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Nucleotide Sequence of 5S Ribosomal Ribonucleic Acid from a Sulfate-Reducing Bacterium, *Desulfovibrio vulgaris*[1)]

KAZUNOBU MIURA,*,[a] JUNJI KAKUCHI,[a] ERIKO ENDO,[a] TOHRU UEDA,[a] KUNIHIKO KOBAYASHI,[b,2)] MASUMI NAKAO[b] and MAKOTO ISHIMOTO[b]

Division of Medicinal Chemistry[a] and Division of Microbiology,[b] Faculty of Pharmaceutical Sciences, Hokkaido University, Nishi-6, Kita-12, Kita-ku, Sapporo 060, Japan

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The 5S ribosomal ribonucleic acid (5S rRNA) isolated from *Desulfovibrio vulgaris* MK was sequenced by three different methods. A comparison of *D. vulgaris* 5S rRNA and 5S rRNAs from other microorganisms in terms of the primary and secondary structures showed that *D. vulgaris* 5S rRNA possessed higher homology with *Bacillus* 5S rRNAs than with other prokaryotic 5S rRNAs examined. This suggests that *D. vulgaris* is phylogenically related to gram-positive bacteria, such as *Bacillus* species.

Keywords——5S rRNA; sulfate-reducing bacterium; *Desulfovibrio vulgaris*; *Bacillus*; evolution

Introduction

As a strategy to study the phylogenic relationships of organisms, the systematic structural analysis of the 5S ribosomal ribonucleic acids (5S rRNAs) is reasonable because 5S rRNA is a common constituent of the ribosome, which occurs in all organisms, and structure determinations of 5S rRNAs from a number of organisms have already been performed. Hori and Osawa have constructed a phylogenic tree by the comparative structural analysis of 5S rRNAs from numerous organisms.[3)]

In this paper, we describe the sequence analysis of 5S rRNA from a sulfate-reducing bacterium, *Desulfovibrio vulgaris* and we compare the results with those for 5S rRNAs from numerous bacterial sources. The results indicate that *Desulfovibrio vulgaris* is related to a number of gram-positive bacteria in terms of the structures of 5S rRNA.

Materials and Methods

Ribonuclease T_2 was purchased from Sankyo Co., Ltd. T4 RNA ligase and ribonucleases kit for RNA sequencing were purchased from P-L Biochemical Co. T4 polynucleotide kinase was from Takara Shuzo Co., Ltd. [^{32}P]pCp and [^{32}P]ATP were products of New England Nuclear.

Preparation of 5S rRNA from *D. vulgaris*——*Desulfovibrio vulgaris* MK[4)] was cultured in the medium containing sulfate and lactate under anaerobic conditions. The 5S rRNA was extracted as a small-molecular RNA fraction from the bacterial cells by the procedure used to obtain transfer ribonucleic acids (tRNAs).[5)] The isolation of 5S rRNA from small molecular RNA fractions was performed by Sephadex G-100 gel filtration (column size; 2.5×94 cm). Further purification of 5S rRNA was performed by polyacrylamide gel electrophoresis after post-labeling.

Sequence Analysis of 5S rRNA——Post-isotope labeling at the 3′- or 5′-terminus of 5S rRNA was performed by ligation of [^{32}P]pCp with T4 RNA ligase to 5S rRNA or kination of 5S rRNA with [^{32}P]ATP and polynucleotide kinase after alkaline treatment, respectively, and labeled 5S rRNAs were further purified by two-dimensional polyacrylamide gel electrophoresis.[6)]

The sequence analysis of *D. vulgaris* 5S rRNA was carried out by three different methods, a chemical degradation method using 3′-end-labeled 5S rRNA,[7)] a ribonuclease degradation method[8)] and mobility shift analysis[9] using 5′-end-labeled 5S rRNA.

Structural Comparison of 5S rRNAs——The comparative structural analysis of 5S rRNAs was carried out by alignment to give maximal sequence homology by introducing a minimal number of gaps according to the report by Hori and Osawa.[3] Furthermore, the homology of 5S rRNAs from different origins in terms of the secondary

