Deoxyribonucleic Acid Homology Among the Caulobacters

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Deoxyribonucleic acid (DNA) homologies among 26 strains of bacteria including representative type species of the genera *Caulobacter*, *Asticcacaulis*, and *Prostheco bacter* were determined by DNA/DNA reassociation in solution with S_1 endonuclease for removing unpaired structures. The relatedness of the strains observed by this procedure are discussed together with previous findings made from numerical taxonomy and phage typing studies.

The caulobacters are single-appendaged prosthecate bacteria represented by the three genera *Caulobacter*, *Asticcacaulis*, and *Prostheco bacter*. The taxonomy of these bacteria has been based upon phenotypic characteristics including morphological and life cycle differences, carbon source utilization patterns, vitamin requirements, colony pigmentation, and so forth. Bacteria in the genus *Caulobacter* have polar flagella and polar stalks with apical holdfasts, whereas members of the genus *Asticcacaulis* have subpolar flagella and subpolar prosthecae with basal holdfasts (14). Strains of the recently proposed genus *Prostheco bacter* resemble *Caulobacter* species morphologically, but are non-motile.

The flagellated caulobacters have been classified by numerical taxonomic procedures into 10 species of *Caulobacter* and 1 species of *Asticcacaulis* based upon 78 characteristics (14). In addition, a double-appendaged species of *Asticcacaulis*, *A. biprosthecum*, has been named (13). The fusiform caulobacter is represented by the single species *Prostheco bacter fusiformis* (18).

A number of phages have been isolated that are effective parasites of motile caulobacters, and these have been used for phage typing studies (1, 14, 16). Carter and Schmidt (5) recently examined the fatty acid composition of a number of caulobacters and other prosthecate bacteria as a means of comparing relatedness among these organisms.

Although the caulobacters comprise the most thoroughly studied and characterized of the prosthecate bacteria, they also represent the only major family of bacteria in this group yet to be subjected to genotypic comparison using deoxyribonucleic acid (DNA) homology procedures. Both the hyphomicrobia (10, 11) and prosthecomicrobia (12) have been the subjects of such studies. Thus, the primary objective of

this investigation was to determine the extent of interrelatedness among the caulobacters using DNA hybridization procedures.

MATERIALS AND METHODS

Bacterial strains. The strains of bacteria utilized in this investigation are listed in Table 1. Except for strains S 3 and C 19, which were kindly provided by Jack Pate, all strains are from the culture collections of the respective authors. The only type species not included in the study were the marine isolates *C. maris* and *C. halobacteroides*.

Each strain was revived from lyophilized or frozen (10% glycerol, -60 or -70°C) cultures, streaked out, and checked with respect to colonial morphology, and its cell morphology was determined with phase-contrast microscopy.

Growth conditions and DNA labeling. For extraction of unlabeled DNA, *Caulobacter* and *Asticcacaulis* strains were grown in PYE medium containing 0.2% peptone (Difco), 0.1% yeast extract (Difco), and 1% vitamin-free Hutner mineral base (6) at 25°C , with aeration, to the late exponential phase of growth. *Prostheco bacter fusiformis* strains were grown on MMB medium (19) containing 0.2% glucose. Each individual culture was observed with phase-contrast photomicroscopy to check for typical morphology and to avoid inclusion of any contaminants. Bacteria were harvested (nonaseptically) by centrifugation. At least one wash with distilled water was employed for each cell preparation. Cell pellets were frozen until the DNA could be extracted.

Type strains of the caulobacters were labeled with [$G^3\text{H}$]adenine (New England Nuclear Corp., Boston, Mass.; specific activity, 27.4 Ci/mmol). Cultures were grown on a medium containing (per liter): 1 g of peptone (Difco), 1 g of glucose, 0.15 g of yeast extract (Difco), 0.25 g of ammonium sulfate, 20 ml of a modified Hutner salts solution (20) except that sodium molybdate was included at 19 mg/liter of stock solution, and 10 ml of a vitamin solution (17). A 100- to 200-ml volume of logarithmically growing cells was labeled with 1.5 mCi of tritiated adenine early enough in the growth cycle to permit two to three subsequent doublings in optical density.

