Nucleotide base sequence of vibrionaceae 5 S rRNA

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Nucleotide base sequences of 5 S rRNAs isolated from *Vibrio vulnificus*, *Vibrio anguillarum*, and *Aeromonas hydrophila* were determined. Comparisons among these and sequences of 5 S rRNAs from other species of Vibrionaceae provide information useful in the evaluation of the evolution of bacterial species.

5 S rRNA *RNA sequence* *Sequence analysis* *Vibrionaceae*

1. INTRODUCTION

Comparisons among 5 S rRNA sequences provide a means for the estimation of evolutionary relatedness among species. The 5 S rRNA nucleotide base sequences have been reported for 6 species of the family Vibrionaceae: *Photobacterium phosphoreum* [1], *Vibrio harveyi* [2], *V. cholerae* [3], *V. parahaemolyticus* (in review), *V. fluvialis* (in review), and *V. marinus* (in review). We report herein the nucleotide base sequences of 5 S rRNA isolated from *V. anguillarum*, *V. vulnificus*, and *Aeromonas hydrophila*, and compare these with previously reported sequences of 5 S rRNAs isolated from species of the Vibrionaceae, and RNA Superfamily I [4]. The base sequences of 5 S rRNAs prepared from *V. anguillarum* and *V. vulnificus* reveal moderately high levels of similarity with those from other species of the family Vibrionaceae, while that of *A. hydrophila* differs significantly, and contains a region with a unique secondary structural implication, novel to 5 S rRNAs from species of RNA Superfamily I.

2. EXPERIMENTAL

Bacterial cells were lysed by the freeze-thaw method [5], and nucleic acid was obtained by phenol extraction. Total cellular RNA was isolated as in [6], with modifications described in [3]. RNA was fractionated on DEAE–cellulose and the fraction containing 4 S to 8 S RNA was separated by electrophoresis on bisacrylylcystamine cross-linked acrylamide gels [7]. The 5 S rRNA band was located by staining with ethidium bromide, viewed on a UV transilluminator (Fotodyne, New Berlin, WI), excised, and recovered from the thiol-solubilized gel on DEAE–cellulose, as in [7]. Separate aliquots of the 5 S rRNAs were end-labeled on 3′- and 5′-termini using [5′-^{32}P]cytidine bisphosphate and RNA ligase or [γ-^{32}P]ATP and T4 polynucleotide kinase, and purified electrophoretically before sequence analysis. Terminal bases were identified by exhaustive digestion of the 3′-end-labeled RNA with RNase T2 and of the 5′-end-labeled RNA with nuclease P1, followed by thin-layer chromatography of the digests on PEI–cellulose, using the methods in [8]. Nucleotide sequences were determined by the enzymatic method from composites of numerous sequence ladders, generated using the methods in [9], and modified in [10]. Endoribonucleases employed in 5 S rRNA sequence determinations were T1 (G), U2 (A), Phy M (A = U), B.c. (C = U), and M1 ('minus C'). All enzymes were purchased from P-L Biochemicals (Milwaukee, WI).

Bacterial strains employed in this study were: *V. anguillarum* ATCC 19264; *V. vulnificus* ATCC 27562; *A. hydrophila* ATCC 9071.

