Phylogenetic Evidence for the Transfer of *Caseobacter polymorphus* (Crombach) to the Genus *Corynebacterium*

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Reverse transcriptase sequencing of 16S ribosomal ribonucleic acid of *Caseobacter polymorphus* was performed in order to determine the relationship of this organism to other mycolic acid-containing actinomycetes and coryneform bacteria. The sequence data clearly demonstrate that *Caseobacter polymorphus* is a member of the genus *Corynebacterium*. Furthermore, *Caseobacter polymorphus* showed 100% sequence homology in a large stretch of 1,458 nucleotides with *Corynebacterium variabilis*. On the basis of the present and previous data we formally propose that *Caseobacter polymorphus* be considered a subjective synonym of *Corynebacterium variabilis*.

The genus *Caseobacter* was proposed by Crombach in 1978 (7) to accommodate some gray-white cheese coryneform bacteria originally described by Mulder and Antheunisse (16). However, the taxonomic position of the genus *Caseobacter* is equivocal (3, 8). Although it is generally accepted that *Caseobacter polymorphus* is a member of the "CMN (*Corynebacterium-Mycobacterium-Nocardia*) group" of organisms, its precise relationship, particularly with respect to corynebacteria and rhodococci, remains unclear. Caseobacters are similar to corynebacteria in possessing relatively short-chain mycolic acids (ca. 30 to 36 carbon atoms) (4). Further support for a possible relationship between *Caseobacter polymorphus* and the genus *Co-*

that the relatively high guanine-plus-cytosine content of *Caseobacter polymorphus* (ca. 60 to 67 mol%) is more in keeping with an affinity with the genus *Rhodococcus*, although several authentic *Corynebacterium* species also possess comparably high values (17). Similarly, caseobacters more closely resemble rhodococci in producing tuberculo-stearic (10-methyl octadecanoic) acid (5). However, a number of *Corynebacterium* species are now also known to contain this fatty acid (e.g., *Corynebacterium ammoniagenes*, *Corynebacterium bovis*, *Corynebacterium variabilis*) (2, 3, 5).

In this study we determined long stretches of the 16S ribosomal ribonucleic acid (rRNA) primary structure of

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UUUGAUGGAG AGUUUGAUCC UGGCUCAGGA CCAACGCUUG CGGCGUGCUU AACACAUGCA AGUCGAACGG AAAGGCCUUG CUUGCAGGG.GGC
GAACGSGUGAG UAACACGUNG GUGAUCUGCC CUGCAGUUGG GGAUAAGCCU GGGAAACUGG GUCUAAUACC GGAUAGGACC AUCGUUUAAU GUCGGUGGUG
GAAAGUUUUU CGSUGCAGGA UGAGCUNGCG GCCUAUCAGC UUGUUGSUGG GGAUAUGGCC UACCAAGGCG UCGACGGGUA GCCGGCCUGA GAGGGUGGAC
GGCCACAUUG GGACUGAGAC ACGGCCAGGA CUCCUACCGG AGGCGAGAGU GGGGAUAUUG GCACAAGUGG CGCAAGCCUG AUGCAGCGAC GCCCGUGGGG
GGAUGAAGGC CUUCGGGUGG UAAACUNCUN UCAACCAUGA CGAAGCAUUA UGUGACGGUA GUGGUGAAGG AAGCACCGGC UAACUACGUG CCAGCAGGCCG
CGGUAUACG UAGGGUGCNA GCGUUGCCG GAAUUCUGG GCGUAAGAG CUCGUAGGUN GUUUGUCGCG UCGUCUGUGA AAUUCGGGG CUUACUCCG
GNCUGGACGG CBAUACGGGC AUAACUUNAG UGUGUGAGGG GAGACUGGAA UUCUUGUGU AGCGGUGAAA UGCGCAGAAU UCAGGAGGAA CACCGAUGGC
GAAGGCGAGU CUCUGGGCAG UAACUGACGC UGAGGAGCGA AAGCAUGGGU AGCGAAGCAG AUUAGAUACC CUNGUAGUCC AUGCCGUAAA CGSUGGGCGC
UAGGUGUGGG GGUUUUCCAC GACUUCUGUG CCGUAGCUAA CGCAUUAAGC GCGCCGCGUG GGGAGUACGG CCGCAAGGCU AAACUCAAA GGAUUGAGC
GGGGCCCGCA CAAGCGGCGG AGCAUGUGGA UUAUUCGAU GNAACGCBAA GAACCUUACC UGGGCUUGAC AUUAGCCGGA UCGNCGCAGA GAUGCGUNUU
CCCUNGUNU CGGUUAUACG GUNGUGCAG GUUGUGUCA GCUGUGUGG UGAGAGUUGG GGUUAAGUCC CGCAACGAGC GCAACCCUNG UCUUGUGUUG
CCAGCACGUU AUGGUGGGGA CUCGCGAGAG ACUGCCGGGG UNAACUCGGA GGAAGGUGGG GNUGACGUA AAUACUAGU CCCCUGUUGU CCAGGGCUUC
ACACAUGCUA CAUUGGCGG UACAGUGGGU UGCGACACCG UNAGGUNGUG CUAAUCCUUN AAAGCCGGUC UNAGUUCGGA UUGGAGUCUG CAACUNGACU
CCAUGAAGUC GGAGUGGCUA GUAUUGCAG AUCAGCAACG CUGCGGUGAA UACGUUCCCG GGCCUUGUAC ACACCGNCCG UCACGUAUG AAAGUUGGUA
ACACCCGAAG CCAGUGGCC AAACUGGUU GGGAGCUGUC GAAGGUGGGA UGCGCGAU