TABLE 1. *Bacterial strains*

Species	Strain no.	Reference and comments
<i>Caulobacter crescentus</i>	CB 2	Type strain, ATCC 15252
	CB 15	ATCC 19089
<i>Caulobacter vibrioides</i>	CB 51	Neotype strain, <i>C. vibrioides</i> subsp. <i>limonus</i>
	CB 16	
	CB G	
<i>Caulobacter subvibrioides</i>	CB 88	Type strain, ATCC 15265, <i>C. subvibrioides</i> subsp. <i>albus</i>
	CB 81	ATCC 15264
<i>Caulobacter fusiformis</i>	CB 27	Type strain, ATCC 15257
<i>Caulobacter bacteroides</i>	CB 7	Type strain, ATCC 15254
	CB 11a	ATCC 19090
	CB 9	
	CB 10	
<i>Caulobacter henricii</i>	CB 4	Type strain, ATCC 15253
	CB R	Type strain, ATCC 15266, <i>C. henricii</i> subsp. <i>aurantiacus</i>
<i>Caulobacter leidy</i>	CB 37	Type strain, ATCC 15260
<i>Caulobacter variabilis</i>	CB 17	Type strain, ATCC 15255
<i>Caulobacter intermedius</i>	CB 63	Type strain, ATCC 15262
<i>Caulobacter</i> sp. type V	CB 28	Type strain, ATCC 15258
<i>Asticcacaulis excentricus</i>	AC 48	Type strain, ATCC 15261
	AC 12	
	S 3	
<i>Asticcacaulis biprosthecum</i>	C 19	Type strain, ATCC 27554
<i>Prostheco bacter fusiformis</i>	FC 4	Type strain, ATCC 25309
	FC 1	ATCC 27091
	FC 2	
	FC 3	

DNA extraction, purification, and hybridization. DNA was extracted and purified as described previously (12).

Reaction mixtures for DNA/DNA reassociation contained 150 µg of heat-dissociated unlabeled and 0.1 µg of labeled DNA in 1.0 ml of 0.17 M NaCl. Reassociation was carried out at 71°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 57°C. Under these conditions, hydrolysis of double-stranded DNA was less than 1%. Reacted DNA was precipitated with trichloroacetic acid and collected by filtration. The radioactivity was measured with a Beckman LS 330 liquid scintillation counter.

RESULTS AND DISCUSSION

As can be seen from the results (Table 2), there is considerable homology among the strains of *C. vibrioides* and *C. crescentus*. The respective type strains, CB 51 and CB 2, are more closely related by this procedure than are any other strains included in the study. In addition, other strains within these species show a high degree of reassociation with one another. Indeed, using the guidelines proposed for the

enteric bacteria by Brenner (3) whereby strains are placed into the same species if they show 70% or greater DNA homology with one another, one could argue that, with the exception of CB G, all of the strains in these two species should be placed in a single species. Bacteriophage host range studies also imply that these two species are very similar (2, 14, 16). In the numerical taxonomic study (14), CB 2 and CB 51 exhibited a 65% similarity value (*S*) to one another. CB 16 had an *S* of 66% with CB 51 and 55% with CB 2, whereas CB 15 showed a 58% *S* with CB 51 and 83% with CB 2. CB G was also more distantly related by the numerical taxonomic study, showing an *S* of less than 50% with both type strains.

Although the type strains of *C. vibrioides* and *C. crescentus* were 97% homologous to one another, their homologies with CB 15, CB 16, and CB 81 differed by 14, 13, and 19%, respectively. In addition to possible experimental variation, these discrepancies could be due to differences in genome size among these strains (4).

The third frequently isolated vibrioid species of *Caulobacter*, *C. henricii*, showed a more distant, but still significant homology with all strains of *C. vibrioides* and *C. crescentus*. This is consistent with the bacteriophage host range studies by Schmidt and Stanier (16) and with the findings of Driggers and Schmidt (8), that

TABLE 2. DNA homology of caulobacters.

