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The nucleotide sequence of the 5S rRNA from *Spiroplasma* species BC3 and *Mycoplasma mycoides* sp. *capri* PG3

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ABSTRACT

Using in vitro labelling techniques, the complete nucleotide sequence of the 5S ribosomal RNAs isolated from the honeybee pathogen, Spiroplasma species BC3 and Mycoplasma mycoides sp. capri PG3, have been determined. The latter shows only 3 differences from the reported sequence of M. capricolum 5S rRNA, indicating that these two species are very closely related. The Spiroplasma sequence is also 107 nucleotides long and a comparative analysis of the sequence confirms that this Spiroplasma species is closely related to the Mycoplasma species and that they and the Gram-positive eubacteria have descended from a common ancestor and in the process the cell wall-less organisms have lost a large percentage of their genome.

INTRODUCTION

Members of the family Mycoplasmataceae have been known for nearly a century but the first member of the family Spiroplasmataceae was only identified in 1967[1] when a cell wall-less organism was isolated from a plant source. Since then, these helical, highly motile mycoplasmas have been recognised to be associated with a variety of pathological conditions such as citrus stubborn disease, corn stunt disease and to cause cataracts, neurological disturbances and death in experimental infections of suckling rats[2]. More recently, in 1976, a new species was identified in diseased honeybees[3]. The relatedness among the individual Spiroplasma species is far from clear although four distinct serological groups have been identified[4]. Even less clear is the relationship between the spiroplasma and other members of the order Mycoplasmatales, although comparison of 16S rRNA T_1-RNase catalogues has indicated that the Spiroplasma, like the Mycoplasma, are related to Gram-positive eubacteria[5].

Previously, evidence we obtained concerning the 5S rRNA sequence from Thermoplasma acidophilum [6], helped establish that this species is not even a Eubacteria but it in fact a member of the Archaebacteriae and because of the unique size of the 5S rRNA of the only Mycoplasma species so far reported [7]

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(M. capricolum ATCC 27343, 107 nucleotides) , we decided to investigate the nucleotide sequence of the 5S rRNA for phylogenetic purposes of M. mycoides sp. capri PG3 and of Spiroplasma species BC3 isolated from the honeybee.

MATERIALS AND METHODS

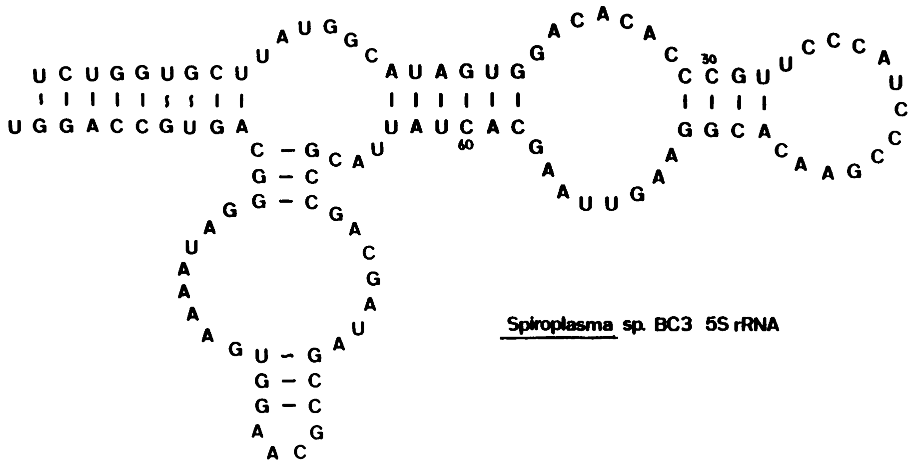
Cells of Mycoplasma mycoides sp. capri PG3 were grown and the low molecular weight RNA isolated as previously described[8]. Spiroplasma cells (grown under MAFF licence number PHF 78/13 and 78/52) were obtained from a 48 hr incubation of a 10% inoculum at 30° in PPLO broth (Difco) 2%; glucose, 0.2%; fructose, 0.2%; sucrose, 0.2%; sorbitol, 7.0% and horse serum, 5%. An optical density at 600 nm of around 0.1 was obtained which is equivalent to about 10^9 cells/ml Growth was in full 500 ml bottles, the cells were harvested in the usual way and the low molecular weight RNA (100 µg/l) isolated as previously described[8]. The RNA samples were then initially fractionated on a BD-cellulose column but whereas normally 5S rRNA is eluted first from this column[6], these 5S rRNAs were considerably retarded and could be found in the main UV-absorbing peak. Fractions of this peak were then subjected to polyacrylamide gel electrophoresis (1.0 kV for 7 hr on a 40 cm x 20 cm x 0.02 cm 8% slab) and the band corresponding to 5S rRNA eluted and used for sequencing.

