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Sulfobacillus thermosulfidooxidans: a new lineage of bacterial evolution?

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The nucleotide sequence of 5 S ribosomal RNA (rRNA) of type strain *Sulfobacillus thermosulfidooxidans* VKM B-1269 was determined. This organism represents a group of moderately thermophilic acidophilic chemolithotrophic bacteria, able to use ferrous and sulfur compounds as the sole energy source. 5 S rRNA of this bacterium is drastically different from all other known bacterial 5 S rRNA sequences. It is suggested that *S. thermosulfidooxidans* represents a new lineage of bacterial evolution, that diverged from other bacteria at an early step of their evolution.

Ribosomal, 5 S, RNA sequencing; Bacterial taxonomy

1. INTRODUCTION

Among chemolithotrophs of the sulfur and ferrous cycle there are bacteria that use S^0 and ferrous ion as the sole energy source. As these microorganisms are able to oxidize sulfide minerals they may be used in biohydrometallurgy of rare and noble metals. These bacteria are phylogenetically diverse and belong to different evolutionary lineages, including both eubacteria and archaebacteria. *Thiobacillus ferrooxidans* [1], and yet unnamed strains similar to *Thiobacillus* [2-5] represent the former urkingdom, and *Acidianus brierleyi* [6], earlier called *Sulfolobus brierleyi* [7,8], represents the latter.

Another representative of these chemolithotrophs is *Sulfobacillus* with the only species *S. thermosulfidooxidans* [9-11]. *Sulfobacillus* genus includes Gram-positive aerobic non-motile facultative autotrophic bacteria able to use ferrous ion and elemental sulfur, or its reduced compounds as the sole energy source. *Sulfobacillus* is moderately thermophilic (temperature optimum 50°C, maximum 58°C) aerobic acidophylic (pH optimum 1.9-2.4) non-motile spore-forming bacterium. Its morphology somewhat resembles that of *Bacillus*, but is different in its ability of cell branching and complex aggregate formation, similar to coryneform bacteria. The double-layer cell wall is 40-45 nm thick and the cytoplasmic membrane is 7 nm thick. GC content of DNA varies from 45.5 molar % in *asporogenes* subspecies to 49.3 in *thermotolerans* one. Genome size is 3.7×10^9 Da in type strain VKM B-1269, and somewhat less (3.0×10^9) in *asporogenes*, suggesting a possible deletion in DNA of the latter strain.

DNA/DNA hybridization study [12] failed to reveal the phylogenetic position and taxonomic status of this genus. This paper describes an attempt to solve this problem by sequencing 5 S ribosomal RNA of the type strain *S. thermosulfidooxidans* VKM B-1269.

2. MATERIALS AND METHODS

S. thermosulfidooxidans strain VKM B-1269 was grown at 50°C on modified Manning medium [13] containing 0.1% fructose and 0.02% yeast extract instead of $FeSO_4$. Total RNA preparation was obtained by hot (60°C) phenol extraction [14] of ca 0.2 g of wet cells grown to the end of the exponential phase. RNA was precipitated and washed with ethanol and 3′-end labelled in vitro with [^{32}P]cytidine biphosphate (pCp) by use of T4 RNA ligase [15]. Labelled 5 S ribosomal RNA was purified by means of electrophoresis through 8% polyacrylamide gel [16]. RNA was sequenced by the chemical method of Peattie [17], with minor changes [14]. The 5 S RNA sequence of *S. thermosulfidooxidans* was compared to other published bacterial sequences [18].

3. RESULTS AND DISCUSSION

To reveal the phylogenetic status of *Sulfobacillus*, the 5 S rRNA sequence was determined. Fig.1 presents the tentative secondary structure of this molecule, which can adopt conventional Y-shaped conformation, characteristic of eubacterial 5 S rRNAs. Some unusual features of this structure can be mentioned: stem E and the adjacent loop are shortened, andno residue in the B stem is bulged out. These features can also be found in several other bacteria and do not stand for the uniqueness of the structure of *Sulfobacillus* 5 S rRNA.

Comparison of the primary structure of this RNA with sequenced 5 S rRNAs of all other bacteria indicates

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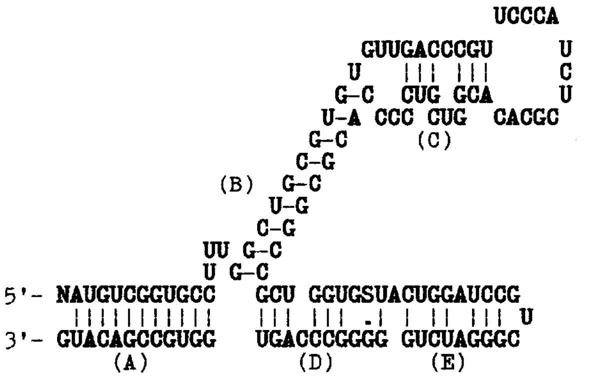