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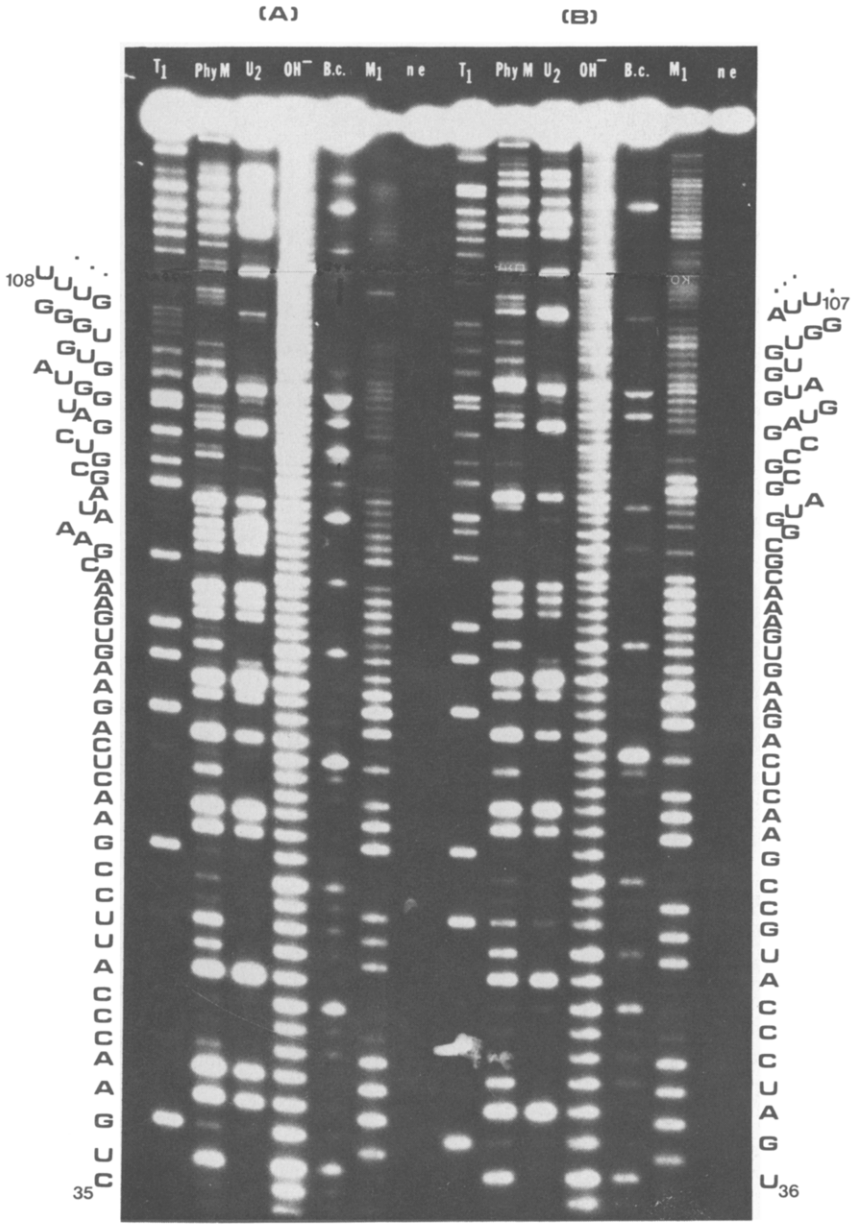
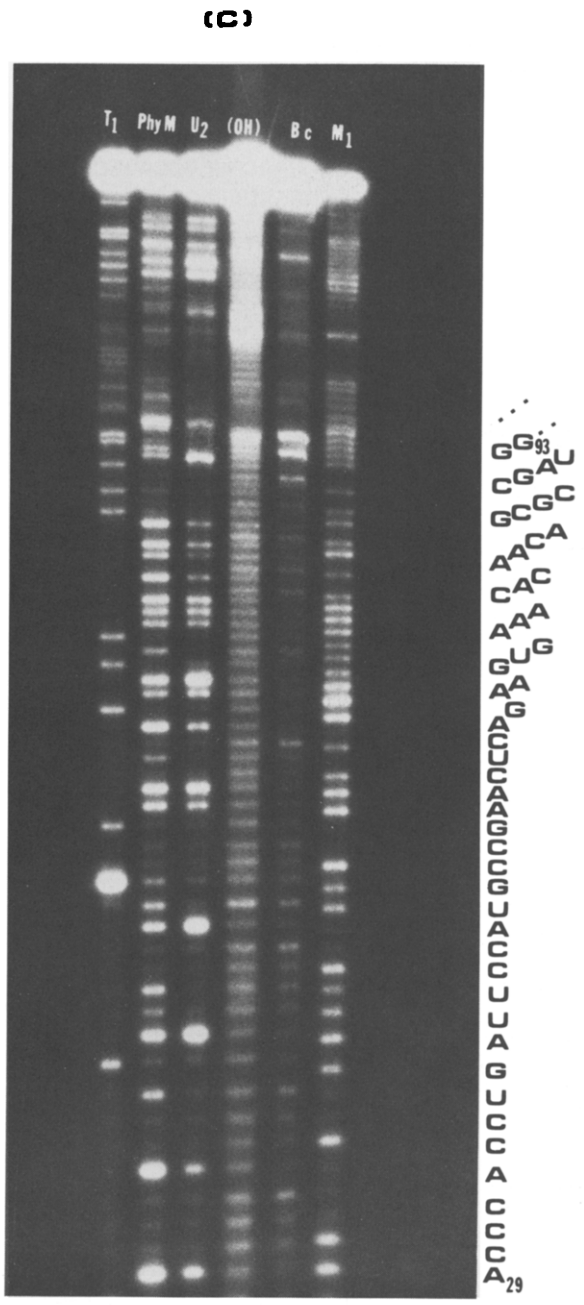


