NOTE

DNA sequencing reveals limited heterogeneity in the 16S rRNA gene from the rrnB operon among five Mycoplasma hominis isolates

Tina Mygind, Svend Birkelund and Gunna Christiansen

To investigate the intraspecies heterogeneity within the 16S rRNA gene of Mycoplasma hominis, five isolates with diverse antigenic profiles, variable/identical P120 hypervariable domains, and different 16S rRNA gene RFLP patterns were analysed. The 16S rRNA gene from the rrnB operon was amplified by PCR and the PCR products were sequenced. Three isolates had identical 16S rRNA sequences and two isolates had sequences that differed from the others by only one nucleotide.

Keywords: Mycoplasma hominis, 16S rRNA, variation, RFLP analysis, intraspecies heterogeneity

Mycoplasmas are among the smallest self-replicating prokaryotes known. They differ from other prokaryotes in that they lack a cell wall, have low genomic DNA G+C content (Razin & Freundt, 1984), and have a small genome that varies in size from 580 kbp in Mycoplasma genitalium (Su & Baseman, 1990) to 1300 kbp in Mycoplasma iowae (Grau et al., 1991). Furthermore, mycoplasmas have a special use for the codon UGA, which is used as a stop codon in other bacteria but encodes tryptophan in mycoplasmas. Because of their small coding capacity, mycoplasmas have a low copy-number of rRNA genes, with one or two copies of the operon (Amikam et al., 1984). The order of genes in each operon is from 5' to 3': 16S, 23S and 5S.

Mycoplasma hominis is usually found as part of the normal flora of the human urinogenital tract. It has been associated with neonatal infection (Quinn et al., 1985) and is a risk factor for pre-term delivery (Hillier et al., 1995). In spite of the association of M. hominis with the above-mentioned diseases, it has not been possible to reveal specific pathogenicity factors. This is partly due to the fact that M. hominis isolates show considerable heterogeneity, predominantly in their membrane proteins (Christiansen et al., 1990; Olson et al., 1991). Moreover, at present three variable surface antigens have been described for M. hominis: Lmp (Jensen et al., 1995; Ladefoged et al., 1995, 1996), P120 (Nyvold et al., 1997) and Vaa (Zhang & Wise, 1997). These proteins are probably involved in defence against the host immune system. In a study conducted by Nyvold et al. (1997), the surface antigen P120 was found to be composed of one hypervariable domain, two semivariable domains and four constant domains. The hypervariable domain was sequenced for several strains; four strains showed pairwise identity (strains P2/SC4 and 7488/DC63).

Considerable intraspecies heterogeneity has been described within the 16S rRNA genes (Christiansen & Andersen, 1988; Blanchard et al., 1993). Christiansen & Andersen (1988) found pronounced heterogeneity among M. hominis strains when 26 strains were examined for heterogeneity by RFLP analysis using parts of rRNA genes as probes; pairwise analysis of strains P2/SC4 and 7488/DC63 also revealed differences. Blanchard et al. (1993) found significant differences in the 16S rRNA genes of two M. hominis strains, PG21T and 183. Therefore, the aim of this study was to determine the intraspecies variability of the 16S rRNA genes of M. hominis by automated DNA sequencing and to relate this heterogeneity to the variability in the genes encoding membrane proteins (surface antigens). M. hominis strains P2, SC4, DC63, 7488, 183 and PG21T were selected for the study.

Two Escherichia coli XL-1 Blue clones were obtained from previous investigations. The clone pBMhE6.6 (Özkökmen et al., 1994) has an insert with DNA upstream of the 16S rRNA gene and part of the 16S...


Table 1. *M. hominis* isolates used in this study

<table>
<thead>
<tr>
<th><em>M. hominis</em> strain</th>
<th>Source</th>
<th>Year/place of isolation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG21&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Lower genitalia</td>
<td>1953/England</td>
<td>Nicol &amp; Edward (1953)</td>
</tr>
<tr>
<td>DC63</td>
<td>Cavum oris</td>
<td>1963/England</td>
<td>Taylor-Robinson <em>et al.</em> (1963)</td>
</tr>
<tr>
<td>7488</td>
<td>Cervix</td>
<td>1988/Aarhus, Denmark</td>
<td>This laboratory</td>
</tr>
<tr>
<td>P2</td>
<td>Upper urinary tract</td>
<td>1978/Aarhus, Denmark</td>
<td>Thomsen (1978)</td>
</tr>
</tbody>
</table>