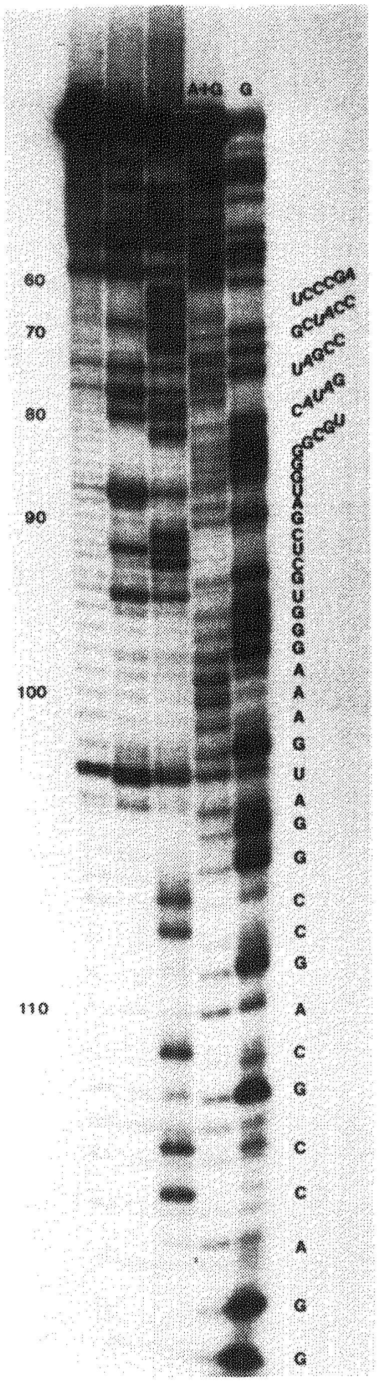

Fig. 1. 10% Polyacrylamide Gel Electrophoresis after Chemical Degradation Reactions of 5S rRNA

U, C+U, A+G and G, and OH represent chemical degradations specific for the indicated nucleobases, and limited alkaline degradation, respectively.

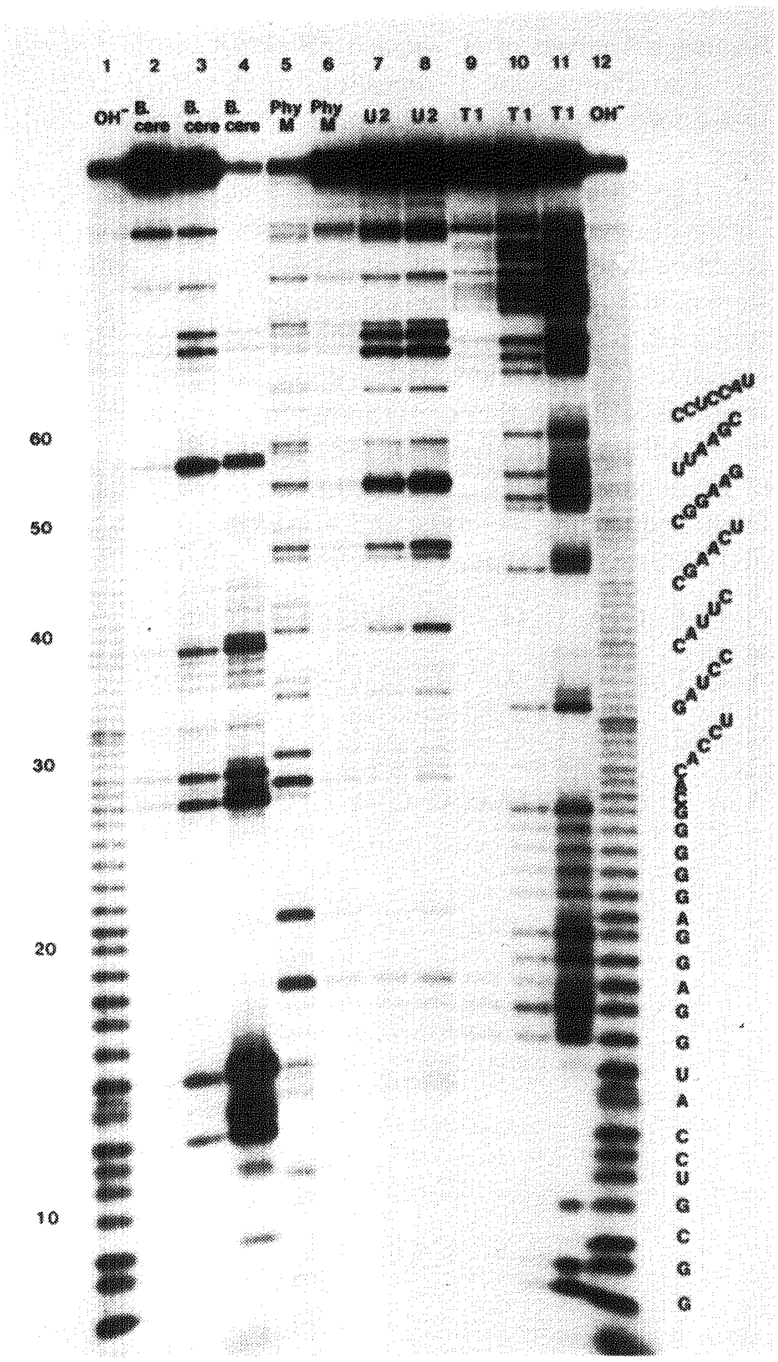

Fig. 2. 10% Polyacrylamide Gel Electrophoresis after Ribonuclease Degradation of 5S rRNA

