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A Unique Type of Eubacterial 5S rRNA in Members of the Order Planctomycetales

Daniel Bomar,[1] Stephen Giovannoni,[2] and Erko Stackebrandt[1]

[1] Institut für Allgemeine Mikrobiologie, Christian Albrechts Universität, Olshausenstr. 40, D-2300 Kiel, FRG
[2] Department of Biology, Indiana University, Jordan Hall, Bloomington, Indiana 47405, USA

Summary. Analysis of the 5S ribosomal RNA from members of the eubacterial order Planctomycetales, i.e., *Planctomyces, Pirella, Gemmata,* and *Isosphaera,* reveals several unexpected features. Firstly, the primary structures are significantly shorter than those of the majority of eubacteria and vary in length between 109 and 111 nucleotides. Secondly, the lack of an insertion at position 66 is a feature not encountered before in prokaryotic 5S rRNAs. Thirdly, as compared to the proposed eubacterial "minimal" 5S rRNA structure (Erdmann and Wolters 1986) the secondary structure contains numerous base-pair transversions. The isolated position of the planctomycetes as an individual eubacterial division and the phylogenetic position of its genera are in accord with the results obtained from 16S rRNA cataloguing.

Key words: 5S rRNA — Phylogeny — Tachytelic evolution — Eubacteria — Planctomycetes

Introduction

Members of the order Planctomycetales (Schlesner and Stackebrandt 1986) constitute a unique group of eubacteria. As inferred from 16S ribosomal RNA catalogs (Stackebrandt et al. 1984, 1986a,b) and 16S rRNA sequences (Weisburg et al. 1986), these budding, nonprosthecate bacteria represent one of about 10 eubacterial divisions (Stackebrandt 1985; Woese et al. 1985a). The high degree of sequence divergence observed between the planctomycetes and other eubacterial groups is suggestive of an early evolutionary origin. One of the most prominent characteristics of the planctomycetes is the presence of a proteinaceous cell envelope devoid of murein (Liesack et al. 1986; Stackebrandt et al. 1986b), a macromolecular structure which is otherwise believed to be a uniting feature of eubacteria (Schleifer and Kandler 1972; Woese and Olsen 1986). Here, analyzing the 5S rRNA from seven strains of *Planctomyces, Pirella, Gemmata,* and *Isosphaera,* we not only confirm the phylogenetic coherency of these organisms and the high degree of unrelatedness of these strains to other eubacteria, but report about striking differences from the minimal model of eubacterial 5S rRNA secondary structure.

Materials and Methods

Materials. The following enzymes were purchased from PL Biochemicals, Milwaukee: RNases T1, U2, Phy M, M1, and RNase from *Bacillus cereus*; nuclease P1 and RNA ligase from T4-infected *Escherichia coli.* Alkaline and bacterial phosphatase were from Boehringer-Mannheim; phosphatase-free 5′-polynucleotide kinase from T4-infected *E. coli* was from New England Nuclear, Boston. [γ-^{32}P]ATP (3000 Ci/mmol) was obtained from New England Nuclear. DEAE-cellulose thin-layer plates and foils (Polygram CEL 300 DEAE/HR-2/15), 20 × 40 cm, were from Macherey & Nagel, Düren. Cellulose acetate strips, 3 × 55 cm, were from Schleicher & Schuell, Dassel. Reagents for PAGE were purchased from Serva, Heidelberg. Sequence analyses were carried out on an LKB electrophoresis system.

Organisms and Culture Techniques. Cultivation of the *Planctomyces, Pirella,* and *Gemmata* strains were as indicated by Schlesner (1986) and Franzmann and Skerman (1984). *Isosphaera pallida* is a phototactic eubacterium resembling planctomycetes in morphology and lack of murein (Giovannoni et al. 1987). Source and strain numbers are listed in Table 1.