FIG. 1. Partial primary structure of the 16S rRNA of *Caseobacter polymorphus* (*Corynebacterium variabilis*) as determined by reverse transcriptase. The dots indicate an unsequenced region of eight nucleotides. n, Undetermined nucleotide. The star at the 5' terminus corresponds to position 1, and the last base at the 3' terminus corresponds to position 1,497 of the *E. coli* sequence (1).

rynebacterium comes from the presence of only *N*-acetyl residues in the glycan moiety of its murein (3). All other mycolic acid-containing genera (i.e., *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Tsukamurella*) contain both *N*-acetyl and *N*-glycolyl residues (18). It has been suggested (8, 14)

Caseobacter polymorphus in an attempt to determine the relationship of this organism to other mycolic acid-containing taxa.

MATERIALS AND METHODS

Cultures. *Caseobacter polymorphus* NCDO 2097^T (T = type strain) and *Corynebacterium variabilis* NCIB 9455^T

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TABLE 1. 16S rRNA sequence homologies between *Caseobacter polymorphus* (*Corynebacterium variabilis*) and reference actinomycetes

Strain	% Homology to:											
	<i>Caseobacter polymorphus</i> NCDO 2097 ^T	<i>Corynebacterium bovis</i> NCDO 1689 ^T	<i>Corynebacterium cystitidis</i> DSM 20524 ^T	<i>Corynebacterium glutamicum</i> DSM 20300 ^T	<i>Corynebacterium renale</i> DSM 20298	<i>Rhodococcus erythropolis</i> DSM 43188	<i>Rhodococcus fascians</i> DSM 20131	<i>Mycobacterium chelonae</i> TMC 1544	<i>Mycobacterium komossense</i> ATCC 33013	<i>Mycobacterium phlei</i> TMC 1516	<i>Mycobacterium tuberculosis</i> H37/RV	<i>Nocardia asteroides</i> DSM 43005
<i>Corynebacterium bovis</i> NCDO 1689 ^T	90.2											
<i>Corynebacterium cystitidis</i> DSM 20524 ^T	80.1	79.9										
<i>Corynebacterium glutamicum</i> DSM 20300 ^T	89.2	86.4	85.3									
<i>Corynebacterium renale</i> DSM 20298	82.0	78.9	84.0	81.5								
<i>Rhodococcus erythropolis</i> DSM 43188	79.4	79.8	77.3	78.7	81.2							
<i>Rhodococcus fascians</i> DSM 20131	78.9	78.7	76.7	79.0	79.1	90.1						
<i>Mycobacterium chelonae</i> TMC 1544	75.1	76.5	73.9	75.9	76.8	82.4	79.8					
<i>Mycobacterium komossense</i> ATCC 33013	78.3	79.9	78.0	79.4	80.0	85.8	79.8	91.5				
<i>Mycobacterium phlei</i> TMC 1516	77.2	79.4	76.5	77.1	79.9	85.3	81.3	87.2	94.3			
<i>Mycobacterium tuberculosis</i> H37/RV	78.3	78.3	76.8	77.6	78.1	82.3	79.4	83.6	92.6	89.9		
<i>Nocardia asteroides</i> DSM 43005	75.8	77.4	73.4	75.8	76.4	88.0	83.5	78.2	83.0	82.3	81.6	
<i>Tsukamurella paurometabolum</i> DSM 20162 ^T	80.8	78.3	79.6	81.1	80.8	87.7	84.8	81.8	87.4	86.2	83.5	84.0

were grown in shake flasks containing corynebacterium broth (casein peptone, 10 g; yeast extract, 5 g; glucose, 5 g; NaCl, 5 g; water, 1 liter; pH 7.4) to late exponential phase at 30°C.