Enzymes, radioactive materials, chemicals and most techniques were all as previously described[9,10].

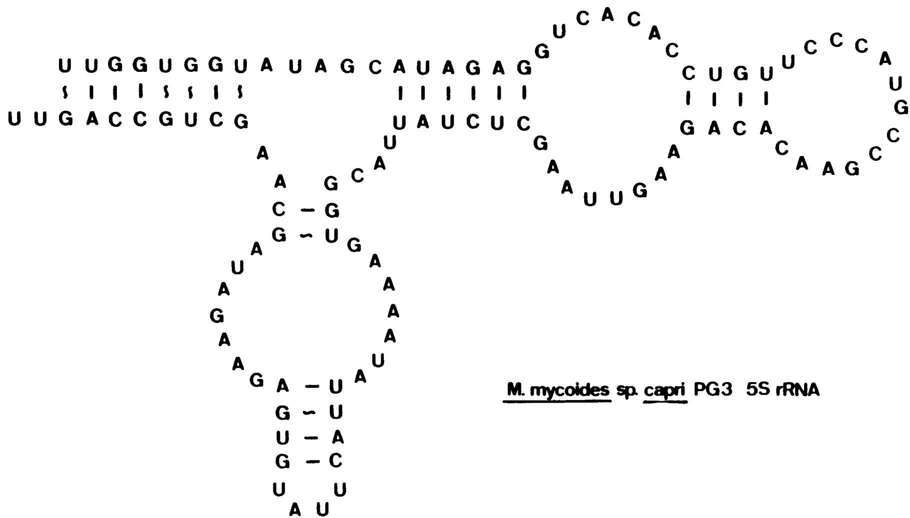
Sequence data was obtained (1) by the use of 5'- and $3'-^{32}P$ in vitro end labelled 5S rRNA[10,11] by (a) nuclease P_1 treatment followed by electrophoresis and homochromatography[10] (b) rapid gel sequencing methods using RNase T_1, U_2, Phy.M and from B. cereus[12]. The 3'-labelled material was previously treated with calf alkaline phosphatase to remove observable heterogeneity in the products of enzymatic digestion (c) rapid gel chemical sequencing[13]. (2) by the method of Stanley and Vassilenko[14] as modified by Tanaka et al[15]. The position of each nucleotide was independently deduced by at least two methods.

RESULTS

The sequences are given in the figure. No length or sequence heterogeneity was observed. No post-transcriptionally-modified nucleotides were detected. The 5S rRNA of Spiroplasma with 107 nucleotides is thus the same size as that of M.capri even though it has a higher mobility upon electrophoresis on a denaturing gel when compared with the Mycoplasma 5S rRNA.



Spiroplasma sp. BC3 5S rRNA



M. mycoides sp. capri PG3 5S rRNA

The secondary structures are shown in the form suggested by De Wachter et al[16]. Even though these mycoplasma 5S rRNAs are very short, they can still be accommodated in this model and when compared with a normal length 5S rRNA, helix E is shortened by deletion of bases from both strands of the helix. Thus when correctly aligned, M. mycoides sp. capri has one more base pair inserted in this helix than is found in the Spiroplasma species. Acholeplasma laidlawii 5S rRNA is 112 nucleotides long and contains three extra base pairs inserted in this helix (C. R. Woese, personal communication). It is known that the genome of these organisms is small and very A-T rich[2]. As they have

evolved with the Gram-positive eubacteria from a common ancestor by loss of genetic material, they are now likely to be under some constraint to conserve the remainder for use in 'essential' functions only. This has already been demonstrated by the relatively low level of tRNA post-transcriptional activity[8,17] and the probable use of $tRNA^{Gly}$ to decode 4 codons CCN[17]. Thus it is tempting to speculate that many of the nucleotides normally found in 5S rRNA in helix E are not essential for the adequate functioning of this molecule.

COMPARISON WITH OTHER 5S rRNAs

The homology shown between the 5S rRNA of M. capricolum (ATCC 27343) and M. mycoides sp. capri is greater than 97%. This infers that these are a minor species variation although serological evidence indicates that they are rather more distinct than this[18]. Considerable homology exists between these two mycoplasmas and the Spiroplasma species BC3. Indeed, nucleotides 14-67 show 90% homology. A comparative sequence analysis (H. Küntzel, personal communication)[19], confirms that the Spiroplasma species is closely related to M. capricolum and that they share a common ancestor with the Gram-positive eubacteria.

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