Fig.1. Nucleotide sequence and the tentative secondary structure of 5 S ribosomal RNA of *Sulfobacillus thermosulfidooxidans* strain VKM B-1269. S = C + G, N = A + C + G + U.

that it is drastically different from all of them. Fig.2 shows the UPGMA cluster dendrogram built by comparison of 5 S rRNA sequences of bacteria belonging to all the main groups. It is obvious from the dendrogram that the mutation distances between *S. thermosulfidooxidans* 5 S rRNA and all other 5 S rRNAs are comparable to the distances separating representatives of the two primary bacterial kingdoms: eubacteria and archaebacteria. Certainly, this dendrogram should not be regarded as the phylogenetic tree, since unequal mutation rates in different evolutionary lineages can invalidate results of the cluster analysis based phylogenetic reconstruction. This dendrogram can be used only to demonstrate the level of dissimilarity between 5 S rRNA of *S. thermosulfidooxidans* and all other bacteria. There are more valid methods for phylogenetic reconstruction, but taking into account the high and approximately uniform level of dissimilarity between *Sulfobacillus* RNA and all others, its precise phylogenetic position can hardly be established unequivocally. This is why we hesitated with construction of evolutionary schemes.

The uniqueness of *S. thermosulfidooxidans* 5 S rRNA sequence suggests that this bacterium has diverged at an early stage of evolution. Certainly, analysis of the single type of molecule, especially one that is as short as the 5 S rRNA, can result only in preliminary conclusions. As mentioned above, the high divergence of 5 S rRNA can reflect not the ancient origin of the bacterium, but rather the high mutation rate in this RNA. To make the final phylogenetic conclusion, it is necessary to determine sequences of other molecules, e.g. 16 S rRNA, and to study the phylogeny of related strains. Nevertheless, we feel that the result described in this paper is important since it shows tentatively the unique phylogenetic position of this organism and suggests an extensive study of this interesting group of bacteria.

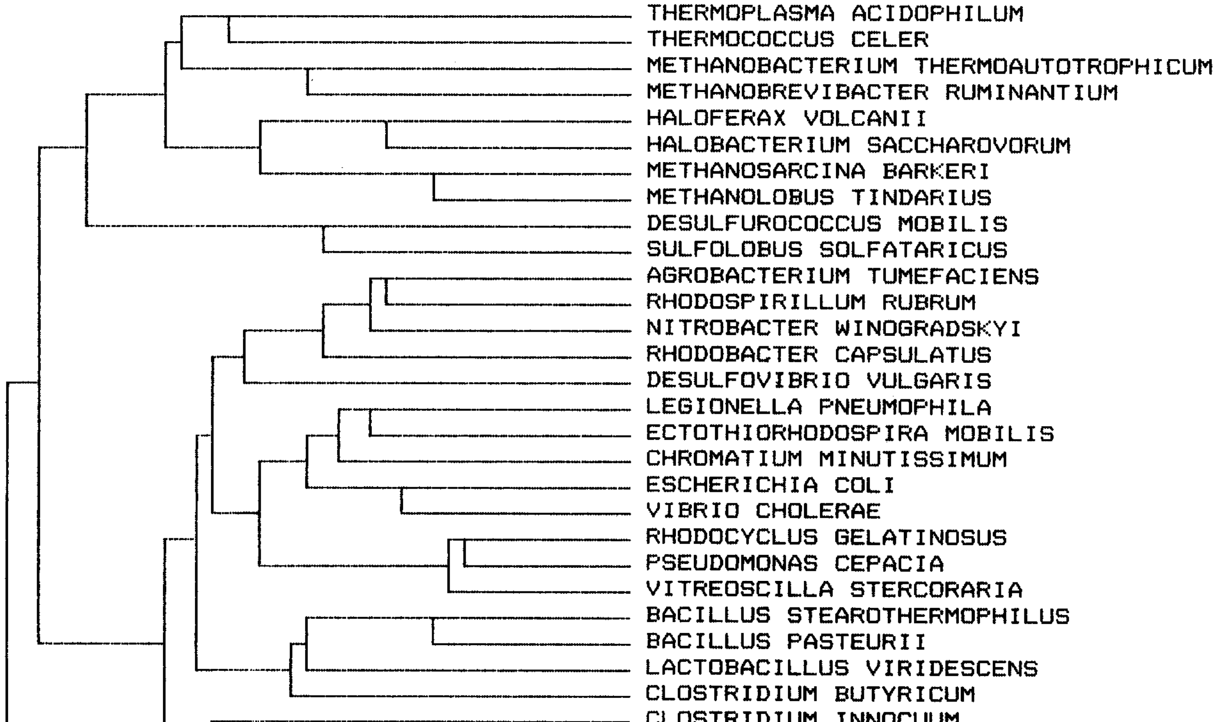

Fig.2. Cluster dendrogram showing relations between 5 S rRNAs of *Sulfobacillus thermosulfidooxidans* and other bacteria.

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