Reverse transcriptase sequencing of ribosomal ribonucleic acid. rRNA was isolated as described previously (10). Analysis of 16S rRNA by using reverse transcriptase was done as described by Lane et al. (15). The sequence of oligonucleotide primers, their target sites, and the electrophoretic separation conditions used for complementary deoxyribonucleic acid (DNA) were as described previously (10). Sequencing ambiguities were resolved by using terminal transferase (9). Selected stretches were aligned with homologous regions of 12 strains representing five mycolic acid-containing genera. Computation of levels of homology and evolutionary distance values (K_{nuc}) and the construction of an unrooted phylogenetic tree followed previously published procedures (12, 13, 18).

DNA hybridization. DNA was purified as described previously (11). DNA was labeled by nick translation, using [α -³²P]deoxycytidine triphosphate (kit N5500; Amersham International, Amersham, United Kingdom). DNA-DNA spot hybridizations were performed on nitrocellulose membranes (type BA85/20; Schleicher & Schuell, Dassel, Federal Republic of Germany) in Denhardt solution (6× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone) at 72°C overnight. The filters were washed serially with 100-ml portions of 2× SSC, 0.2× SSC, and 0.1× SSC by using the Hybaid system (Hybaid Ltd., Cambridge, United Kingdom).

RESULTS AND DISCUSSION

Two long stretches of the 16S rRNAs of *Caseobacter polymorphus* and *Corynebacterium variabilis* were compared. Out of 1,458 base sequences, not a single nucleotide difference between the two species was detected (Fig. 1).

The sequence of *Caseobacter polymorphus* was aligned and compared with the sequences of 12 reference actinomycetes (4; unpublished data). Homology and derived K_{nuc} values were based on 480 nucleotides involving variable positions between points 200 and 1,435 (*Escherichia coli* numbering system [1]) (Table 1). The phylogenetic tree constructed from the latter values is shown in Fig. 2. *Caseobacter polymorphus* clearly clustered within the confines of the genus *Corynebacterium* and was well removed from rhodococci and other mycolic acid-containing taxa. The recovery of *Caseobacter polymorphus* within the radiation of true corynebacteria is consistent with the presence of corynemycolic acids (4) and *N*-acetyl muramic acid (3) within this species. Furthermore, the occurrence of 100% 16S rRNA sequence homology (for 1,458 nucleotides) between the type

FIG. 2. Unrooted phylogenetic tree displaying the position of *Caseobacter polymorphus* (*Corynebacterium variabilis*) with respect to some mycolic acid-containing actinomycete taxa. Bar = 0.04 K_{nuc} unit.

FIG. 3. DNA-DNA spot hybridizations in which [^{32}P]DNA from *Caseobacter polymorphus* NCDO 2097^T was used. Spot 1, *Caseobacter polymorphus* NCDO 2097^T; spot 2, *Corynebacterium variabilis* NCIB 9455^T; spot 3, *Corynebacterium diphtheriae* NCTC 11397^T; spot 4, *Corynebacterium renale* NCTC 7448^T; spot 5, *Caseobacter polymorphus* NCDO 2102.

strains of *Caseobacter polymorphus* and *Corynebacterium variabilis* indicates that these organisms are closely related, a conclusion confirmed by DNA-DNA hybridization data (Fig. 3). *Caseobacter polymorphus* and *Corynebacterium variabilis* both possess a coryneform morphology, and these organisms are biochemically extremely similar (2, 7, 8, 16). These species are also indistinguishable on the basis of chemotaxonomic criteria (e.g., murein, fatty acids, mycolic acids, quinones, etc.) (2–5). On the basis of these phenotypic similarities, together with the high levels of DNA relatedness and 16S rRNA sequence homology, we believe that these taxa represent a single species. Therefore, we propose that *Caseobacter polymorphus* be considered a later subjective synonym of *Corynebacterium variabilis* (2).

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