Deoxyribonucleic Acid Base Compositions and Nucleotide Distributions of 65 Strains of Budding Bacteria

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A total of 65 strains of appendaged or prosthecate, budding bacteria from our culture collection were selected for a study of deoxyribonucleic acid (DNA) base composition and nucleotide distribution. These strains represented 11 genera, including 4 genera of hyphal, budding bacteria which have not been formally described yet. The DNA species were thermally denatured, and absorbance-temperature profiles were recorded. The midpoints, widths, and asymmetries of the melting transitions were determined. When the DNA base compositions and nucleotide distributions were plotted on a dissimilarity map, it became evident that the strains of each genus occupied a distinct area. The distribution of strains within such an area indicated the degree of heterogeneity of a genus. When 16 *Hyphomicrobium* strains were analyzed, they formed five clusters within their generic area. These clusters correlated well with groups which had been previously established by DNA base composition analyses, by DNA-DNA homology studies, and by numerical taxonomy. Nine of the strains investigated were distinguished by melting profiles which were skewed uniquely to the left.

The appendaged or prosthecate, budding bacteria are a diverse group of procaryotes. These organisms may be regarded as part of the even more diverse collection of budding bacteria reviewed by Hirsch (15).

Deoxyribonucleic acid (DNA) base compositions have been reported for 8 strains of *Rhodomicrobium vannielii* (27), for 66 *Hyphomicrobium* and 2 *Hyphomonas polymorpha* strains (25), for 15 *Prosthecomicrobium* and 2 *Ancalomicrobium* strains (46), and for 7 strains of *Pedomicrobium* spp. (10). The distribution of DNA nucleotides has been determined for seven *pedomicrobia* (10). Genome sizes are known for only two strains, *Hyphomicrobium* sp. strain B-522 (M_r , 3.1×10^9) (34) and *Rhodomicrobium vannielii* RM5 (M_r , 2.1×10^9) (40). DNA-DNA base sequence homologies have been reported for a number of *Hyphomicrobium* strains and other budding bacteria (33), for *Prosthecomicrobium*, *Ancalomicrobium*, and *Hyphomicrobium* (35), and for *Pedomicrobium* species (11). Ribosomal ribonucleic acid-DNA hybridizations have been performed only between *Hyphomicrobium* sp. strain B-522 and various other bacteria (32).

To complete the taxonomic scheme based on analyses of the midpoints of the melting profiles (T_m), we selected 65 representative strains from the culture collection of the Institut für Allgemeine Mikrobiologie, Kiel, Federal Republic of Germany, to study DNA base compositions and nucleotide distributions. The latter, expressed as the widths (left plus right standard deviations of compositional nucleotide distribution, $\sigma_l + \sigma_r$ and asymmetries (σ_l/σ_r) of the guanine-plus-cytosine (G+C) frequency curves (6), describe an additional physicochemical quality of the bacterial genome. The DNA nucleotide distributions of some 2,500 bacterial strains were found to be "quite similar within each genus," but varied considerably between genera (6), thus providing us with routinely determined properties of the bacterial genomes which characterize genera, species, and strains.

MATERIALS AND METHODS

Bacterial strains. The strain designations and the sources of isolation of the bacteria used are listed in Table 1.

Cultivation. Most *Hyphomicrobium* strains, as well as strains SW-808 and T-854, were grown in medium 337+1/2 (18); for strain B-522 this medium was supplemented with 2.5 μ g of cyanocobalamin per liter (29). *Hyphomicrobium*-like strains SW-814 and SW-815 were grown in PYGV (44), which was supplemented with artificial seawater (ASW) (24). Strains SX-821 and PC-1356 and most of the *Hyphomonas*-like strains, as well as *Prosthecomicrobium enhydrium* 1187^T (T = type strain) and *Prosthecomicrobium* sp. strain SCH-1316, *Pirella* sp. strain SCH-1313, and *Plantomyces* sp. strain SCH-1317, were grown in medium 387+1/4 ASW, which contained (per liter) 1 g of yeast extract (Difco Laboratories, Detroit, Mich.), 1 g of glucose, 20 ml of Hutner basal salts (3), 250 ml of ASW, and 50 ml of 0.1 M tris(hydroxymethyl)aminomethane(Tris)hydrochloride (pH 7.5); the final pH was 7.2. Strains SCH-1415, SCH-1325, and 1188^T, all strains of "*Stella*," most *Pirella* strains, *Plantomyces maris* 1190^T, and strain SCH-1448 were grown in PYGV supplemented with 250 ml of ASW per liter and 50 ml of Tris per liter; the final pH was 7.2. *Hyphomonas polymorpha* strains PS-728^T and PR-727 and *H. neptunium* LE-670^T were cultivated in medium 383, which contained (per liter of double-distilled water) 1 g of yeast extract, 1 g of glucose, 2 g of Casitone (Difco), and 1 g of $MgCl_2$; the final pH was 8.0. Strains 868, 869, G-1381, ST-1307, and 1008 were grown in PYGV (44). *Pedomicrobium*-like strains ST-1306 and WD-1355 were grown in PSM (9). Genus T sp. strains 1128 and 1300 were grown in medium Y (8). Medium 400, which was used for genus D sp. strains, contained (per liter) 1 g of peptone (Difco), 1 g of yeast extract, 1 g of glucose, 10 ml of a vitamin solution (44), 20 ml of Hutner basal salts, and 970 ml of ASW (2.5 $\times$ concentrated); the final pH was 7.5. Strain SCH-1315 of genus F was grown in medium AC, which contained (per liter) 10 ml of vitamin solution, 20 ml of Hutner basal salts, 250 ml of ASW, 1 g of sodium acetate, 1 g of KNO_3 , and 42 mg of NaH_2PO_4 ; the final pH was 6.9. *Prosthecomicrobium* sp. strain SCH-1314