Fig.1. Autoradiograms of sequence ladders generated from partial enzymatic digests of 5 S rRNAs prepared from (A) *V. vulnificus*, (B) *A. hydrophila*, and (C) *V. anguillarum*. Bands, corresponding to bases in the region of approximately G(37) to U(108), are resolved and may be read directly from the autoradiogram.

3. RESULTS

3.1. *Terminal analyses*

Autoradiograms of the thin-layer chromatograms of exhaustive digests of the [3′-^{32}P]RNA indicated only (Up) on the autoradiogram for all 3 species. Chromatograms of exhaustive digests of the [5′-^{32}P]RNA indicated only (pU) for *V. anguillarum* and *V. vulnificus*, and (pA) for *A. hydrophila*.



3.2. *Sequence analyses*

Autoradiograms of equivalent portions from approximately U(37) to U(108), of the sequence ladders, generated from partial digests of the 5 S rRNA from each of the 3 aquatic *Vibrio* species, are shown in fig.1. Composites of sequence ladders yielded primary structures for 5 S rRNAs prepared from each of the 3 bacterial species, and are listed in fig.2. Comparisons among these and other representatives of RNA Superfamily I indicate a much higher degree of sequence homology (97%) between *V. vulnificus* and *V. cholerae* 5 S rRNAs than for any other pair, suggesting that these species shared a relatively recent common ancestor. By the same criterion, *V. anguillarum* is more closely related to *P. phosphoreum* (92%) than to other species examined to date, while 5 S rRNA from *A. hydrophila* lacks extensive sequence homology with any of the *Vibrio* species.

4. DISCUSSION

Since post-transcriptional modification of nucleotide bases apparently does not occur in bacterial 5 S rRNAs, they represent ideal material for sequence determination by the enzymatic method. Until recently, however, lack of an endoribonuclease with sufficient specificity to differentiate between uridine and cytidine residues prevented enzymatic sequencing methods from being extensively applied, since unequivocal pyrimidine determinations could not be made. RNase M1, possessing equal affinity for adenine, uridine and guanine residues, but no detectable affinity for cytidine, provided a solution to the problem of differentiation of the pyrimidine bases. Unlike other endoribonucleases commonly used for RNA sequence analyses, RNase M1 requires zinc for stability (J.F. Jolly, personal communication). Therefore, the dilemma is posed whereby RNA is incubated either in the presence of zinc, in which case non-specific degradation of the RNA will occur [11], or without zinc, in which case enzyme activity will be lost within a few hours. Since inclusion of zinc in M1 dilution buffers ultimately gives rise to an unreadable sequence ladder, it is advisable to avoid it, limiting waste by keeping volumes of working dilutions of RNase M1 to a minimum.

An alignment, according to the convention of [12], of the 5 S rRNA sequences for selected species of the Vibrionaceae (fig.3), indicates that the base sequence of 5 S rRNA from *A. hydrophila* contains a minor sequence variation of importance in the folding of the molecule. According to current secondary structure models, the

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V. vulnificus   5'-UGCCUGGCGACCAUAGCGUUUUGGACCCACCUGAACCCAUUCCGAACUCAGAAGUGAAA
V. anguillarum  5'-UGCCUGGCGACCAUAGUGUUGUGGACCCACCUGAUUCCAUGCCGAACUCAGAAGUGAAA
A. hydrophila   5'-UGCCUGGCGGCCAUAGCGCCGUGGAACCACCUGAUCCCAUGCCGAACUCAGAAGUGAAA

V. vulnificus   CGAAAUAGCGUCGAUGGUAGUGUGGGGUUUCCCCAUGUGAGAGUAGAACAUCGCCAGGCAU-3'
V. anguillarum  CACAACAGCGCCGAUGGUAGUGUGGGGUCUCCCCAUGUGAGAGUAGGACAUCGCCAGGCAU-3'
A. hydrophila   CGCGGUAGCGCCGAUGGUAGUGUGGCAUUU-GCCAUGCGAGAGUAGGACACUGCCAGGCA--3'
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Fig.2. Nucleotide base sequences of the 5 S rRNAs of *V. vulnificus*, *V. anguillarum* and *A. hydrophila*.

