Phylogenetic evidence for the relationship between the genera Mobiluncus and Actinomyces

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1. SUMMARY

Partial reverse transcriptase sequencing of 16S rRNA from Mobiluncus curtisi and M. mulieris clearly indicate that the genus Mobiluncus is not a member of the Bacteroidaceae but belongs to the order Actinomycetales. The highest degree of relationship is found with the genus Actinomyces which is supported by the presence of common physiological properties.

2. INTRODUCTION

The genus Mobiluncus was described for a group of vibrio-shaped, curved and motile anaerobic bacteria from the vaginae of women with bacterial vaginosis [1]. The two species of Mobiluncus, M. curtisi and M. mulieris exhibit a multilayered cell wall, lack an outer membrane, and cells are resistant to the antibiotics colistin and nalidixic acid, but sensitive to penicillin and vancomycin. On the other hand, cells stained Gram-variable, which, according to Spiegel and Roberts [1] may be due to the thinness of the peptidoglycan. Despite the presence of distinct Gram-positive features the authors tentatively placed Mobiluncus into the family Bacteroidaceae, the reason being the obvious phenotypic differences to any described genus of the order Actinomycetales, Bifidobacterium, Propionibacterium and other Gram-positive genera with a similar DNA G + C content. The present work was carried out to obtain information about the phylogenetic relationship of these organisms, with the ultimate goal to design DNA probes for the rapid identification of these possibly opportunistic pathogens.

3. MATERIALS AND METHODS

Mobiluncus curtisi ATCC 35421 and M. mulieris ATCC 35423 were cultivated on chocolate-haemin agar [2] under anaerobic conditions (CO₂ : N₂ = 10% : 90%) at 37°C. Crude ribosomal RNA was isolated [3] and reverse transcriptase sequencing done as previously described [4,5]. Sequences of oligonucleotide primers, their target
sites and electrophoretic conditions of cDNA followed published procedures [4,5]. Sequences were aligned to homologous regions of several actinomycete reference organisms the sequences of which have been published (see legend to Table 1). The phylogenetic analysis was carried out using the neighborliness method [6,7]. In simulation studies this method has been shown to be one of the 2 best methods for recovering a given tree topology from distance data assuming a varying rate of nucleotide substitution [8]. It has been refined using a value of tree-likeness for every scored quadruple [9]. The algorithm was implemented as part of the program package ‘Sage’ (Technoma GmbH, Heidelberg, F.R.G.) designed for the IBM XT/AT and compatibles.

4. RESULTS AND DISCUSSION

The partial sequences of *M. curtisii* and *M. mulieris* are shown in Fig. 1. Gaps in the sequence are caused by the failure of the primer 530 to optimally bind to the complementary rRNA target and by a non-readable region 5' adjacent to primer position 1200. The quality of sequencing gels was poor for certain regions of the 16S rRNA from *M. mulieris*; consequently these parts were omitted from Fig. 1.

In a preliminary analysis, comparison of putative RNase T1 resistant oligonucleotides of the sequence of *M. curtisii* with published signature oligonucleotides [10] pointed towards the membership of the genus *Mobiluncus* to the actinomycetes.
Table 1

Estimated evolutionary distance values

The lower left part of the matrix shows equally weighted (Hamming) pairwise distances, calculated from an alignment with the total length of 1069 positions. The upper right part shows weighted pairwise distances according to the following rules. Transitions: 0.6, transversions: 1.2; Ns (nucleotide composition not determined): 0.7; gaps: 0.7. Underlined regions indicate those sites which may serve as targets for genus-specific cDNA probes.

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Fig. 2. Rooted tree topology illustrating the phylogenetic position of the genus Mobiluncus within the actinomycetes subdivision of eubacteria. Segment lengths are proportional to evolutionary distances shown in Table 1, upper right half.

subdivision of Gram-positive eubacteria. Determination of pair-wise distances between M. curtisii and representatives of several actinomycete genera, including anaerobic and facultatively anaerobic members, confirmed this relationship (Table 1). rRNA homologies between M. curtisii and Bacteroides fragilis were significantly lower than between the former organism and Gram-positive eubacteria (below 75% and higher than 85%, respectively). The weighted distances (upper right half of Table 1) were used to construct a phylogenetic tree (Fig. 2) which shows Mobiluncus and Actinomyces to possess a common ancestry. The topography of the tree (omitting Mobiluncus) is very similar to the one derived from distance matrix analysis of a slightly different 16S rRNA data base [11,12].

Restricting the analysis to those stretches which are common to the two Mobiluncus species the pairwise homology is 93.8%. This value is higher than those found for the same rRNA regions of the two most unrelated species of the genera Actinomyces (88.9% for A. odontolyticus and A. bovis [12]) and Propionibacterium (91.3% for P. freudenreichii and P. acidipropionici [11]). M. curtisii and M. mulieris can therefore be considered moderately related species of the genus, a conclusion that confirms the finding of low (about 25%) DNA homology for strains of the two species [1]. The phylogenetic relationship between Mobiluncus and Actinomyces species is supported by the spectrum of end-products formed from carbohydrates, i.e. acetic, lactic and succinic, but no propionic acid, and a requirement for carbon dioxide for maximum growth. The DNA G+C content of 49–52 mol% is rather low for a member of the actinomycetes subdivision but similar low values have been reported for propionibacteria [13] and Renibacterium salmoninarum [14]. The combination of other features such as curved cell
shape, the possession of multiple subpolar flagella and a Gram-variable staining behaviour [1] is characteristic for the genus *Mobiluncus*. Once information is available for chemotaxonomic markers of proven taxonomic value, e.g. peptidoglycan type and the composition of fatty acids and polar lipids, the identification of members of this genus will be even more reliable.

The 16S rRNA primary structures unraveled for the two *Mobiluncus* species have at least two regions in common which are sufficiently different to all known eubacterial 16S rRNAs to serve as target sites for DNA probes of complementary nucleotide composition (see Fig. 1). Work is in progress to test the specificity of such probes which hopefully allow rapid identification of these organisms, which may be especially useful when their pathogenic potential has been demonstrated.

**ACKNOWLEDGEMENT**

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**REFERENCES**


