5S rRNA Sequences of Myxobacteria and Radioresistant Bacteria and Implications for Eubacterial Evolution

HILDE VAN DEN EYNDE, YVES VAN DE PEER, HILDE VANDENABEELE, MARK VAN BOGAERT, AND RUPERT DE WACHTER*

Departement Biochemie, Universiteit Antwerpen (UIA), Universiteitsplein 1, B-2610 Antwerp, Belgium

5S rRNA sequences were determined for the myxobacteria Cystobacter fuscus, Myxococcus coralloides, Sorangium cellulosum, and Nannocystis exedens and for the radioresistant bacteria Deinococcus radiodurans and Deinococcus radiophilus. A dendrogram was constructed by using weighted pairwise grouping based on these and all other previously known eubacterial 5S rRNA sequences, and this dendrogram showed differences as well as similarities compared with results derived from 16S rRNA analyses. However, in contrast to the 16S rRNA results, the Deinococcus-Thermus 5S rRNA sequences clustered with 5S rRNA sequences of the genus Thermus, as suggested by the results of 16S rRNA analyses. Therefore, we set out to add more Deinococcus 5S rRNA sequences to the data base.

The basis for this unusual assembly is the presence of a highly conserved segment of the 5S rRNA sequence that has been found to be specifically related to the gram-negative genera Deinococcus and Thermus (14, 42), thus forming one of the 10 major phyla that are discernible on the basis of 16S rRNA analyses (45, 46). These three genera share a common peptidoglycan type (A3β) (31) and have unsaturated menaquinones with eight isoprene units as their principal isoprenoid quinones (9, 14, 24, 48, 50). On the basis of 5S rRNA analyses, the genus Thermus has been connected with two Octopus Spring isolates (36). Since only one Deinococcus 5S rRNA sequence has been available previously (4), it has not been possible to confirm the suggested relationship of the genus Thermus to the genera Deinococcus and Deinobacter un-equivocally (1, 4). Therefore, we set out to add more Deinococcus 5S rRNA sequences to the data base.

On the basis of the 16S rRNA catalogue data (12, 15, 18) and the results of a full sequence analysis (25), the dissimi-latory sulfate- and sulfur-reducing bacteria have been grouped with the myxobacteria and bdellovibrios. This group has been assigned (45) to the recently described (34) class Proteobacteria (formerly the purple bacteria and relatives), one of the 10 major eubacterial taxa (45), as its delta group. Myxobacteria, bdellovibrios, and sulfate- and sulfur-reducing organisms are fairly different in their physiological and ecological properties. For example, myxobacteria are strictly aerobic, organotrophic, gliding bacteria that form fruiting bodies and myxospores (29), hunt prey in groups, and are an important source of antibiotics (30). They are found mainly in soil, dung, decaying plant material, and bark of living and dead trees (29). On the basis of their unique and complex life cycle and their rather narrow genomic guanine-plus-cytosine (G+C) content range (68 to 72 mol%), the myxobacteria appear to be a phylogenetically coherent group (29). The genus Bdellovibrio consists of obligately aerobic bacteria that have a morphologically and physiologically biphasic life cycle and are predacious upon other gram-negative bacteria (3); they exhibit a reasonably broad genomic G+C content range (33.4 to 51.5 mol%). Finally, the sulfur- and sulfate-reducing eubacteria are a morphologically diverse group of strictly anaerobic organisms that are capable of using sulfate or sulfur as external electron acceptors (43).

Until now, the phylogenetic relationship of organisms as different as the organisms described above to one another and to the alpha, beta, and gamma groups of the Proteobacteria (34) has not been supported by molecular data other than 16S rRNA data; confirmation from, for instance, rRNA-DNA hybridization studies and 5S rRNA analyses has been wanting. In this respect it is interesting that the one 5S rRNA sequence available for the group, the Desulfovibrio vulgaris sequence, has been reported to be phylogenetically related to sequences of gram-positive bacteria (specifically, Bacillus sequences) (19). Therefore, we investigated a few more species of the delta group of the Proteobacteria for their 5S
rRNA structures to determine what would happen when a somewhat more substantial data set for the delta group was introduced into the 5S rRNA picture.

In this study, we determined the 5S rRNA sequences of Myxococcus coralloides and Cystobacter fuscus, which are representatives of the suborder Cystobacterinae in the order Myxobacteriales (29), and of Sorangium cellulosum and Nannocystis exedens, which are members of the suborder Sorangineae (29). We also determined the 5S rRNA sequences of Deinococcus radiodurans and Deinococcus radiophilus; this permitted a comparison with the results of 16S rRNA data analyses. The sequences were incorporated into an alignment containing 296 other eubacterial 5S rRNA sequences, which covered 8 of the 10 phyla that are distinguishable on the basis of 16S rRNA data (45), and a dendrogram was constructed by using a pairwise clustering algorithm.

