Evidence for a Close Phylogenetic Relationship between *Melissococcus pluton*, the Causative Agent of European Foulbrood Disease, and the Genus *Enterococcus*

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The 16S rRNA gene sequence of *Melissococcus pluton*, the causative agent of European foulbrood disease, was determined in order to investigate the phylogenetic relationships between this organism and other low-G+C-content gram-positive bacteria. A comparative sequence analysis revealed that *M. pluton* is a close phylogenetic relative of the genus *Enterococcus*.

“*Bacillus pluton*,” the primary etiological agent of European foulbrood of the honey bee, *Apis mellifera*, was originally described by White (14). This bacterium was first cultured and characterized in detail by Bailey (1) and was reclassified as “*Streptococcus pluton*” by him. The taxonomic position of “*S. pluton*” has always been problematic, and the species was not included on the Approved Lists of Bacterial Names (12). Bailey and Collins (3) assigned “*S. pluton*” to a new genus, *Melissococcus*, on the basis of the results of phenotypic studies (2). Our knowledge concerning the genetic interrelationships of streptococci and related low-G+C-content gram-positive taxa has improved considerably in recent years with the application of nucleic acid hybridization and 16S rRNA sequencing techniques. As a result, the genus *Streptococcus* is now primarily restricted to pyogenic and oral species (10). The “lactic” (or Lancefield group N) and “fetal” (Lancefield group D) streptococci have been assigned to two new genera, *Lactococcus* and *Enterococcus*, respectively (9–11). More recent comparative 16S rRNA sequencing data have shown that the genus *Lactococcus* is phylogenetically more closely related to the genus *Streptococcus* than to the genus *Enterococcus* (4). The latter genus is phylogenetically closely related to the genera *Carnobacterium* and *Vagococcus* (4, 13, 15). Until now, there has been no information concerning the phylogenetic affinities of the genus *Melissococcus*. In this paper we describe the 16S rRNA gene sequence of *Melissococcus pluton* and the results of a comparative analysis performed with other catalase-negative, low-G+C-content, gram-positive bacteria. *M. pluton* NCDO 2443T (T = type strain) was grown as described by Bailey and Collins (2), and chromosomal DNA was prepared by using the method of Farrow et al. (6). The 16S rRNA gene of *M. pluton* NCDO 2443T was amplified by a PCR and was sequenced directly by using a Sequenase version 2.0 sequencing kit (U.S. Biochemicals) as described previously (8). Sequences were aligned and similarity values were determined by using the Wisconsin Molecular Biology package (5). Phylogenetic trees were constructed by using neighbor-joining and unweighted pair group with mathematical average methods.

The 16S rRNA gene sequence of *M. pluton* NCDO 2443T which we have determined has been deposited in the GenBank data base under accession number X75751. This sequence consisted of 1,396 nucleotides, representing approximately 93% of the total 16S rRNA primary structure. The derived 16S rRNA sequence of *M. pluton* was aligned with more than 150 other low-G+C-content gram-positive 16S rRNA sequences retrieved from the EMBL and GenBank data bases. For the initial homology analysis approximately 90 nucleotides proximal to the 5′ end of the molecule were removed because of problems in aligning the hypervariable V1 region, which is different lengths in phylogenetically diverse taxa. After we established the closest relatives of *M. pluton*, however, this stretch of sequence could be aligned unambiguously and was

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FIG. 1. Dendrogram showing the relationships of *M. pluton*, enterococci, and other low-G+C-content gram-positive organisms. Clustering was by the unweighted pair group method. *T*. *Tetragenococcus*; *C*. *Carnobacterium*; *V*. *Vagococcus*; *E*. *Enterococcus*. 
TABLE 1. Levels of sequence similarity for a 1,396-nucleotide region of the 16S rRNAs of M. pluton and closely related low-G+C-content gram-positive organisms

<table>
<thead>
<tr>
<th>Strain</th>
<th>Carnobacterium mobilе NCDO 2765T</th>
<th>Carnobacterium piscicola NCDO 2762T</th>
<th>Enterococcus dispar NCIMB 13000T</th>
<th>Enterococcus durans NCDO 596T</th>
<th>Enterococcus faeciacium NCDO 942T</th>
<th>Enterococcus gallinarum NCDO 2313T</th>
<th>Enterococcus hirae NCDO 1258T</th>
<th>Enterococcus malodoratus NCDO 846T</th>
<th>Enterococcus mundii NCDO 2375T</th>
<th>Enterococcus pseudoavium NCDO 2138T</th>
<th>Enterococcus raffinosus NCTC 1219T</th>
<th>Enterococcus saccharolytics NCDO 2594T</th>
<th>Tetragenococcus halophilus NCDO 1635T</th>
<th>Vagococcus fluvialis NCDO 2497T</th>
<th>Vagococcus salmoninarum NCDO 2777T</th>
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<td>92.4 92.2 95.2 96.0 94.0 93.7 95.0 95.3 95.0 95.7 95.5 95.2 95.4 95.2 95.2 95.4 94.9 93.9 93.1 93.2 92.7 92.6 92.4 94.2 94.2 94.2 94.1 94.2 94.1 94.2 94.4 94.3 94.3 94.3 94.2 94.3 94.1 94.1 94.3 94.3 94.3 94.4 92.9 93.9 94.1 93.8 93.6 91.4 92.6 96.9 96.1 96.1 97.4 98.8 96.2 98.8 98.7 99.0 99.6 98.8 99.4 99.5 96.8 96.5 96.5 93.7 94.5 94.3</td>
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<td>98.8 99.3 98.5 98.7 97.1 96.1 93.2 94.0 93.8</td>
<td>98.5 99.5 99.5 97.3 96.7 93.5 94.6 94.1</td>
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<td>99.2 96.7 96.5 93.6 94.7 93.8</td>
<td>97.0 96.3 93.5 94.7 94.0</td>
<td>96.9 94.2 93.8 93.8</td>
<td>93.7 94.0 93.7</td>
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above, and \textit{M. pluton} clustered at the periphery of the genus \textit{Enterococcus} (15). Close, albeit more distant, relationships to the genera \textit{Carnobacterium}, \textit{Vagococcus}, and \textit{Tetragenococcus} (formerly \textit{Pediococcus halophilus}) were also observed (Fig. 1). We found that on the basis of 16S rRNA sequence data \textit{M. pluton} is phylogenetically only remotely related to streptococci. Both evolutionary distance calculations and treeing programs revealed that \textit{M. pluton} and enterococci are closely related. Additional evidence for a close affinity between these taxa comes from the report that Lancefield group D antigen occurs in \textit{M. pluton} (7). Because of the close relationship between \textit{M. pluton} and enterococci, it could be argued that these organisms should be placed in a single genus. However, we consider such a change in nomenclature to be unwise, as the monospecific genus \textit{Melissococcus} (Bailey and Collins 1983) has nomenclatural priority over the genus \textit{Enterococcus} (Schleifer and Kilpper-Báč 1984). The genus \textit{Enterococcus} currently contains 18 species, and this genus name is widely used and accepted by the scientific community. A change of name would in our opinion serve no useful purpose and would create unnecessary nomenclatural confusion. Hence, we believe that the fastidious cultural requirements and low DNA G+C content of \textit{M. pluton} (2), together with the branch point of \textit{M. pluton} at the periphery of the \textit{Enterococcus} cluster (Fig. 1), are sufficient to justify retention of \textit{Melissococcus} as a separate genus.

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\textbf{REFERENCES}