Strain	Percent hybridization with DNA from ^a :										
	<i>Caulobacter vibrioides</i> CB 51 (7,139) ^b	<i>C. crescentus</i> CB 2 (18,893)	<i>C. henricii</i> CB 4 (17,429)	<i>C. bacteroides</i> CB 7 (9,248)	<i>C. subvibrioides</i> CB 81 (59,500)	<i>C. variabilis</i> CB 17 (58,810)	<i>C. intermedius</i> CB 63 (14,607)	<i>C. leidyi</i> CB 37 (5,000)	<i>C. fusiformis</i> CB 27 (42,441)	<i>Asticcacaulis eccentricus</i> AC 48 (38,840)	<i>Prosthecomicrobium fusiformis</i> FC 4 (26,320)
<i>Caulobacter vibrioides</i>											
CB 51	100	(97)	14	6	1	3	2	(2)	(6)	(<1)	1
CB G	60	58	19	4	2	3	3	3	<1	<1	4
CB 16	90	77	34	<1	5	2	4	2	<1	<1	<1
<i>C. crescentus</i>											
CB 2 (ATCC 15252)	97	(100)	22	6	20	2	3	2	(6)	1	<1
CB 15 (ATCC 19089)	85	71	16	2	1	<1	1	<1	<1	<1	<1
<i>C. henricii</i>											
CB 4 (ATCC 15253)	16	(22)	100	(9)	(2)	4	(2)	(<1)	(2)	(2)	(<1)
CB R (ATCC 15266)	2	3	4	6	9	6	33	3	<1	<1	<1
<i>C. bacteroides</i>											
CB 7 (ATCC 15254)	9	9	100	19	13	15	(2)	(6)	3	3	
CB 10	1	2	<1	30	10	7	8	<1	<1	<1	<1
CB 9	2	2	3	29	15	8	6	1	<1	<1	<1
CB 11a (ATCC 19090)	2	2	<1	24	19	8	8	3	<1	<1	<1
<i>C. intermedius</i> CB 63 (ATCC 15262)	2	3	2	11	(9)	8	100	(<1)	(3)	<1	<1
<i>C. fusiformis</i> CB 27 (ATCC 15257)	6	6	2	6	<1	1	3	3	100	<1	<1
<i>C. subvibrioides</i>											
CB 81 (ATCC 15264)	1	(20)	2	(19)	100	10	9	(<1)	(<1)	(<1)	(<1)
CB 88 (ATCC 15265)	10	1	8	12	12	<1	15	(2)	(6)	3	3
<i>C. variabilis</i> CB 17 (ATCC 15255)	(3)	(2)	7	(13)	(10)	100	(8)	(4)	(4)	3	(<1)
<i>C. leidyi</i> CB 37 (ATCC 15260)	2	(2)	4	2	<1	<1	<1	100	(3)	<1	1
<i>Caulobacter</i> sp. type V CB 28 (ATCC 15261)	<1	<1	<1	29	11	9	8	<1	<1	1	<1
<i>Asticcacaulis eccentricus</i>											
AC 48 (ATCC 15261)	<1	(1)	2	(3)	<1	<1	<1	2	(<1)	100	<1
S 3	<1	<1	<1	<1	<1	2	<1	<1	<1	86	<1
AC 12	<1	<1	3	<1	1	<1	<1	2	<1	33	<1
<i>A. biprosthecum</i> C 19 (ATCC 127554)	—	—	3	0	—	5	0	—	1	—	1
<i>Prosthecomicrobium fusiformis</i>											
FC 4 (ATCC 25309)	(1)	(<1)	<1	(3)	<1	<1	<1	2	(<1)	<1	100
FC 1 (ATCC 27091)	—	—	—	—	—	9	—	<1	—	—	5
FC 2	—	—	—	—	—	—	—	3	—	—	1
FC 3	—	—	—	—	—	—	—	<1	—	—	3

^a The hybridization values are the average of assays performed in triplicate and have been corrected for background counts obtained with DNA from *Prosthecomicrobium enhydrium* (7.6% of input). Eighty percent of the input ³H-labeled DNA used in the homologous reactions underwent reassociation. Values listed in parentheses were obtained in the reciprocal reaction and are listed for comparison. — indicates that the analysis was not performed.

