THE NUCLEOTIDE SEQUENCE OF A MINOR 5 S RNA SPECIES FROM *LACTOBACILLUS VIRIDESCENS*

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1. Introduction

Sequence heterogeneity has been reported in a number of prokaryotic 5 S RNA sequences [1–5] that vary from one base in *Escherichia coli* MRE 600 and CA265 [1] and *Bacillus stearothermophilus* [3] to as many as 8 bases in *B. licheniformis* [4], *B. subtilis* and *Bacillus* Q [5]. The proportion of these 5 S RNA variants range from 50% in *E. coli* MRE 600 and CA265 [1] to 12% of the total 5 S RNA in *B. subtilis* and *Bacillus* Q [5]. In the *Bacillus* species it has been proposed that one of the 6–8 5 S RNA cistrons codes for these 'minor' 5 S RNAs [5]. The nucleotide sequences of the *Bacilli* minor species are highly conserved, varying from each other in only 3 positions.

During our studies of 5 S RNA from *Lactobacillus viridescens* ATCC 12706 [6], we observed a minor species of 5 S RNA that migrated slower than the major species on denaturing polyacrylamide gel electrophoresis. The sequence of the major 5 S RNA from *L. viridescens* showed greater similarities to *Bacillus* 5 S RNAs than to the 5 S RNA from *Lactobacillus brevis* [7]. Therefore it was of interest to compare the sequence of the minor 5 S RNAs from *L. viridescens* to the conserved sequences of the minor 5 S RNAs from the *Bacillus* species. Here, we report the sequence of this minor 5 S RNA from *L. viridescens* which varies from the major species in 3 positions.

2. Materials and methods

The minor species of 5 S RNA from *L. viridescens* was isolated using two-dimensional polyacrylamide gel electrophoresis with the first dimension being a 10% native gel and the second dimension 20% gel, containing 6 M urea, run at 4°C until the xylene cyanol FF marker had migrated an equivalent distance of 80 cm.

The sequencing techniques were identical to those used for the major 5 S RNA in [6] where both uniformly ^{32}P-labelled samples were analysed and rapid gel sequencing techniques were used.

3. Results

The separation of major and minor 5 S RNA by two-dimensional polyacrylamide gel electrophoresis is shown in fig.1. In uniformly ^{32}P-labelled preparations, the minor species constitutes 12% of the total 5 S RNA.

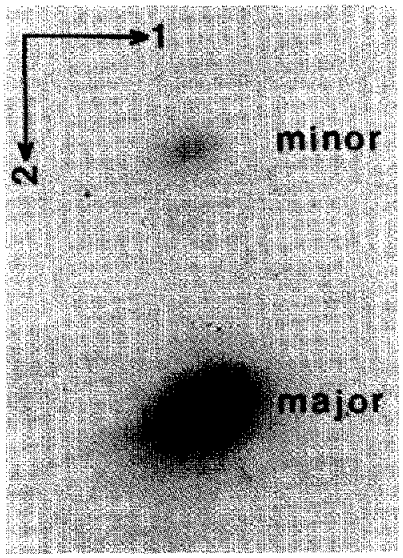

Fig.1. Autoradiograph of ^{32}P-labelled 5 S RNA species separated by two-dimensional polyacrylamide gel electrophoresis. For details see section 2.

