Reclassification of *Mesoplasma pleciae* as *Acholeplasma pleciae* comb. nov. on the basis of 16S rRNA and *gyrB* gene sequence data

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Genomic DNA sequence data for the 16S rRNA gene and the *gyrB* gene of *Mesoplasma pleciae* PS-1^T (= ATCC 49582^T = NBRC 100476^T) demonstrate a much closer relationship to *Acholeplasma laidlawii* and *Acholeplasma oculi* than to other species in the order *Entomoplasmatales*. In addition, the preferred use of UGG rather than UGA as the codon for tryptophan in the *gyrB* sequence probably places the organism outside the order *Entomoplasmatales*. It is proposed that *M. pleciae* be reclassified in the genus *Acholeplasma*, as *Acholeplasma pleciae* comb. nov.

The classification of mollicute species based on phenotypic traits has been difficult and error-prone. The sterol requirement that separates the *Acholeplasma*, *Mesoplasma* and other mollicute species has been an effective classification tool (Tully *et al.*, 1993), but its testing and interpretation are difficult and require experience and skill. Strain PS-1^T, originally classified as '*Acholeplasma pleciae*', was classified in the genus *Mesoplasma* based on its lack of sterol requirement and its need for fatty acid supplements. Increasingly, classification is based on genotype, especially the sequence data for the 16S rRNA gene, and, less widely, on the protein coding sequence of the DNA gyrase beta subunit gene (*gyrB*) (Watanabe *et al.*, 2001).

Two differently dated lyophilized samples of *Mesoplasma pleciae* PS-1^T (Tully, 1983; Clark & Whitcomb, 1984; Clark *et al.*, 1986; Tully *et al.*, 1994) obtained from the mollicute collection at the University of Florida, and a lyophilized sample of the equivalent ATCC culture, ATCC 49582^T, were grown at 30 °C in M1D or SP-4 medium (Whitcomb, 1983).

Growth was quite slow (5 days) in both media, in marked contrast to my experience of much more rapid (1–2 days) growth with other *Mesoplasma* and *Entomoplasma* species.

Lysis and phenol/chloroform extraction of genomic DNA were performed using standard techniques.

PCR amplification of the 16S rRNA gene was performed (Johansson *et al.*, 1998) using primers U1F and U8R,

Qiagen Quantitect PCR mix, and cycling consisting of an initial 15 min hot start at 95 °C and 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 66 °C for 3 min. The unusually low extension temperature was found necessary to successfully amplify the very low G + C content regions found in some of the sequences (Su *et al.*, 1996).

The approximately 1500 bp rRNA gene product was gel-purified and sequenced using primers U1F, U8R, U2F, U5R, U2R, U4R, U6F and U6R to achieve complete coverage in both directions of the entire fragment. Sequencing was done on an Applied Biosystems 310 instrument with BigDye v3.1 chemistry. Assembly was done with ABI Autoassembler software.

Novel degenerate primers for the *gyrB* gene were designed for the *Entomoplasmatales* by CLUSTAL W alignment of existing mollicute *gyrB* sequences, and careful selection of primers from highly conserved regions. PCR of the *gyrB* sequence was performed using degenerate primers *gyrB*-F (5'-gac ttg agg ctg tta gaa aam ghc cwg gna tgt a-3') and *gyrB*-R (5'-tgt ttg tca taa ttg atc wgv rty cat ytc wcc wa-3'). The resulting approximately 1700 bp fragment was gel-purified and sequenced with primers *gyrB*-seq-F (5'-gac ttg agg ctg tta gaa-3') and *gyrB*-seq-R (5'-tgt ttg tca taa ttg atc-3'). Four additional species-specific primers were chosen and synthesized to complete forward and reverse sequencing of the *gyrB* fragment.

16S and *gyrB* sequencing was performed on all three samples obtained, with identical results.

BLAST analysis of the 16S rRNA gene sequence showed closest alignment with *Acholeplasma laidlawii* (98 % sequence identity). All other top matches were *Acholeplasma* or *Phytoplasma* species from the order *Acholeplasmatales*,

Published online ahead of print on 16 April 2004 as DOI 10.1099/ijs.0.02904-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Mesoplasma pleciae* PS-1^T is AY257485; that for the partial *gyrB* sequence of *M. pleciae* PS-1^T is AY257486.

with no matches above 90 % identity to species outside this order. Other *Mesoplasma* species matched at the 82 % level.

The nucleotide alignment of the *gyrB* gene showed an 85 % identity to the nucleotide sequence of *A. laidlawii* with no similarly significant matches to any other GenBank entries.

The amino acid alignment of the partial *gyrB* sequence showed a 93 % identity to the sequence for *A. laidlawii*. Despite the presence of ten sequenced *gyrB* genes from other *Mesoplasma* and *Entomoplasma* species in GenBank, the next most significant match was 62 %, to the *gyrB* sequence of *Staphylococcus epidermidis*, a species quite outside the mollicute clade.

The partial amino acid sequence of the *gyrB* gene shows three occurrences of the amino acid tryptophan. In each case, the codon used for this amino acid is UGG, the standard bacterial codon. Coding sequences in the order *Entomoplasmatales* almost always use UGA as the codon for tryptophan. For example, other *gyrB* sequences from the *Entomoplasmatales* show 18 tryptophan codons, of which 17 use UGA as the codon. The single use of UGG occurs in *Entomoplasma luminosum* (GenBank/EMBL/DDBJ accession no. AY196995), which also has an occurrence of UGA in the same sequence.

The three occurrences of UGG and the lack of any UGA codons in the *M. pleciae* coding sequence strongly suggest that the organism uses UGA as a stop codon, rather than as a tryptophan codon, as would be expected from a species of the *Acholeplasmatales*.

I believe that the combination of 16S rRNA and *gyrB* nucleotide sequence similarity to *Acholeplasmatales* species, the lack of similarity of nucleotide and amino acid sequences to *Entomoplasmatales* species, and the use of UGG rather than UGA as the preferred codon for tryptophan make a compelling argument that this species is properly classified as a member of the genus *Acholeplasma*.

Description of *Acholeplasma pleciae* comb. nov.

The description is based upon that given by Tully *et al.* (1993) for *Mesoplasma pleciae*.

The type strain is PS-1^T (=ATCC 49582^T=NBRC 100476^T); its 16S rRNA and *gyrB* gene sequences are

available from GenBank/EMBL/DDBJ under accession numbers AY257485 and AY257486, respectively.

Acknowledgements

This work was supported in part by DARPA/ONR grant N00014-01-1-1060. I thank G. Gasparich and R. Whitcomb for providing genomic DNA and valuable discussions, M. Davidson for supplying the lyophilized strains and I. Lawhorn for assistance in sequencing and assembly.

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