MATERIALS AND METHODS

The following strains were used: Deinococcus radiodurans ATCC 13939T (T = type strain), Deinococcus radiophilus ATCC 27603T, M. coralloides ATCC 25202T, Cystobacter fuscus strain Cbf2 (18), N. exedens DSM 71T, and S. cellulosum ATCC 25532. The medium in which Deinococcus radiodurans was grown contained (per liter) 10 g of tryptone (Difco Laboratories, Detroit, Mich.), 5 g of yeast extract (Difco), 5 g of glucose, and 5 g of sodium chloride. The pH was adjusted to 7.3. Deinococcus radiodurans growth medium contained 30 g of tryptic soy broth (Difco) per liter, and the pH was adjusted to 7.4. Both Deinococcus radiodurans and Deinococcus radiophilus were grown at 30°C with mild aeration. M. coralloides was grown aerobically at 30°C in a medium containing (per liter) 1 g of D(+)-ribose pentahydrate (Janssen Chimica, Beerse, Belgium), 1 g of sucrose (E. Merck AG, Darmstadt, Federal Republic of Germany), 1 g of D(+)-galactose (Janssen Chimica), 5 g of soluble starch (Merck), 2.5 g of Casitone (Difco), 0.5 g of magnesium sulfate heptahydrate, and 0.25 g of dipotassium hydrogen phosphate. The pH was adjusted to 7.4. N. exedens, S. cellulosum, and Cystobacter fuscus were cultured as described previously (18). Cultivated organisms were harvested by centrifugation and stored until further use by freezing or freeze-drying.

Cells of Deinococcus radiodurans and Deinococcus radiophilus were ruptured by using lysozyme, DNase treatment, and a freeze-thaw cycle, as described previously (26). Cells of M. coralloides were broken with glass beads by using a cell mill as described previously (44). N. exedens, S. cellulosum, and Cystobacter fuscus were ground in a mortar with alumina (40). Sheared cells were then subjected to phenol extraction (44) in order to obtain the rRNA fraction. 5S rRNA was prepared by polyacrylamide gel electrophoresis as described previously (10). 5S rRNA that was 3'-terminally labeled with cytidine 3', 5'-[3H]-bisphosphate was purified on 8% polyacrylamide gels and subjected to the Paezit partial chemical degradation method (28). Whenever necessary, the nucleotide pattern was confirmed by partial enzymatic degradation (6). When there was length heterogeneity, components of different chain lengths were sequenced separately. The entire M. coralloides sequence was elucidated by electrophoresis of the partial degradation products on 8 to 20% polyacrylamide gels that were kept at a constant temperature of 65°C. The 5'-terminal nucleotides of the 5S rRNAs of Deinococcus radiodurans, Deinococcus radiophilus, N. exedens, and S. cellulosum were determined by comparing the partial chemical degradation products of, on the one hand, (Ap),A, and, on the other hand, (Up),U, extended (6) and 3'-terminally labeled 5S rRNA. We found that Cystobacter fuscus possesses at least two 5S rRNA components, one only of which was extendable by using (Ap),A and (Up),U. The sequences of both components were determined and are shown here below. The European Molecular Biology Laboratory data library and GenBank accession numbers of the sequences which we determined are as follows: X52297 (Cystobacter fuscus 1), X52298 (Cystobacter fuscus 2), X52299 (Deinococcus radiophilus), X52300 (Deinococcus radiodurans), X52301 (M. coralloides), X52302 (N. exedens), and X52303 (S. cellulosum).
Dendrograms were constructed by using a weighted pairwise clustering algorithm that has been described previously (7, 16). Briefly, a dissimilarity matrix that took into account multiple and back mutations served as the input for the tree construction algorithm. A correction for the effects of unequal evolutionary rates along different eubacterial lineages was incorporated (7). For this purpose, 10 cytoplasmic 5S rRNA sequences from red algae (39, 47) were used as a reference outgroup. The standard deviations for the substitution terms of the computed dissimilarities are indicated on all of the branching points of one of the resulting dendrograms (see Fig. 2).
RESULTS AND DISCUSSION

Figure 1 shows part of an alignment (available from us), in which the sequences reported in this paper were aligned with all previously published 5S rRNA sequences; the majority of these previously published sequences can be found in a recent update (47). In Fig. 1 putative double-stranded areas are enclosed in boxes; this was done according to a well-established secondary-structure proposal (8) that is applicable to the 5S rRNAs of all phylogenetic lineages.

**General topology of the dendrogram.** Figure 2 shows the complete dendrogram constructed from an alignment of 303 eubacterial 5S rRNA sequences supplemented with 10 cytoplasmic 5S rRNA sequences from red algae (data not shown) in order to allow correction for unequal evolutionary rates in different bacterial lineages. This dendrogram has been published and its general topology has been discussed previously (41). It is shown here to allow readers to localize the clusters containing the species for which the 5S rRNA sequences are reported in this paper. The radioresistant bacteria form a separate cluster (indicated in Fig. 2), whereas three of the four myxobacteria group together with other species in a cluster labeled miscellaneous in Fig. 2.

**Radioresistant bacteria and their relatives.** The detailed structure of the cluster based on the 5S rRNA sequences from radioresistant bacteria and their relatives is shown in Fig. 3a. The presence of the newly determined 5S rRNA sequences from *Deinococcus radiodurans* and *Deinococcus radiophilus* along with the sequences of *Thermus sp.*, *Thermus aquaticus*, and an unspecified strain of *Deinococcus radiodurans* is in complete agreement with the results obtained from 16S rRNA studies (14, 42). The placement of the cluster containing radioresistant organisms and their relatives among the alpha, beta, and gamma groups of the Proteobacteria need not worry us, in view of the large standard deviations indicated on the branches in Fig. 2.

