophic bacteria have been reported among Gram-positive actinomycetes (O'Donnell *et al.*, 1993; Kim *et al.*, 1998) in addition to the previously known species of Gram-positive genera, *Arthrobacter* and *Bacillus* (Meyer *et al.*, 1986). This suggests that carboxydotrophy may be widespread across Gram-positive bacteria as well.

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Acinetobacter sp. strain JC1 DSM 3803 is a carboxydobacterium, which was isolated from a soil sample in Seoul, Korea (Cho *et al.*, 1985). Earlier studies on carbon monoxide dehydrogenase (CO-DH) of this bacterium, the key enzyme responsible for the oxidation of carbon monoxide, showed that the CO-DH is similar to CO-DHs from other carboxydobacteria in molecular weight, subunit structure, cofactor composition and range of artificial electron acceptors for CO oxidation (Kim *et al.*, 1989). However, the enzyme was found to have no immunological relationship with those from other carboxydobacteria, suggesting that the basis for CO oxidation might be diverse among carboxydobacteria (Kim *et al.*, 1989). In addition, it has been shown that this bacterium is capable of growing on methanol and possesses a novel mechanism for the assimilation of methanol (Ro *et al.*, 1997). This strongly implies that *Acinetobacter* sp. strain JC1 DSM 3803 is quite unique among known members of the carboxydobacteria. In this report we present evidence that *Acinetobacter* sp. strain JC1 DSM 3803 should be transferred to the genus *Mycobacterium*, as *Mycobacterium* sp. strain JC1 DSM 3803.

Many years after the isolate had been classified as a member of the genus *Acinetobacter* (Cho *et al.*, 1985), it has been found that the bacterium has different characteristics from other members of the genus. Nucleotide sequences of many genes of the bacterium showed an

unusually high mol% G+C compared to the known values for *Acinetobacter*, 38~47%, and it grows in the presence of CO, a strong inhibitor of aerobic respiration with oxygen as the terminal electron acceptor, while species of *Acinetobacter* have a strictly respiratory type of metabolism requiring oxygen (Juni, 1984).

Analyses for bacteriological and biochemical properties were repeated to determine the exact taxanomic position of the bacterium. In addition to the staining property of the bacterium being Gram-negative (Cho *et al.*, 1985), the bacterium was also found to be acid-fast, which is one of the unique features among acid-fast actinomycetes including *Mycobacterium*, *Nocardia*, *Rhodococcus* and *Corynebacterium*. Such a staining property, being strongly acid-fast but weakly stainable in Gram stain is one of the characteristics of members of the genus *Mycobacterium*, which are considered as Gram-positive. This misinterpretation of the result of Gram stain was the major reason which made confusion in our initial classification of this bacterium as an *Acinetobacter* species. The G+C content of the DNA also supports the placement of this bacterium among actinomycetes. The mol% G+C of the isolate estimated by the method of Mandel and Marmur (1968) is about 73%. Considering the mol% G+C of sequenced regions of this bacterium, 66.1% over 19 kb, and the possible error range of the experimental method, the mol% G+C of the bacterium is comparable to those of actinomycetes, including mycobacterial species whose mol% G+C is 62-70% (Wayne and Kubica, 1986). Along with these results, the bacteriological and biochemical properties of this bacterium, being rod-shaped or slightly curved with no mycelium, acid-alcohol-fast, nonmotile, aerobic, oxidase-negative, catalase-positive and nitrate reductase-positive, relocated the isolate from the genus *Acinetobacter* to the genus *Mycobacterium* according to the description of Wayne and Kubica (1986).

To further confirm the placement of the bacterium in the genus *Mycobacterium*, its fatty acid pattern was investigated. Fatty acid methyl esters were obtained from cells grown on trypticase soy broth agar that were saponicated and methylated, and separated using Hewlett Packard 6890 gas chromatography. Fatty acids were identified by the Microbial Identification System (Microbial ID, Inc, Newark, Del.). Tuberculostearic acid (10-methyl $C_{18:0}$) which is unique in actinomycetes including mycobacteria was detected. The fatty acid profile composed of various unbranched, saturated and unsaturated fatty acids, and tuberculostearic acid (Table 1) shows a typical pattern of mycobacterial fatty acids, verifying the taxanomic position of this bacterium in the genus *Mycobacterium*.