1 and 12, limited alkaline degradation; 2—4, *B. cereus* ribonuclease digestion (0.1, 0.5 and 1 unit/reaction, respectively); 5 and 6, ribonuclease Phy M digestion (5 and 1 unit/reaction, respectively); 7 and 8, ribonuclease U_2 digestion (1 and 5 unit/reaction, respectively); 9—11, ribonuclease T_1 digestion (0.1, 0.5 and 1 unit/reaction, respectively).

structure was determined based on the construction of a secondary structure by arrangement of the sequence of 5S rRNA into a universal model,[10] followed by comparison of the number and position of the bulge structures and the size of loops and the length of stems.

Results and Discussion

Sequence Analysis of *D. vulgaris* 5S rRNA

The ribonuclease T_2 digestions of 5S rRNAs labeled at the 5′- or 3′-termini followed by two-dimensional thin layer chromatography showed that the 5′- and 3′-terminal nucleobases were both uracil (data not shown).

The 5S rRNA labeled with [^{32}P]pCp at the 3′-terminus was sequenced by Peattie's chemical degradation method.[7] The sequence from the 3′-end up to about 90 nucleotides could be determined by this method. A typical polyacrylamide gel electrophoretic pattern is shown in Fig. 1. For the sequencing from the 5′-end, the 5′-end-labeled 5S rRNA was subjected to the enzymatic degradation technique.[8] The sequence can be read from the 5′-end to about 100 nucleotides with a slight ambiguity within several nucleotides of the 5′-terminal region (Fig. 2). Further confirmation of the sequence in the 5′-terminal region was performed by mobility shift analysis[9] of the 5′-end labeled 5S rRNA (Fig. 3). Then, the total sequence of *D. vulgaris* 5S rRNA was deduced by alignment of the sequence data from the three different sequencing methods, and *D. vulgaris* 5S rRNA was found to belong to the 5S rRNA of 120-nucleotide type. The nucleotide sequence determined is pU–A–G–C–C–U–G–G–C–

10 20 30
G–U–C–C–A–U–G–G–A–G–G–A–G–G–G–G–G–C–A–C–A–C–C–U–G–A–U–C–C–C–

40 50 60
A–U–U–C–C–G–A–A–C–U–C–G–G–A–A–G–U–U–A–A–G–C–C–C–U–C–C–A–U–C–

70 80 90
G–C–C–G–A–U–G–A–U–A–C–U–G–C–G–G–G–G–U–A–G–C–U–C–G–U–G–G–G–A–

100 110 120
A–A–G–U–A–G–G–C–C–G–A–C–G–C–C–A–G–G–A–C–U_{OH}.