The genera *Deinococcus* and *Deinobacter* are not unique in exhibiting extraordinary degrees of radiation resistance. Recently, a radiation-resistant *Acinetobacter* strain has been isolated from cotton and soil (22), and its level of DNA relatedness to other *Acinetobacter* strains has been found to be such (23) that the organon of a new species (22), *Acinetobacter radioresistens*, is warranted. The *A. radioresistens* ubiquinone system was found to be Q-9 (22). Another highly radiotolerant organism, *Rubrobacter radiotolerans* (37), has been extensively characterized chemotaxonomically (37). This organism can be distinguished from the genera *Deinococcus, Deinobacter*, and *Thermus* by its A3-a peptidoglycan type (31, 37). At the same time, its major isoprenoid quinones are menaquinones with eight isoprene units (37), as is the case for members of the genera *Deinococcus, Deinobacter*, and *Thermus*. It might be interesting to determine the phylogenetic positions of *A. radioresistens* and *R. radiotolerans* in relation to the phylum of radioresistant bacteria and their relatives by using 16S rRNA sequencing or 5S rRNA sequencing or both.

Two microorganisms isolated from a Yellowstone National Park hot spring, designated Octopus Spring isolates 2 and 3, were thought to be distantly related to *T. aquaticus* and *Thermus* sp. as determined by 5S rRNA analyses (36) at a time when fewer 5S rRNA sequences were available. We could not confirm this suggested relationship, since the Octopus Spring isolate 2 5S rRNA sequence was recovered with the miscellaneous cluster in Fig. 3b, and the 5S rRNA of Octopus Spring isolate 3 was found to be distantly related to the 5S rRNAs of peptidoglycanless eubacteria (Fig. 2) (41).
Delta subdivision of the Proteobacteria. As Fig. 3b shows, we did not confirm the existence of a delta subdivision of the class Proteobacteria. Instead, as pointed out previously (19), an association of the so-called delta organisms with Firmicuta bacteria (gram-positive bacteria and relatives) having low genomic G+C contents was suggested by the 5S rRNA data. Figure 3b shows that *Desulfovibrio vulgaris* SS rRNA groups specifically with one of the Octopus Spring isolate 5S rRNA sequences. Although the 5S rRNA sequences of the species of *Cystobacterinae* (29) which we investigated appear to be more closely related to one another (Fig. 3b) than the species of *Sorangineae* (29) are (41) (Fig. 3b), the relationship is not complete, for also included in the cluster are the 5S rRNA sequences of *Cytophaga heparina* and "Herpetosiphon" sp. strain Senghas Wie 2 (40), which are representatives of the *Bacteroides*-Cytophaga-Flavobacterium phylum (27) and the phylum of green nonsulfur bacteria and their relatives (13, 46), respectively, as suggested by 16S rRNA analyses. At least two genes coding for 5S rRNA are found in *Cystobacter fuscus*. They appear to have diverged from one another well before the divergence of *M. coralloides* and Cystobacter fuscus. As for the *Sorangineae*, the NC-1052 SS rRNA sequence groups with a cluster which includes sequences of *Flexibacter* sp. (41), *Bacillus brevis*, and *Bacillus acidocaldarius* (Fig. 3b), whereas the *S. cellulosum* sequence is distantly related to the cluster containing sequences of Firmicuta bacteria having high genomic G+C contents (Fig. 2).

Thus, 5S rRNA sequence analysis clearly failed to confirm the existence of a delta group of the Proteobacteria. As Fig. 2 shows, many features of eubacterial evolution as deduced from 16S rRNA analyses are corroborated by the results of 5S rRNA analysis. The existence of the alpha, beta, and gamma subdivisions of the class Proteobacteria is corroborated by information from 5S rRNA sequence analysis and rRNA-DNA hybridization studies (summarized in reference 34). In contrast, the existence of a delta subdivision is supported only by 16S rRNA data. Conclusions about evolutionary relationships among higher taxa based on sequence comparisons of 5S rRNA molecules are problematic because of the scarcity of informative characters and the inherent limitations of the tree construction method. Furthermore, only four 5S rRNA sequences of myxobacteria, one sequence of a sulfate reducer, and no sequences of members of the genus *Bdellovibrio* have been examined. We believe that it would be desirable to test the homogeneity and affiliation of the delta subdivision of the Proteobacteria by examining additional molecular markers, such as 23S rRNA.

ACKNOWLEDGMENTS

We thank E. Stackebrandt for providing cells of *Cystobacter fuscus*, *N. exedens*, and *S. cellulosum*. Our research was supported in part by a grant from the Fonds voor Kollektief Fundamenteel Onderzoek. Y.V. received a scholarship from the Instituut tot aanmoediging van het Wetenschappelijker Onderzoek in Nijverheid en Landbouw.

LITERATURE CITED