DD′ helix of known Superfamily I 5 S rRNAs terminates in $(G/C)_4$ and a 3-membered pyrimidine hairpin loop, while the 5S rRNA from *A. hydrophila* terminates in $(G/C)_3$ C/G, and a 4-membered hairpin loop, reducing the maximum possible base pairs in helix DD′ from 8 to 7. The proposed secondary structures of 5 S rRNAs of *A. hydrophila, V. vulnificus* and *V. anguillarum* are shown in fig.4. The secondary structure suggested is identical to the universal '5-helix' model of [13], except that helix AA′ is re-oriented to reflect results of recent NMR [14], X-ray scattering [15], and nuclear Overhauser [16] studies, which indicate that helix segments AA′, DD′ and EE′ comprise one single continuous helix.

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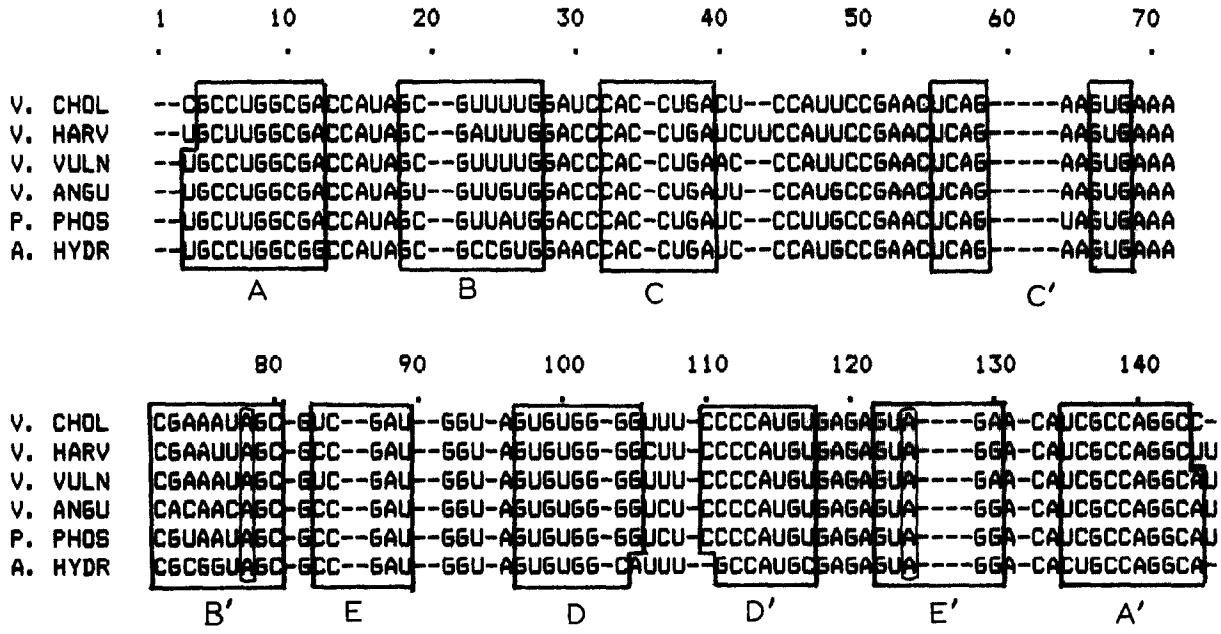

Fig.3. Alignment of 5 S rRNA sequences according to the convention of [12]. Boxed-in areas indicate base-paired regions. Letters beneath the boxed regions are helix designations. V. CHOL, *V. cholerae*; V. HARV, *V. harveyi*; V. VULN, *V. vulnificus*; V. ANGU, *V. anguillarum*; P. PHOS, *P. phosphoreum*; A. HYDR, *A. hydrophila*.