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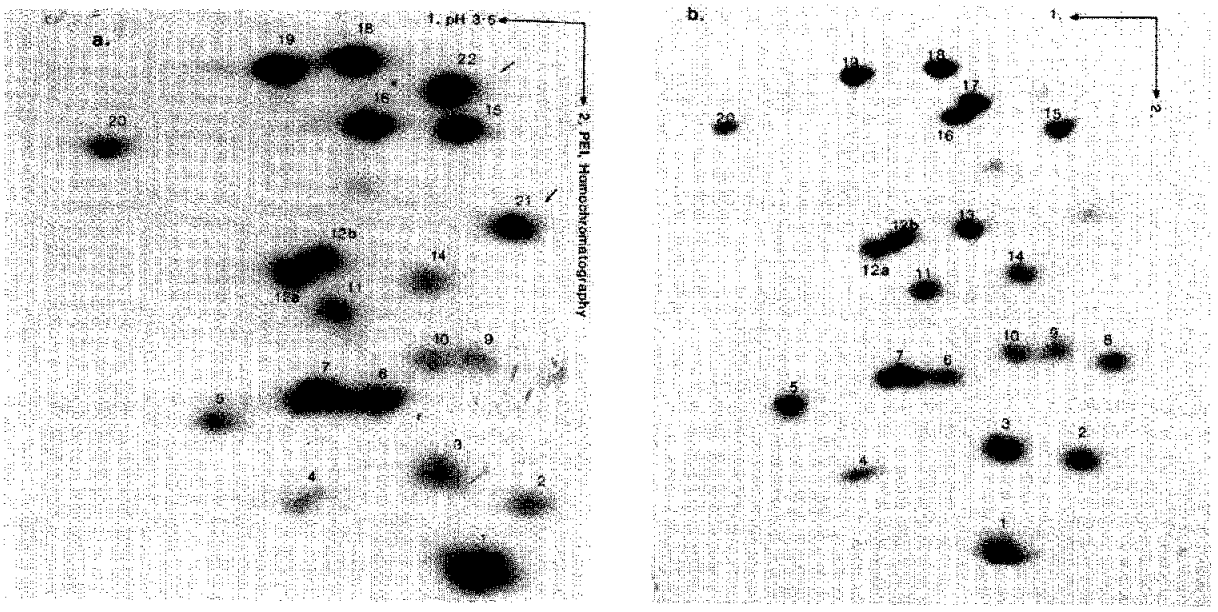

Fig.2. Autoradiograph of the fingerprint of a complete RNase T_1 digest of (a) *L. viridescens* 5 S RNA (minor) and (b) *L. viridescens* 5 S RNA (major). Arrows indicate the new fragments that appear in the minor species (a) when compared with the major species (b).

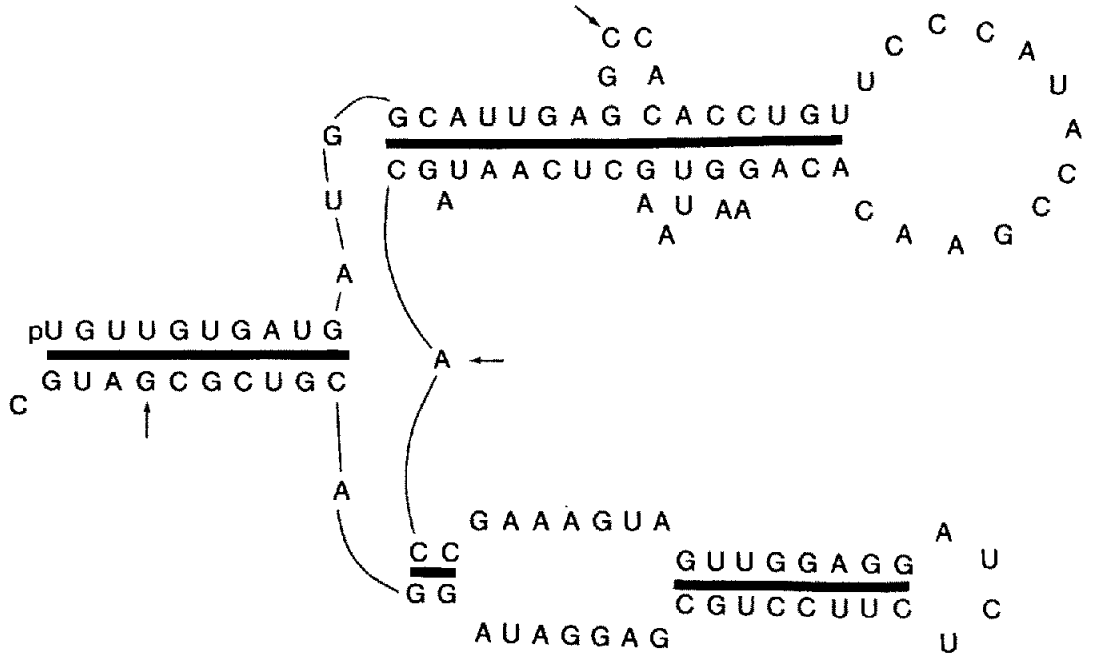

Fig.3. The sequence drawn in the 'wishbone' model for secondary structure proposed in [8].