Comparison of sequences of 16s rDNA has been a valuable tool for identification of unknown bacterial isolates. A DNA fragment of 16s rDNA of the isolate was amplified by PCR (Kirschner *et al.*, 1993) using two oligonucleotide primers, 5'-gagtttgatcctggctcag-3' and 5'-agaaaggaggtgatccagcc-3', and the sequence of the 16s rDNA was determined using fluorescence-labelled Dideoxy Dye terminators on an ABI 377 automated DNA sequencer (Perkin-Elmer). The nucleotide sequences of the 16s rDNA was deposited in the GeneBank database under the accession number AY147261. The sequence of 1,475 nucleotides corresponding to position 31-1,517 of 16s rDNA sequence of *M. tuberculosis* H37Rv (GeneBank, Z83862) was compared with those of other mycobacteria using CLUSTAL W program (Thomson *et al.*, 1994). Analysis of the sequences revealed that the 16s rDNA sequence of the isolate is more than 96% similar to those of other mycobacteria, confirming the identity of the isolate as a member of the genus *Mycobacterium*. One of the unique features of the sequence is deletion of 12 nucleotides corresponding to position 459-470 of the *M. tuberculosis* H37Rv sequence, which is the signature of fast-growing mycobacteria in accordance with the growth rate of the isolate, t_d=6 h when heterotrophically grown with succinate. Phylogenetic analysis also located the isolate among fast-growing mycobacteria, grouped with two other species, *M. peregrinum* (Kusunoki and Ezaki, 1992) and *M. wolinsky* (Brown *et al.*, 1999) with sequence similarities of 99.7% and 99.4%, respectively (Fig. 1).

As the isolate was not clearly separated from *M. peregrinum* and *M. wolinsky* based on 16s rDNA sequences, more detailed analyses were carried out with different methods. In addition to 16s rDNA sequence, a part of *rpoB* gene served as a molecular marker to precisely differentiate closely related species of mycobacteria by PCR-restriction fragment length polymorphism analysis (Lee *et al.*, 2000). Genomic DNAs from the three strains of interest, *Mycobacterium* sp. JC1 DSM 3803, *M. peregrinum* ATCC 14467 and *M. wolinsky* ATCC 700010 were used as templates to amplify a 360-bp region of *rpoB* gene by PCR, and the amplified DNAs were subsequently digested

Table 1. Fatty acid content of *Mycobacterium* sp. strain JC1 DSM 3803

Fatty acid	Content (%)
14:0	7.04
15:0	0.86
16:1 *cis* 9	1.93
16:0	34.07
16:0 10-methyl	0.67
17:1 *cis* 8	0.94
17:0	0.63
18:1 *cis* 9	21.17
18:0	4.21
18:0 10-methyl	11.24
Summed feature 4*	13.75
Summed feature 7*	3.51

*Summed features represent groups of fatty acids which were not individually identified: summed feature 4, 15:0 iso 2-OH and 16:1 *cis* 7; summed feature 7, 18:1 *cis* 9, 18:1 *trans* 12, and 18:1 *cis* 7.

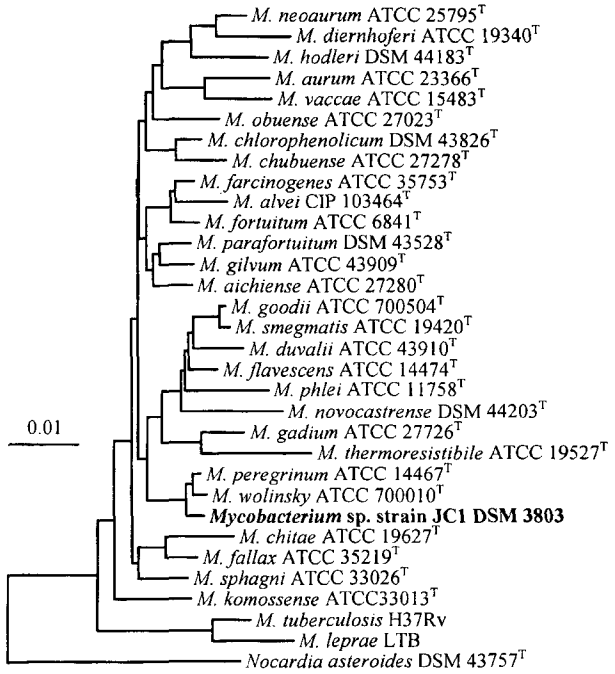

Fig. 1. Phylogenetic tree based on 16s rDNA sequences. Scale bar represents 0.01 substitution per nucleotide.