Offprint requests to: E. Stackebrandt

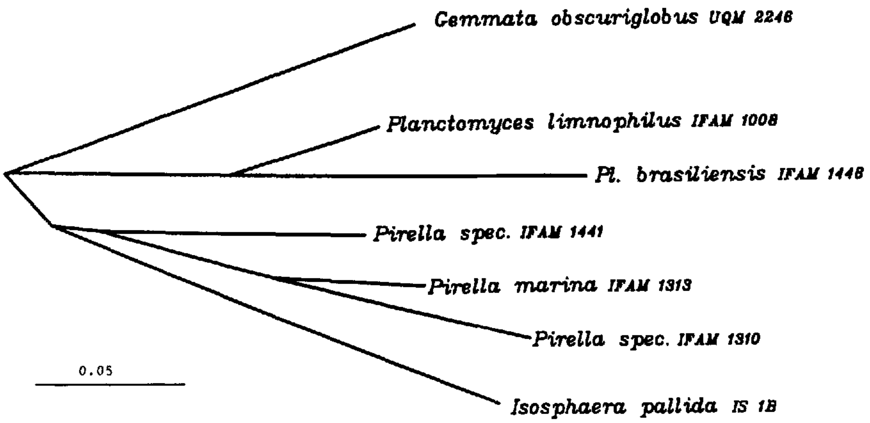

Fig. 1. Evolutionary tree of 5S rRNA sequences of members of the order Planctomycetales. The scale bar represents an evolutionary distance of 0.05 D.

Sequence Determination. Analysis of 5S rRNAs from *Planctomyces, Pirella,* and *Gemmata* strains followed the enzymatic methods described by Donis-Keller et al. (1977). Fragments of trimer size and larger were verified by determination of RNase T1-resistant oligonucleotides (Stackebrandt et al. 1985). The nucleotide sequence from *Isosphaera pallida* was determined by both the enzymatic (Donis-Keller et al. 1977) and the chemical (Peattie 1984) sequencing protocols.

Data Analysis. The Felsenstein PHYLIP program, version 2.9 1986, was used to determine the tree topology that best fit, by the least-square distance method (Fitch and Margoliash 1967), the homology values. Evolutionary distances, D, were calculated according to De Wachter et al. (1985).

Results and Discussion

Table 1 is an alignment of the 5S rRNA sequences from the strains investigated. Similarities for these organisms range between 72 and 85% (Table 2).

Table 1. Complete 5S rRNA sequences for members of the order Planctomycetales. Numeration and stem designations follow the proposal of Erdmann and Wolters (1986)

	5'	
	111111111122222222223333333333444444444455555555556	
	123456789012345678901234567890123456789012345678901234567890	
Pirella marina IFAM 1313	-UUCCGGUGACCAUAUCGAAAAGGUC AUACCUGUUCCCAUUCCGAACACAGCCGUCAAGC	
Pirella spec. IFAM 1310	-UUCCGGCGAUCAUAUCUUAAAGGUU AUACCUGUUCCCAUUCCGAACACAGCAGUCAAGC	
Pirella spec. IFAM 1441	-UUCCGGUGACCAUAUGGUUGUGGAA ACACCUGUUCCCUUUCCGAACACAGCAGUUAAGC	
Planctomyces limnophilus IFAM 1008	-UUCCGGUGACUUUACGCGUGAGGAA ACACUCGUUCCCAUUCCGAACACGACAGUUAAGC	
Planctomyces brasiliensis IFAM 1448	-UUCUGGUGACUUUACGUCUGGGGAA ACACUCGUUCCCAUUCCGAACACGACAGUUAAGC	
Isosphaera pallida IS 1B	-CUCCGGUGACCAUACCGUCGGGGUC CUACCCGUUCCCAUUCCGAACACGGCCGUCAAGC	
Gemmata obscuriglobus UQM 2246	-UUCCGGUGACCAUACCCAAACGGAA ACACCCGUUCCCAUUCCGAACACGGCCGUGAAA	
	A B C C'	
	111111111111111111111	
	666666666777777777788888888889999999999000000000011111111112	
	123456789012345678901234567890123456789012345678901234567890	3'
Pirella marina IFAM 1313	UUUUC-GAGCCGAUGAUAGUACC--- ACA---AGUGUGAAAGUAGGU-AUCGCCGGAUC-	
Pirella spec. IFAM 1310	UUUAA-GAGCCGAUGAUAGUGCC--C ACC---AGCGUGAAAGUAGGU-CUUGCCGGAUC-	
Pirella spec. IFAM 1441	ACAAC-CAGCCGAUGAUAGUGCC--C ACC---AGUGCGAAAGUAGGU-AUCGCCGGAUC-	
Planctomyces limnophilus IFAM 1008	UCCCG-CGGCCGAUGAUAGUGCC--C ACC---AGCGUGAAAGUAGGUUAUCGCCGGAC--	
Planctomyces brasiliensis IFAM 1448	CCUGA-CGGCCGAUGAUAGUACU--- GCA---AGUGUGAAAGUAGGUGAUUGCCAGAC--	
Isosphaera pallida IS 1B	CCGCCGGGCCUAUGAUAGUGCG---- UCA----GCGCGAAAGUCGGUGAUCGCCGGAUCU	
Gemmata obscuriglobus UQM 2246	GUUUG-AGGCCGAUGGUAGUGCG--- UCC---AGCGCGAGAGUAGGU-AUCGCCGGCC--	
	B' D E E' D' A'	