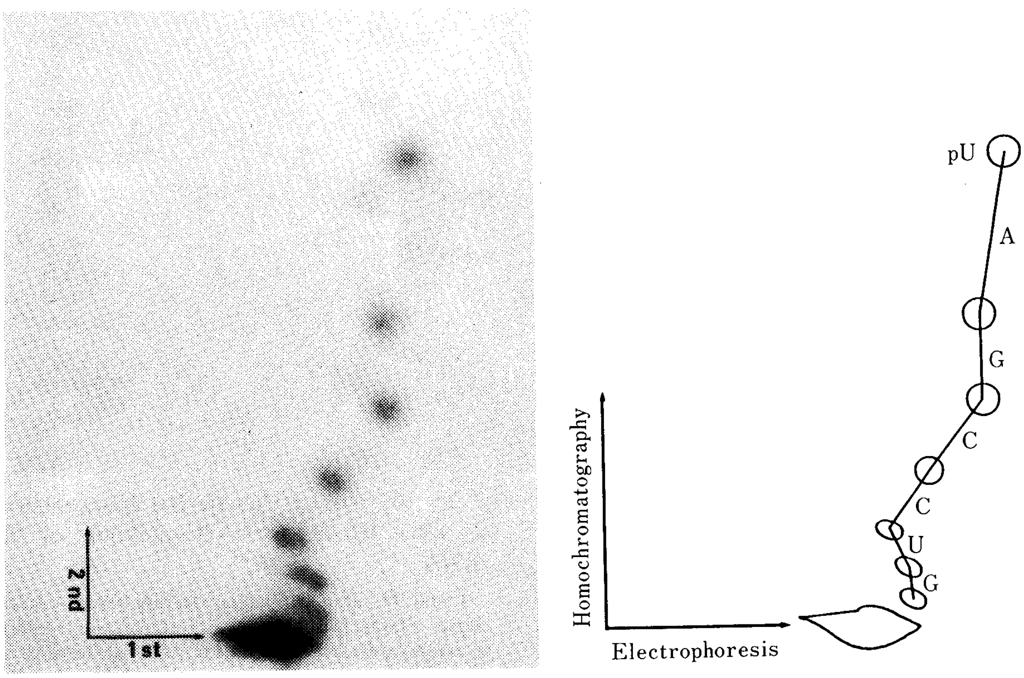

Fig. 3. Mobility Shift Analysis of the 5′-Terminal Region of 5S rRNA
5S rRNA was partially hydrolyzed in boiling water.

Structural Comparison between 5S rRNAs from *D. vulgaris* and Other Bacteria and the Phylogenic Relationships

The percent homologies were estimated by primary sequence alignment between *D. vulgaris* 5S rRNA sequence and published bacterial 5S rRNA sequences.[11] Table I lists representative results. It appears that *Bacillus* 5S rRNA showed the highest percent homology with *D. vulgaris* 5S rRNA.

For the comparative analysis of the secondary structures of 5S rRNAs, *D. vulgaris* 5S rRNA sequence and other bacterial 5S rRNA sequences were arranged into the universal secondary structure model proposed by Wachter *et al.*[10] As shown in Fig. 4, the secondary

Table I. Sequence Homologies between 5S rRNAs of *D. vulgaris* and Other Bacteria

Bacteria	Percent homology (%)
Bacillus acidocaldarius	76
Bacillus subtilis	73
Bacillus brevis	73
Bacillus stearothermophilus	70
Thermus thermophilus	69
Proteus vulgaris	69
Pseudomonas fluorescens	68
Clostridium pasteurianum	67
Streptomyces griseus	67
Escherichia coli	67