rRNA gene. The clone pBMhE1.2 has an insert with part of the 16S rRNA gene and DNA downstream of the 16S rRNA gene. Plasmid DNA from the two *E. coli* clones was prepared by the alkaline lysis method as described by Sambrook *et al.* (1989), and the sequence was determined with primers 5' GCGTTCATCCTGGAGC 3' and 5' GTAATCGACTCACTATAGGCGAATTGGG 3'. The sequences were obtained with the Thermo Sequenase Dye Terminator Cycle Sequencing kit (Amersham) using an ABI Prism 377 fluorescent sequencing instrument. *M. hominis* strains PG21<sup>T</sup>, P2, SC4, 7488, DC63 and 183 (Table 1) were cultivated in BEa medium (Christiansen & Andersen, 1988) and genomic DNA was isolated as described by McClenaghan *et al.* (1984).

PCR was carried out both directly on proteinase-K-treated culture and on genomic DNA. The following primers were used for PCR: 5' CCGAGAGATAAACATTCTTCTCAAACATCTGGATAC 3' and 5' GGAATATGAATACGTCGCTTTTGG 3'. These primers (DNA Technology) were designed to amplify a fragment of approximately 2 kbp containing the entire 16S rRNA gene. PCR was performed in a total volume of 100 µl containing 5 U AmpliTaq DNA polymerase, 100 pmol each primer, 0.2 mM each of dATP, dTTP, dGTP and dCTP, 10 µl 10× PCR buffer II, 10 µl 25 mM MgCl<sub>2</sub> solution, and 2.5 µl purified genomic DNA or 20 µl proteinase-K-treated culture (made up to 100 µl with water). The enzyme and buffers were purchased from Perkin Elmer. The reaction mixture was thermocycled in a GeneAmp PCR system 9600 thermal cycler from Perkin Elmer under the following cycling conditions: denaturation of template, 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min. The PCR products were purified with PCR Preps DNA Purification Resin and Wizard Minicolumns (Promega). The purification was carried out in accordance with the instructions of the manufacturer. The forward PCR primer is specific for the *rrnB* operon (Christiansen & Andersen, 1988) as *rrnA* and *rrnB* have different upstream regions. The reverse PCR primer is not specific as it is situated inside the operon and the two operons are very similar. Therefore the 16S rRNA genes amplified from genomic DNA are from *rrnB*.

The PCR products were sequenced with the primers described above in addition to the following primers: 5' GAAAACCTGGATACGAATACCATAACGTCGAC 3'; 5' GCCGGGCTCGAGAGACTGAAC 3'; 5' GCGTTCGAGCTTGTTAAGTGC 3'; 5' CGCATTAATGATCCGCTGAG 3'; 5' GCCTTTACGAGTGGGGCC 3'; 5' CTGCGATTACTAGGCATTCCCGAC 3'; 5' CCATCTGTCACTCCGATAACCCTCC 3'; 5' GCTAAGCTGCTTGCCTTTTGG 3'; 5' CGAAAGACCTTACCCAGC 3'; 5' GCGTCATCCTGGAGC 3'; and 5' GCGTCATCAAGCTTGGC 3'. These primers (DNA Technology) were designed to sequence the entire 16S rRNA gene as there were approximately 300 bp separating each primer. To eliminate errors introduced by PCR, different PCR amplification products were sequenced.

DNA sequences were assembled and analysed with software from the Genetic Computer Group sequence analysis software package. The program FASTA was used to search for similar sequences in the EMBL database. In addition, the GenBank database at The National Center for Biotechnology Information was searched via its text-searching system.