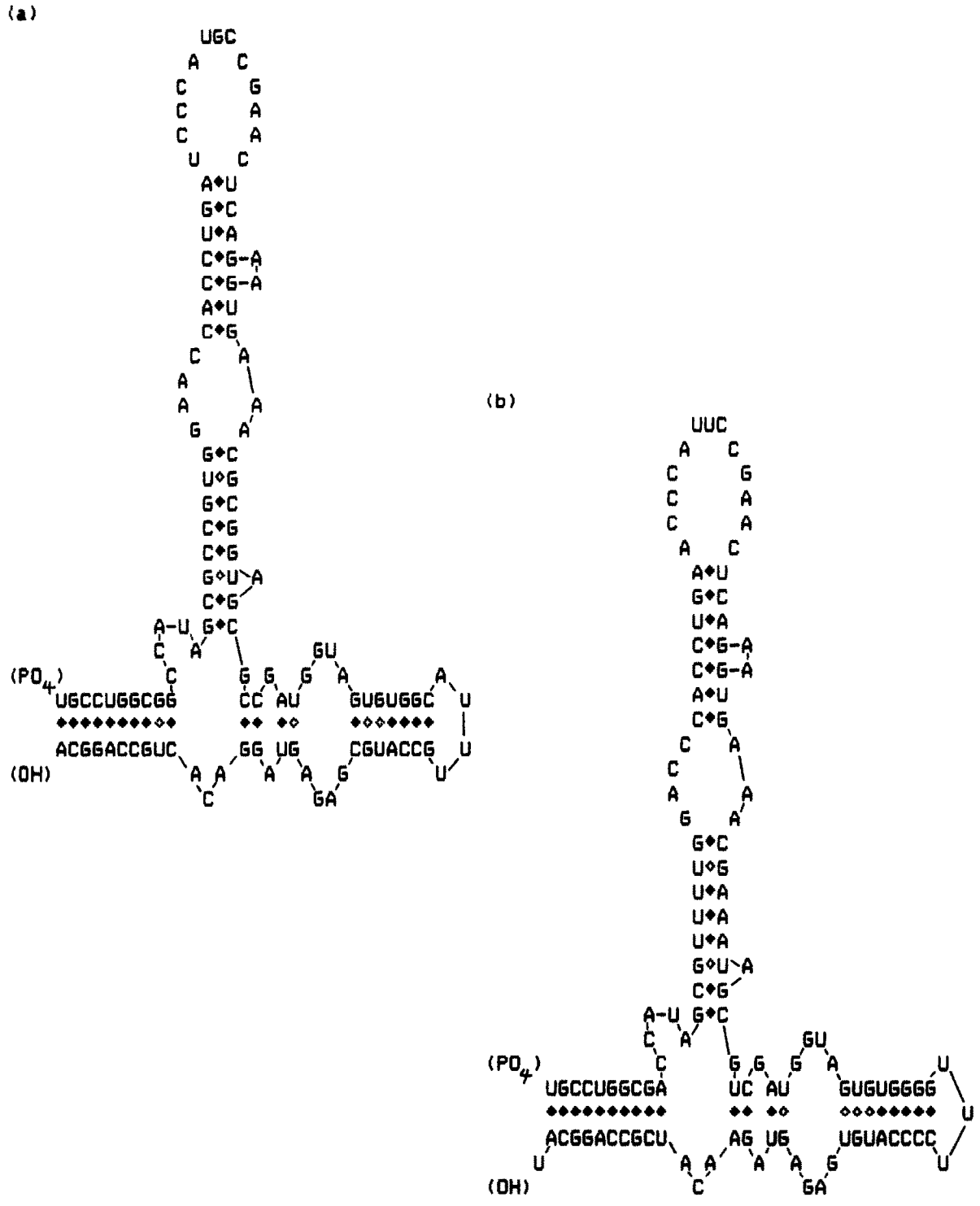

Fig.4. Proposed secondary structures for (a) *A. hydrophila* and (b) *V. vulnificus*. The secondary structure shown here is a minor modification of the universal '5-helix' model [13] (see text).

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