IFAM, Institut für Allgemeine Mikrobiologie, Christian-Albrechts-Universität, 2300 Kiel, FRG; IS, Culture Collection of R.W. Castenholz, Department of Biology, University of Oregon, Eugene, USA; UQM, Department of Microbiology, University of Queensland, St. Lucia 4067, Queensland, Australia

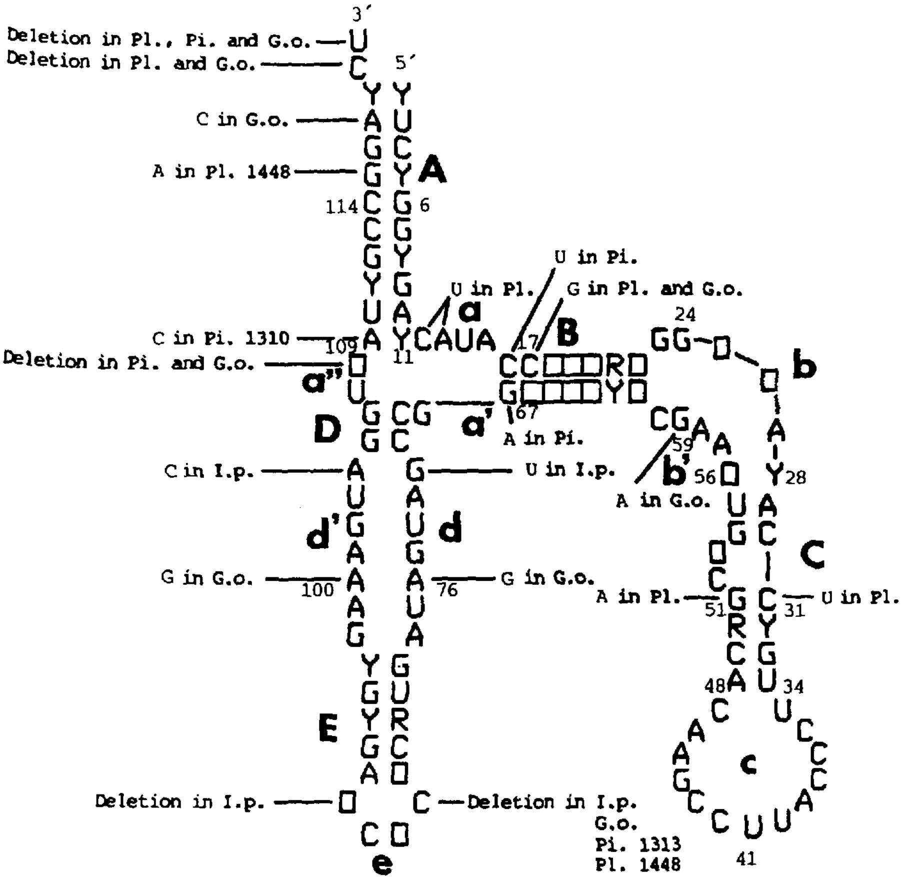

Fig. 2. "Minimal" secondary structure model of the 5S rRNA of members of the order Planctomycetales, aligned to the model derived for eubacteria (Erdmann and Wolters 1986). Also indicated are significant deviations occurring in certain planctomycetes strains. Loop and stem designations correspond to those indicated by Erdmann and Wolters (1986). Abbreviations of strains as in Table 2; Y—pyrimidines; R—purines; squares—hypervariable positions.

Isosphaera pallida, whose values with the six Planctomycetales strains fell within the range of the group, should also be considered a member of this order. This is consistent with unpublished results on the analysis of the 16S rRNA primary structure (S. Giovannoni et al., unpublished). Homology values between 5S rRNAs from planctomycetes and other eubacterial divisions as defined by 16S rRNA anal-

Table 2. Percent similarities among the 5S rRNA sequences. The Beckman Microgenie Sequence Analysis Program (1985) was used for calculation of homologies

	Planctomycetes							Other eubacteria[a]						Archaeobacteria[a]	
Organism	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. *Pl. limnophilus* IFAM 1008	—	83	72	75	82	73	76	54	65	58	62	52	61	47	35
2. *Pl. brasiliensis* IFAM 1448		—	71	68	75	72	72	58	65	59	61	52	63	46	38
3. *Pi. marina* IFAM 1313			—	85	80	80	80	58	65	62	68	57	59	43	35
4. *Pirella* sp. IFAM 1310				—	81	73	74	56	59	60	60	53	56	43	36
5. *Pirella* sp. IFAM 1441					—	75	75	54	64	61	61	59	62	41	36
6. *I. pallida* IS 1B						—	78	59	66	67	63	56	66	48	39
7. *G. obscuriglobus* UQM 2246							—	59	66	68	64	57	65	45	40