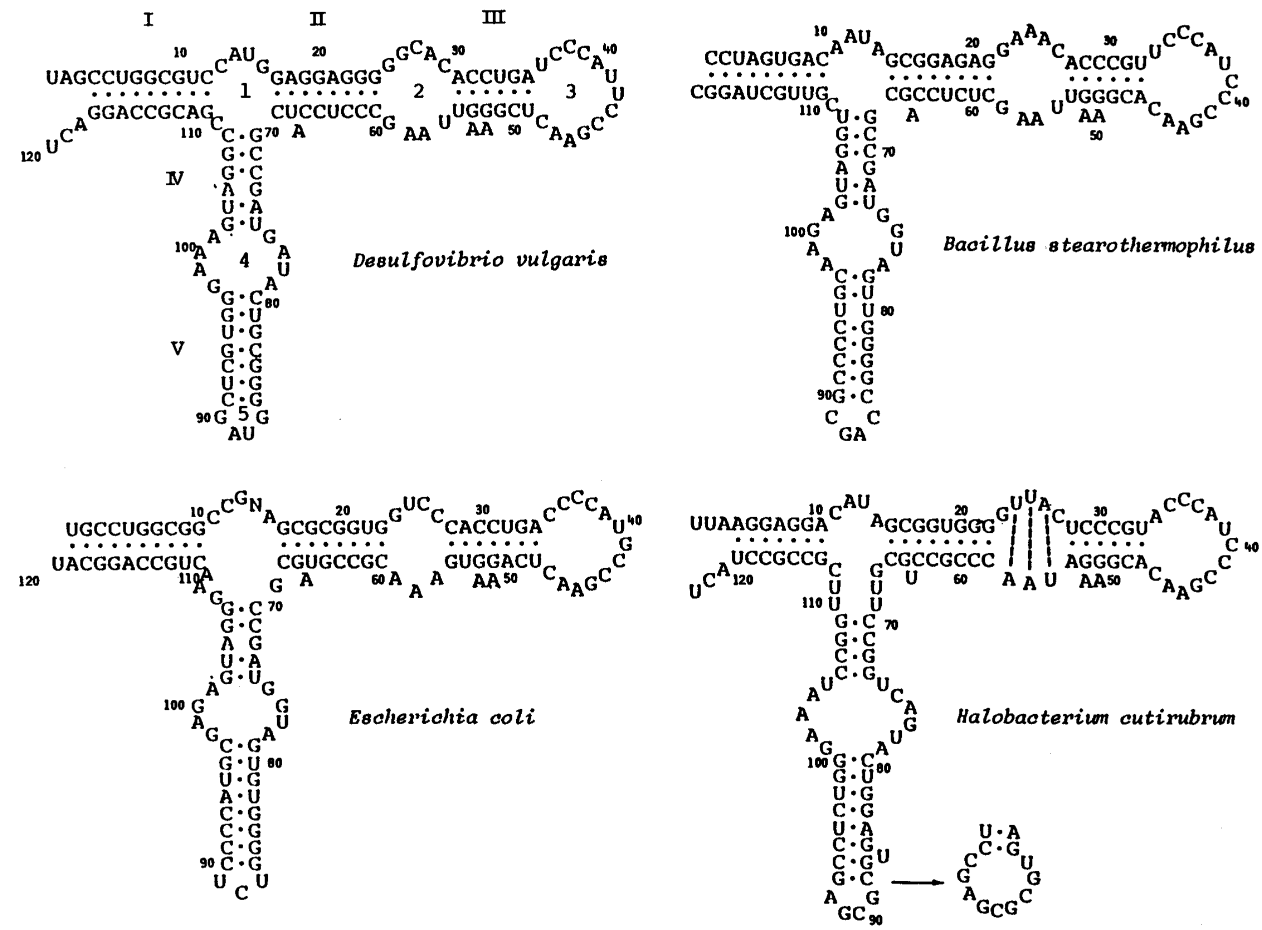

Fig. 4. Secondary Structures of 5S rRNAs

structure of *D. vulgaris* constituted from helices I (9 base pairs), II (8 base pairs), III (6 base pairs), IV (5 base pairs) and V (7 base pairs), loops 1 (number of nucleotides including the base-paired nucleotides, 11), 2 (13), 3 (15), 4 (12) and 5 (6) and two bulge structures. The secondary structures of typical bacterial 5S rRNAs (from *Escherichia coli, Bacillus stearothermophilus* and *Halobacterium cutirubrum*) are also shown in Fig. 4.

Next, we considered the type of the bulge structures, the number of nucleotides in the loops, and the number of base-pairs in the helices. In these structures, the common structural features are that two bulge structures are present in helices II (adenine or uracil) and III (major nucleotides, adenine and adenine), the sequence C–G–A–A–C, assumed to be a tRNA binding site,[12] is conserved in loop 3, and the size of loop 3 is conserved.

On the other hand, some differences were found in the size of loops 1, 2 and 5 and the number of base-pairs of the stems I, III, IV and V, so that the secondary structures of 5S rRNAs from numerous bacteria could be classified into several major types. As shown in Fig. 4, the secondary structure of *E. coli* 5S rRNA represents the first type of secondary structure, the characteristics of which are the larger loop 1, smaller loop 2 and one nucleotide longer stem III as compared with the other types. The second type includes 5S rRNAs from seven species of *Bacillus*, and two species each of *Lactobacillus* and *Micrococcus*, and others. The 5S rRNAs from archaebacteria form the third type of the secondary structure.

A comparison of the structures showed that the secondary structure of *D. vulgaris* 5S rRNA was similar to the second type of structure as represented by 5S rRNA from *B. stearothermophilus* in Fig. 4.

The primary sequence homologies of the 5S rRNAs from *Thermus thermophilus*, *Proteus vulgaris* and *Pseudomonas fluorescens* are relatively high (Table I). However, the secondary structures of the 5S rRNAs of such bacteria are different from that of *D. vulgaris*. Therefore, the comparison of the primary and secondary structures of 5S rRNAs suggests that *D. vulgaris* is phylogenically closely related to *Bacillus*, in spite of the fact that *D. vulgaris* is a gram-negative and strictly anaerobic bacterium. Schwartz and Dayhoff have constructed a phylogenic tree based on the structure of ferredoxins, showing that *Desulfovibrio* diverged from *Bacillus*.[13]

In conclusion, the results of the present study on the structure of 5S rRNA from *D. vulgaris* suggests that *D. vulgaris* is phylogenically related to gram-positive bacteria such as *Bacillus*.

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References and Notes

1) This paper is dedicated to Professor Morio Ikehara on the occasion of his retirement from Osaka University in March, 1986.
2) Present address: *Department of Anatomy, Nagoya University School of Medicine, Showa-ku, Nagoya 466, Japan.*

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