Deoxyribonucleic Acid Homology of *Prosthecomicrobium* and *Ancalomicrobium* Strains

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Deoxyribonucleic 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. enhydrium*, *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. enhydrium* 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. enhydrium* 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. enhydrium* has 10 to 30 short (i.e., <0.5- μ m), conical prosthecae and is motile and non-gas vacuolate. *P. pneumaticum* has 10 to 30 somewhat longer conical prosthecae (ca. 1.0 μ m) and is immotile and gas vacuolate. *A. adetum* has two to eight cylindrical prosthecae that are considerably longer (ca. 3 μ m) 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. enhy-*

drum 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-ethylenediaminetetraacetic 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 $\times$ SSC (SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate) with 100 μ g of Pronase per ml and 0.2% sodium lauryl sulfate 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 μ g of heat-dissociated, non-labeled and 0.1 μ g 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 μ g 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 prosthecae 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. enhydrium* 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

TABLE 1. Characteristics of *Prosthecomicrobium* and *Ancalomicrobium* strains and their relatedness to other prosthecae bacteria as revealed by DNA homology

Strain	Morphology			DNA base composition ^a	Source and sp act (counts/min per μ g) of tritium-labeled DNA and % hybridization with DNA from various strains ^b			
	Prosthecae ^c	Motility	Gas vacuoles		<i>P. enhydrium</i> 94,700	<i>P. pneumaticum</i> 29,700	16 79,400	<i>A. adetum</i> 58,800
<i>P. enhydrium</i> (ATCC 23634)	Short	+	—	66.1	100	2	4	0
1 (ATCC 27825)	Short	—	—	66.8	7	2	4	0
6 (ATCC 27827)	Short and long	+	—	67.9	5	1	90	9
5 (ATCC 27826)	Short	+	—	68.4	4	— ^d	—	—
10 (ATCC 27829)	Short and long	+	—	68.9	6	4	—	—
11 (ATCC 27830)	Short and long	+	—	68.9	4	0.4	—	—
12 (ATCC 27831)	Short and long	+	—	68.9	7	2	90	8
16 (ATCC 27832)	Short and long	+	—	68.9	0	4	100	—
17 (ATCC 27833)	Short	—	—	68.9	0.5	5	2	7
<i>P. pneumaticum</i> (ATCC 23633)	Short (rarely long)	—	+	69.4	5	100	4	4
7 (ATCC 27828)	Short and long	+	—	69.9	2	3	86	6
19 (ATCC 27835)	Short	—	+	70.4	—	31	4	4
<i>A. adetum</i> (ATCC 23632)	Long	—	+	70.4	0	5	4	100
18 (ATCC 27834)	Long	—	—	71.4	4	2	7	51
<i>Caulobacter crescentus</i> (ATCC 15252)	Long	+	—	67.0	3	0	2	5
<i>Hyphomicrobium</i>								
NQ-521	Long	+	—	66.8	3	2	—	5
NM-765	Long	+	—	64.3	4	2	0	3
B-522	Long	+	—	60.2	8	2	3	2
H-526	Long	+	—	60.2	3	5	—	7
(ATCC 15444; LE-670)	Long	+	—	61.7	4	0.1	1	0

^a Determination for *Prosthecomicrobium* and *Ancalomicrobium* strains from reference 9, and *Caulobacter* and *Hyphomicrobium* strains from references 3 and 5.

^b Average of at least two analyses. Values were corrected for background counts obtained with coliphage T4 DNA, which amounted to 12% or less of homologous binding values. Eighty percent or greater of the input ³H-labeled DNA underwent duplex formation in the homologous reactions.

^c Short prosthecae are conical in shape and less than 2 μ m long; long prosthecae are cylindrical and greater than 2 μ m in length.

^d Dash indicates that the hybridization reaction was not conducted.

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.

This research was supported by National Science Foundation grant GB-30313.

R. Bigford and E. DiBlasio provided excellent technical assistance.

REPRINT REQUESTS

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