8. *E. coli*[a]								—	72	66	73	60	68	50	41
9. *R. gelatinosus*[a]									—	65	72	58	63	47	38
10. *A. tumefaciens*[a]										—	67	60	73	53	44
11. *B. subtilis*[a]											—	56	72	60	48
12. *An. nidulans*[a]												—	57	42	35
13. *T. aquaticus*[a]													—	55	48
14. *M. hungatei*[a]														—	55
15. *S. acidocaldarius*[a]															—

Abbreviations. Pl., Planctomyces; Pi., Pirella; I., Isosphaera; G., Gemmata; E., Escherichia; R., Rhodocyclus; A., Agrobacterium; B., Bacillus; An., Anacystis; T., Thermus; M., Methanospirillum; S., Sulfolobus

[a] Sequences are taken from Erdmann and Wolters (1986)

ysis (Stackebrandt 1985; Woese et al. 1985a), e.g., gram-positives excepting the mycoplasmas, purple bacteria, and their nonphototrophic relatives (rhodobacteria), cyanobacteria, *Chlorobium,* and *Thermus,* as well as archaebacteria are lower, ranging between 35 and 48% and 52 and 68%, respectively.

The isolated position of the planctomycetes within the kingdom of eubacteria as derived from 16S rRNA cataloguing (Stackebrandt et al. 1984, 1986a,b) is verified by the analysis of 5S rRNA. This agreement reaches beyond the order level: both molecules very clearly separate members of the genera *Planctomyces, Pirella,* and *Gemmata* (Fig. 1); *I. pallida* appears as an early branch of the *Pirella* line of descent, a position that is consistent with morphological similarities of these organisms; *I. pallida* and *Pirella* strains both lack stalks. The root of the tree could not be accurately determined due to inherent statistical uncertainty; however, analyses indicate the root lies in the vicinity of the node connecting *G. obscuriglobus* (unpublished). This finding is also in accord with 16S rRNA analysis placing this species as the deepest branching organism within the division of planctomycetes (Stackebrandt et al. 1986b).