The PG21<sup>T</sup> 16S rDNA sequence is given in Fig. 1; the Prisbrow box and the approximate positions of the mature rRNA molecule are shown. Table 2 shows that PG21<sup>T</sup>, P2 and SC4 have identical sequences. The 16S rRNA sequence of strain DC63 differed from these three sequences by one nucleotide; position 1300 is thymine in DC63 and cytosine in the rest of the sequences. The sequence of strain 7488 also differed from the three identical sequences by one nucleotide; position 258 is thymine in 7488 and cytosine in the rest of the sequences including that of strain DC63. Furthermore, it is notable that, in strains PG21<sup>T</sup> and SC4, the region upstream of the 16S rRNA gene differed from the rest of the sequences at one and four positions, respectively (position -107 in PG21<sup>T</sup> and positions -233, -140, -123 and -86 in SC4). It is also notable that all of the strains have an *EcoRI* site at positions 763–768. Finding this site in the sequence of strain DC63 and the lack of variation was not expected according to Christiansen & Andersen (1988), even though lack of recognition at a specific restriction endonuclease site only reflects variation within a six nucleotide region. From the results of this previous study, the 16S rRNA sequence of strain DC63 was expected to lack the *EcoRI* site. This result was
Fig. 1. DNA sequence of the 16S rRNA gene of the *rrnB* operon from PG21T. The Pribnow box and EcoRI site are shown. The approximate beginning and end of the mature rRNA molecule are marked with bold brackets.

Table 2. Nucleotide differences in the 16S rRNA sequence of *M. hominis* strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Nucleotide at position:</th>
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<tbody>
<tr>
<td></td>
<td>-233</td>
</tr>
<tr>
<td>PG21T</td>
<td>T</td>
</tr>
<tr>
<td>SC4</td>
<td>C</td>
</tr>
<tr>
<td>P2</td>
<td>T</td>
</tr>
<tr>
<td>7488</td>
<td>T</td>
</tr>
<tr>
<td>DC63</td>
<td>T</td>
</tr>
</tbody>
</table>

examined as follows. A freeze-dried culture of the original DC63 strain was propagated to ensure that no mutations had accumulated. The cells were subjected to proteinase K treatment and PCR was performed directly on the proteinase K-treated cells. The PCR product was cleaved with EcoRI and the expected fragments were obtained (results not shown). Christiansen & Andersen (1988) conducted their restriction analysis by Southern blotting on DC63 genomic DNA, which probably was modified at the EcoRI site, thus preventing the restriction endonuclease from cleaving the sequence into the expected fragments.

In a study conducted by Weisburg et al. (1989), the 16S rRNA sequence from *M. hominis* PG21T was obtained,
although it is not a full-length sequence and it contains a long stretch of undetermined nucleotides. In a later study conducted by Blanchard et al. (1993), the 16S rRNA sequence from *M. hominis* 183 was obtained (this sequence was not full-length either) and compared with the PG21*°* sequence obtained by Weisburg et al. (1989). On the basis of this comparison, Blanchard et al. (1993) concluded that significant heterogeneity is found in *M. hominis* 16S rRNA genes, verifying the results obtained by Christiansen & Andersen (1988). These sequence data have now been updated as the part of the strain 183 16S rDNA sequence which aligns with the long stretch of N nucleotides in the Weisburg PG21*°* sequence has also been determined (positions 602–1109). The new sequence stretch from strain 183 is identical to that of the other strains.

The *M. hominis* strains selected for this study were chosen to represent isolates that had been previously shown to have differences within their 16S rRNA genes (Christiansen & Andersen, 1988); isolates showing pairwise identity within the hypervariable region of the P120 gene were also included. No correlation between heterogeneities in the 16S rRNA gene and differences within the hypervariable domain of the P120 gene was seen, indicating that different genetic mechanisms are involved in the development of genetic heterogeneity in *M. hominis*.

In conclusion, these results show that the *M. hominis* 16S rRNA gene is not as variable as previously thought. Strains PG21*°*, P2 and SC4 had identical 16S rRNA sequences and strains DC63 and 7488 differed from the other sequences by one nucleotide. These results are in agreement with the results obtained by Pettersson et al. (1996), who studied the heterogeneity of the 16S rRNA genes within the *Mycoplasma mycoides* cluster. In this previous study, the phylogeny of the six species in the cluster was investigated. The 16S rRNA genes from both operons were amplified by PCR and sequenced. From some of the species, several strains were included. When comparing differences between genes from homologous operons, the largest number of nucleotides differing between two strains from one species was four, and the lowest number was zero. When comparing these results with the results obtained in this study, where the largest number of differences is two and the lowest is zero, it is obvious that the degree of intraspecies heterogeneity in the 16S rRNA genes is of the same order of magnitude in *M. hominis* and in the members of the *M. mycoides* cluster. It seems that, although *M. hominis* is a heterogeneous species, only limited variation of the 16S rRNA genes is permitted, as seen in most other organisms. This is reasonable if it is considered that a certain structure of the 16S rRNA has to be maintained in order for it to take part in protein synthesis.

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**References**


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