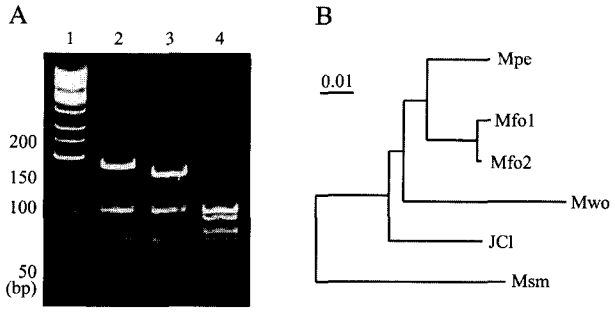

Fig. 2. Differentiation of *Mycobacterium* sp. strain JC1 from other related mycobacterial species. A) *Msp*I-derived PRA band patterns of a 360-bp fragment of *rpoB* gene. Lanes: 1, DNA size marker (50-bp ladder); 2, *M. wolinsky* ATCC 700010; 3, *Mycobacterium* sp. strain JC1 DSM 3803; 4, *M. peregrinum* ATCC 14467. B) Phylogenetic relationship among *Mycobacterium* sp. strain JC1 and other mycobacterial species based on the sequence of a part of *rpoB* gene. Scale bar represents 0.01 substitution per nucleotide. Msm, *M. smegmatis* ATCC 19420; JC1, *Mycobacterium* sp. strain JC1 DSM 3803; Mwo, *M. wolinsky* ATCC 700010; Mfo1, *M. fortuitum* ATCC 6841; Mfo2, *M. fortuitum* ATCC 49404.

with *Msp*I. As shown in Fig. 2A, distribution of DNA fragments generated by *Msp*I digestion of the PCR-amplified DNAs clearly differentiates *Mycobacterium* sp. JC1 from *M. peregrinum* ATCC 14467 and *M. wolinsky* ATCC 700010. Even though these three strains share two DNA fragments of the same size, reflecting the phylogenetic relationship among them, *Mycobacterium* sp. JC1 has a unique DNA fragment pattern that separates the strain from the other two strains.

When sequences of a part of the *rpoB* gene were compared it became more evident that *Mycobacterium* sp. JC1 is divergent from other related strains (Fig. 2B). A sequence of a part of the 360-bp DNA fragment from each strain was determined. The nucleotide sequences of *rpoB* gene of *Mycobacterium* sp. JC1 was deposited in the GeneBank database under the accession number AY147262. To determine the phylogenic location of the isolate corresponding sequences of *rpoB* gene from *M. smegmatis* and two *M. fortuitum* strains, from which *M. wolinsky* and *M. peregrinum* diverged (Kusunoki and Ezaki, 1992; Brown *et al.*, 1999) were included. In contrast to the analysis of 16s rDNA sequences (Fig. 1), two *M. fortuitum* strains and *M. peregrinum*, which are related to each other localize in a single branch, which validates usage of the sequences of *rpoB* gene in phylogenetic analysis. In the phylogenetic tree, *Mycobacterium* sp. JC1 is placed in a separate branch implying that this strain is unique among these bacteria.

A few other characteristics of the bacterium differentiate *Mycobacterium* sp. JC1 from both *M. wolinsky* and *M. peregrinum*. While *M. wolinsky* is deficient of arylsulfatase activity and *M. peregrinum* can not grow at 42°C (Wayne and Kubica, 1986), *Mycobacterium* sp. JC1 is arylsulfatase-positive and capable of growing at the elevated temperature. Together with the molecular phylogenetic criteria, these bacteriological and biochemical properties of the bacterium suggest that the isolate, formerly reported as *Acinetobacter* sp. strain JC1 should be reclassified as *Mycobacterium* sp. strain JC1.

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