The primary and secondary structure of the 5S rRNAs from planctomycetes exhibit unusual features unique for members of the eubacterial kingdom. The primary structures, varying in length between 109 and 111 bases, differ considerably from the "minimal" eubacterial and archaebacterial sequences of 118 bases (Erdmann and Wolters 1986). Shorter versions of this molecule have so far only been found in the highly derived members of the Mycoplasmatales (Erdmann and Wolters 1986).

The secondary structure, depicted as a "minimal" model in Fig. 2 has prokaryotic-specific features, e.g., the base-pair R-Y at position 6-114 [according to the sequential numbering system (Erdmann and Wolters 1986); see also Table 1] and an insertion at position 41. Eubacterial-specific traits include a G–C pair at position 6-114, a short (two base pairs) helix D, a long unpaired region dd', and the lack of inserted bases at positions 74.1 and 84.1, present in archaebacterial and eukaryotic 5S rRNAs. Although the similarities between the secondary structures of planctomycetes and members of other eubacterial divisions are obvious, there are several unique differences not encountered before in eubacterial 5S rRNAs: the lack of an insertion at position 66, present in all prokaryotic 5S rRNAs, a shorter helix E consisting of only 4–5 base pairs and containing an A–C noncanonical base pair in four strains, and a reduction of the bulged loop a″ to a single base in four strains. The rhodobacterium "*Vibrio*" *marinus* is the only other eubacterium reported to possess a one-base loop a″ (MacDonell and Colwell 1984), characteristic of eukaryotes and most archaebacteria.

There are two possible evolutionary explanations for the highly divergent 5S molecules of the planctomycetes: either this group represents an early divergence from the eubacterial line, or they have rapidly evolving (tachytelic) rRNAs. Comparison of the 5S rRNA sequences to the recently proposed eubacterial "minimal" 5S rRNA secondary structure reveals numerous deviations. The most significant changes are two transversions at positions 16-68 and 34-48. The first one changes a G–C pair either into a C–G pair, also found in eukaryotes and certain gram-negative eubacteria, or into a U–A pair, present in several mycoplasmas. In the second example the A–U pair is transversed into a U–A as in rhodobacteria of the alpha and gamma subdivisions. Position 17-67, conserved in eubacteria, is highly variable in planctomycetes: the ancestral pair, G–C, present in three strains, occurs as C–G in three other strains (as also found in a sulfur-dependent archaebacterium and many lower eukaryotes) or as C–A (as present in some lactobacilli and mycoplasmas). The similarities of the Planctomycetales 5S rRNA structure to some members of the division of rhodobacteria are further demonstrated by common features, e.g., A–C odd base pair at position 11-109, G–A at position 24-59, and C–G at position 28-56 (*G. obscuriglobus*), and U–A at position 31-51 (two strains). On the other hand, certain features are shared among planctomycetes and gram-positive eubacteria, including mycoplasmas, e.g., Y–R at position 18-65 (four strains), and A–A at position 76-100 (all strains).

The unusual "minimal" 5S rRNA structure of the *Planctomycetes,* which differs from the "Ur" 5S rRNA sequence (Wolters and Erdmann 1986), as well as the eubacterial "minimal" sequence, is most easily explained by ascribing unusually rapid changes to ribosomal RNA molecules from this group. Otherwise, convergence between the "Ur" and "minimal" eubacterial models is implied. The data presented here are not sufficient to decide between these two possibilities, although an early evolutionary origin is consistent with the lack of murein among members of this group.

Mycoplasmas are the only other eubacterial group known to have undergone such a drastic reduction at the 5S rRNA. In contrast to the mycoplasmas, which are intracellular parasites, planctomycetes are nonparasitic bacteria living in brackish or fresh water. Thus, there is no obvious explanation for tachytelic evolution of those organisms. Whereas mycoplasmas are known to have evolved from gram-positive eubacteria (Woese et al. 1985b), the origin of the planctomycetes is not as clearcut. However, certain common features of the 5S rRNA, shared by planctomycetes and rhodobacteria, may be viewed in terms of a common genealogical origin.

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