^b Numbers in parentheses in column headings indicate specific activity (in counts per minute per microgram) of tritium-labeled DNA.

latent bacteriophages isolated from either of the first two vibrioid species were lytic for *C. henricii* strains. Further, in Poindexter's numerical analysis, some strain pairs of *C. henricii* and *C. vibrioides* displayed *S* values greater than 60%. Interestingly, *C. henricii* CB R was very distantly related to the type strain of the species, but showed considerable homology to *C. intermedius*. This is consistent with the numerical taxonomic analysis, in which this strain exhibited 57% *S* with the type strain of *C. henricii* and 74% with the type strain of *C. intermedius*. Strain CB R was originally classified as a subspecies, *C. henricii* subsp. *aurantiacus* (14).

The remaining vibrioid species, *C. intermedius*, displayed the widest range of generally low, but possibly significant homologies. This was similar to its intermediate phenotypic position among the isolates used in the numerical analysis.

Accordingly, the vibrioid *Caulobacter* species appear to consist of two possibly redundant species (*C. vibrioides* and *C. crescentus*), a third moderately related species (*C. henricii*), and a fourth (*C. intermedius*) no more closely related to those three than to nonvibrioid species.

C. bacteroides comprised a group of strains related to the type strain (CB 7) in a relatively distant but consistent pattern. *Caulobacter* sp. type V was the most closely related strain outside the group, and by DNA homology was more closely related to this group than to any other. Type V strains were phenotypically similar to *C. bacteroides*, but not so designated by Poindexter because of their equal similarity to *C. variabilis*. The homologies of *C. subvibrioides* strain CB 81 and *C. variabilis* CB 17 were also greater than 10% with *C. bacteroides* CB 7. In addition to showing a significant but low level of homology to CB 7, *C. subvibrioides* showed a similar degree of homology to other strains in that species as well as to *C. crescentus* CB 2. The phenotypic analysis had revealed similarity between *C. subvibrioides* and *C. variabilis*, but not between either of these species and *C. bacteroides*, or between *C. subvibrioides* and *C. crescentus* (14).

Some type species of *Caulobacter*, e.g., *C. leidy* and *C. fusiformis*, exhibited little or no homology with any of the other strains studied. Thus, there is strong justification for separate species in these cases.

Correspondingly distant relatedness was shown by the intergeneric DNA hybridizations conducted. For example, although strains of *Asticcacaulis excentricus* were 33 and 86% homologous with the type strain, only low levels of homology (<3%) were detected with all other strains of *Asticcacaulis* as well as strains of *Caulobacter* and *Prostheco bacter*. Such low ho-

mologies were found even between strains now known to share susceptibility to certain phages (9). *A. biprosthecum* was not closely related to any of the strains with which it was compared. This is in agreement with bacteriophage host range studies and with unpublished results of DNA homology studies of five *Asticcacaulis* strains by John Johnson and Jack Pate (personal communication). Similarly, *P. fusiformis* FC 4 exhibited little, if any, homology either to the motile caulobacters or to the other nonmotile strains in this species.

It is of interest to compare the results of taxonomic studies of the caulobacter family with those of the more thoroughly studied family of enteric bacteria. Adansonian analysis of the *Enterobacteriaceae* indicates that 75% *S* or higher comprises a species cluster using 100 or more characteristics (4). The genus level relationship is circumscribed by percent *S* of between 70 and 75. Although a slightly smaller number of characteristics, 78, has been used in the caulobacter group, the similarity values were markedly lower for this group, running generally greater than only 60% for the species level and less than 60% for the genus. With one exception, namely, *C. crescentus* and *C. vibrioides*, the phenotypic criteria for delineating these taxa are consistent with the DNA homology data, indicating that there is greater genetic and phenotypic diversity in a species of the genus *Caulobacter* and in the genus *Caulobacter* than in a species and genus in the family of enteric bacteria. This observation is in agreement with the comment of S. T. Cowan (7): "... the ranking within the family [*Enterobacteriaceae*] has come out of register with that of other areas of bacterial classification, so that taxa which in other families are ranked as species, in this family occur as genera or in some cases as tribes." Therefore, although it would a priori seem reasonable that genetic analysis of relatedness might provide a common basis for all bacteria in circumscribing taxa at the species and genus levels, there are other pragmatic considerations that will determine the practicality of using this approach. The designation of a given level of DNA homology as determinative of a particular taxon will continue to be qualified by considerations of phenotypic similarity, and so can be expected to vary from one bacterial group to another.

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REPRINT REQUESTS

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