The complete RNase T_1 fingerprint of the minor 5 S RNA is shown in fig.2a. This fingerprint is very similar to the RNase T_1 fingerprint of the major 5 S RNA shown in fig.2b. The major 5 S RNA products, t13 (CAAUG) and t17 (UCACACCUG) are absent whilst t8 (CCG) is present in significantly reduced amounts (0.1 mol). Two new RNase T_1 products are present, t21 (CACCG) and t22 (CCACACCUG) with another fragment, t7 (AUG), present in an increased amount. Ordering of the RNase T_1 fragments was assisted by analysis of the RNase A digestion fragments together with rapid gel sequencing of post-labelled samples to yield the final sequence. Fig.3 shows the sequence arranged in the 'wishbone' model, proposed [8] for 5 S RNA secondary structure.

Three base changes between the major and minor 5 S RNA species from *L. viridescens* are found occurring at positions C_{23} (changed from U), A_{67}(G) and G_{113}(A). These changes do not affect the number of base-pairs in the 'wishbone' model of secondary structure (fig.3) nor in the Fox and Woese model [9] of secondary structure. There is, however, an A:U base-pair replaced by a G:U base-pair in the molecular stalk region of the molecule.

4. Discussion

Of major interest is the comparison of the sequence of the minor 5 S RNA from *L. viridescens* with that of the highly conserved minor 5 S RNA species from several *Bacillus* strains. The phylogenetic tree, constructed by computer analysis of 5 S RNA sequences [10], shows *L. viridescens* to be derived by direct divergence from the *Bacillus* family. Although the proportion of minor species in the total 5 S RNA is very similar in *L. viridescens* and the *Bacillus* strains [5], the sequences of the minor 5 S RNA species do not show close homology (78.6%). In fact, the major 5 S RNA from *L. viridescens* has slightly greater homology with the *Bacillus* minor 5 S RNA sequences (80.1%). It is evident that the minor 5 S RNA from *L. viridescens* is derived by mutations in a normal *L. viridescens* 5 S RNA gene and not from a separate gene for minor 5 S RNA species evolved because of an unknown functional advantage.

In relation to 'wishbone' models for the secondary structure of 5 S RNA [8], the positions of the base changes in the minor 5 S RNA are worthy of comment. The change at position 23 (U to C) is shown in the general model of 5 S RNA from Gram-positive bacteria as a non-conserved base, appearing in a single stranded region of the structure. The base change at position 67 (G to A), although not affecting base-pairing interactions, occupies a strategic position between the two 'arms' of the wishbone. In all models of secondary structure, this position is invariably occupied by a G residue. The final base change at position 113 (A to G), resulting in a G:U base-pair, occurs in the helical region formed by base-pairing between the 5′- and 3′-ends of the molecule (referred to as the molecular stalk). Six of 8 base changes occur in this region of the *B. licheniformis* minor 5 S RNA [5]. If one looks at the type of base-pairing present in each of the molecular stalks for both the *Bacillus* and the *L. viridescens* minor 5 S RNA species, one can see that there is a decrease in the stability of this region in each minor species when compared with the respective major 5 S RNA. It may be that denaturation of this region of the molecule is the basis of the separation on polyacrylamide gel electrophoresis in the presence of urea.

In [11] it was shown, using artificially constructed 5 S RNA molecules, that high stability in the molecular stalk is not required for biological function, although assembly of the larger ribosomal subunit is impaired if the stability of the molecular stalk is reduced. Thus it is likely that the minor 5 S RNA species can still function in intact ribosomes and the question remains unanswered as to the biological significance of these molecules.

Acknowledgement

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