Deoxribonucleic Acid Homology of Prosthecomicrobium and Ancalomicrobium Strains

R. L. Moore and J. T. Staley

Division of Pathology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 1N4, and Department of Microbiology and Immunology, University of Washington, Seattle, Washington 98195

Deoxribonucleic acid base sequence homology studies have been conducted on 14 strains of the Prosthecomicrobium-Ancalomicrobium group as well as selected strains of other prosthecate bacteria in the genera Hyphomicrobium and Caulobacter. By this procedure Prosthecomicrobium and Ancalomicrobium are not closely related to either the Hyphomicrobium or Caulobacter strains tested. The type strains of P. enhydrum, P. pneumaticum, and A. adetum are discrete from one another, therefore justifying the current taxonomic treatment of the group. In addition to these species, there are at least two additional groups of strains fitting the description of Prosthecomicrobium and Ancalomicrobium but differing from the type strains. One group is comprised of short-appendaged motile and nonmotile strains which morphologically resemble P. enhydrum but show insignificant polynucleotide reassociation with it. The other group, represented by at least four strains, has both short and long appendages and in other respects shares properties with both P. enhydrum and A. adetum.

Bacteria in the Prosthecomicrobium-Ancalomicrobium group have numerous prosthecae radiating from the cell. Present identification within the group is based upon the length, shape, and number of prosthecae and the occurrence of motility and gas vacuolation. Three species are currently recognized (6-8). P. enhydrum has 10 to 30 short (i.e., <0.5-μm), conical prosthecae and is motile and non-gas vacuolate. P. pneumaticum has 10 to 30 somewhat longer conical prosthecae (ca. 1.0 μm) and is immotile and gas vacuolate. A. adetum has two to eight cylindrical prosthecae that are considerably longer (ca. 3 μm) but, like P. pneumaticum, it is immotile and gas vacuolate.

Recently, 14 additional strains having multiple prosthecae and in other respects fitting the description of bacteria in these two genera were isolated and briefly characterized (9). The deoxyribonucleic acid (DNA) base composition of all strains was analyzed and found to range from 66.1 to 71.4%.

This investigation was undertaken to elucidate the interrelatedness of strains within these two genera as well as to assess their relationship to other prosthecate bacteria by DNA base sequence homology. All strains of Prosthecomicrobium and Ancalomicrobium were grown in a dilute, complex, organic medium (MMB broth) (9) in shake cultures or in large fermentors. The growth of other strains used in this study has been previously described (5). The type strains P. enhydrum ATCC 23634, P. pneumaticum ATCC 23633, and A. adetum ATCC 23632 and strain 16 were labeled with [G-3H]adenine (New England Nuclear Corp., Boston, Mass.; specific activity, 27.35 Ci/mmol). A 2-mCi amount was added to 125-ml cultures growing in MMB. Both labeled and nonlabeled cells were harvested in the early stationary phase by centrifugation and washed twice with saline-ethylendiaminetetraacetic acid (0.15 M sodium chloride-0.1 M EDTA, pH 8.0). The pellets were frozen and stored before extraction. The cells were suspended in 1× SSC (SSC is 0.15 M sodium chloride plus 0.1 M sodium citrate) with 1.0 ml of NaCl and incubated for 16 h at 37°C. The sodium lauryl sulfate concentration was increased to 1% and the cells were lysed at 60°C. The DNA was purified by the method of Marmur (4). All DNAs were sheared to 300,000 daltons by passage through a French pressure cell at 15,000 lb/in². Radiolabeled DNA was further purified by hydroxyapatite column chromatography (1, 2).

Reaction mixtures for DNA-DNA reassociation contained 150 μg of heat-dissociated, nonlabeled and 0.1 μg of labeled DNA in 1.0 ml of 0.17 M NaCl. Reassociation was carried out at 72°C for 16 h. Unreacted DNA was hydrolyzed by adding the reaction mixture to 1.0 ml of enzyme solution (pH 4.5) containing 0.2 mM ZnSO₄, 0.06 M sodium acetate, 0.17 M NaCl, 40 μg of single-stranded salmon DNA per ml, and
sufficient S1 endonuclease (Miles Laboratories Inc., Kankakee, Ill.) to hydrolyze 96% of the single-stranded DNA in 20 min at 58 C. Under these conditions, less than 5% of the double-stranded DNA was hydrolyzed. Reacted DNA was precipitated with trichloroacetic acid and collected by filtration, and the radioactivity was measured with a Beckman LS 330 liquid scintillation counter.

Table 1 includes a brief description of the strains used in these experiments and the results of the hybridization studies. Strains are listed in order of their DNA base composition values. Very low, nonsignificant values were obtained between the type strains and strains of other genera. Thus, in confirmation of previous work (5), it is evident that only a very distant relationship can exist between the Prosthecomicrobium-Ancalomicrobium group and other prosthecate bacteria in the genera Caulobacter and Hyphomicrobium.

Indeed, there appears to be considerable heterogeneity even within the collection of Prosthecomicrobium and Ancalomicrobium strains. Thus, P. enhydrum did not appear to be related to any of the other strains tested including strain 5, which resembles it closely phenotypically, as well as 1 and 17, which, although immotile, also bear a strong resemblance to the type strain. These unnamed, short-appendaged strains (i.e., 5, 1, and 17) show insignificant duplex formation with any of the labeled DNAs, suggesting that at least one more species is probably warranted for short-appendaged strains in the genus Prosthecomicrobium.

A. adetum showed significant base sequence homology with only one other strain, no. 18, which, except for the absence of gas vacuoles, bears a strong phenotypic resemblance to the type strain. Likewise, P. pneumaticum exhibited significant homology with only one
strain, no. 19. Both of these strains are gas vacuolate and immotile and have short appendages.

Strain 16 was selected as one of the new isolates which typified the new "intermediate" strains that are motile and avacuolate and have both short and long appendages and DNA base composition values between those of P. enhydrium and A. adetum. Significant hybridization occurred between strain 16 and all of the other short- and long-appendaged strains that were tested (i.e., 6, 7, and 12). In contrast, none of the other strains tested appeared to be related to this group.

The results of this study confirm the current taxonomic treatment of the bacteria in this group (6-8) in that the type strains show appreciable differences from one another. In addition, it appears that the "intermediate" strains could comprise a new species of either Prosthecomicrobium or Ancalomicrobium, and at least one additional species of short-appendaged strains would be justifiable in the genus Prosthecomicrobium. However, no formal taxonomic proposals will be made until further investigations of the general biological properties of the new isolates are completed.

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REPRINT REQUESTS
Address reprint requests to: Dr. J. T. Staley, Department of Microbiology and Immunology, SC-42, University of Washington, G305 Health Sciences Bldg., Seattle, Wash. 98195.

LITERATURE CITED