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TABLE 1. Sources of isolation and cell disintegration methods for bacterial strains

Strain ^a	Source of isolation	Cell disintegration method ^b
<i>Hyphomicrobium</i>		
NQ-521gr (= ATCC 27483)	Brackish water (14, 30) ^c	Cell mill A, 10
B-522 (= ATCC 27484)	Soil (17)	Cell mill A, 10
H-526 (= ATCC 27485)	Soil (17)	Enzyme A
MEV-533gr (= ATCC 27488)	Brackish water (14, 30)	Cell mill A, 10
F-550	Soil (25)	Enzyme A
I-551 (= ATCC 27489)	Soil (25)	Cell mill A, 10
CO-558 (= ATCC 27491)	Soil (14)	Cell mill A, 10
CO-559	Soil (25)	Enzyme A
WH-563	Brackish water (20)	Enzyme D
ZV-580	Swamp soil ^d	Cell mill A, 10
CO-582 (= ATCC 27492)	Soil (25)	Enzyme A
EA-617	Brackish water (20, 30)	Enzyme A
MC-651 (= ATCC 27497)	Soil (25)	Cell mill A, 5
KB-677 (= ATCC 27498)	Sewage (22)	Cell mill A, 10
MC-750 (= ATCC 27500)	Construction soil (25)	Cell mill A, 10
Wi-926 (= W54)	Freshwater pond ^e	Enzyme D
<i>Hyphomicrobium</i> -like		
SW-808	Seawater (14)	Cell mill A, 10
SW-814	Seawater (14)	Enzyme A
SW-815	Seawater (14)	Enzyme A
SX-821	Seawater (14)	Enzyme E
T-854 (= T-37)	Freshwater Mn deposits (48, 49)	Cell mill A, 10
PC-1356 (= PC-5)	Freshwater reservoir (47)	Enzyme A
SCH-1415 (= Schl-37)	Brackish water ^f	Enzyme B
<i>Hyphomonas</i> ^g		
PR-727 (= ATCC 33880)	Purulent nasal mucus (39)	Enzyme A
PS-728 ^T (= ATCC 33881 ^T)	Purulent nasal mucus (39)	Enzyme A
LE-670 ^T (= 14-15 ^T = ATCC 15444 ^T)	Seawater (23)	Enzyme B
<i>Hyphomonas</i> -like		
SCH-1325 (= Schl-89)	Brackish water (19)	Enzyme A
H-1354 (= H-13)	Brackish water (51)	Enzyme E
VP-1382 (= VP-1)	Deep sea thermal vents (21)	Enzyme E
VP-1383 (= VP-2)	Deep sea thermal vents (21)	Enzyme E
VP-1384 (= VP-3)	Deep sea thermal vents (21)	Enzyme E
VP-1385 (= VP-4)	Deep sea thermal vents (21)	Enzyme E
VP-1386 (= VP-5)	Deep sea thermal vents (21)	Enzyme E
SCH-1416 (= Schl-92)	Brackish water ^f	Enzyme E
SCH-1417 (= Schl-135)	Brackish water ^f	Enzyme E
<i>Pedomicrobium</i> -like		
868 (= Hy-1)	Temporary freshwater pond ^h	Enzyme A
869 (= Hy-2)	Temporary freshwater pond ^h	Enzyme A
ST-1306	Freshwater reservoir (47)	Enzyme A
WD-1355 (= WD-4)	Freshwater reservoir (47)	Cell mill A, 10
G-1381 (= SSED-4)	Freshwater pond ⁱ	Cell mill A, 10
<i>Rhodomicrobium</i> sp. strain P-1093 (= P-1)	Acid forest pond (8)	Enzyme A
Genus T		
1128 (= F-1)	Quartzite rock pool (8)	Enzyme A
1300 (= F-2)	Freshwater pond ^j	Enzyme A
ST-1307	Freshwater reservoir (47)	Enzyme D
Genus D		
954 (= 41/7)	Hypersaline, hyperthermal lake (16, 19)	Cell mill B, 5
958 (= 41/2)	Hypersaline, hyperthermal lake (16, 19)	Cell mill B, 5
1185	Hypersaline, hyperthermal lake ^k	Cell mill B, 5
Genus F sp. strain SCH-1315 (= Schl-128)	Brackish water (19)	Enzyme A
<i>Prosthecomicrobium</i>		
1187 ^T (= 9b ^T = ATCC 23634 ^T)	Freshwater creek (44)	Detergent A
1188 ^T (= 3a ^T = ATCC 23633 ^T)	Freshwater creek (44)	Enzyme A
SCH-1314 (= Schl-127)	Brackish water (19)	Detergent A
SCH-1316 (= Schl-129)	Brackish water (19)	Detergent A
"Stella"		
1203 (= VKM 1137)	Cultivated black soil (50)	Enzyme C
SCH-1312 (= Schl-41)	Brackish water (19)	Detergent A
SCH-1320 (= Schl-141)	Sewage-polluted freshwater creek ^f	Detergent A

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TABLE 1—Continued

Strain ^a	Source of isolation	Cell disintegration method ^b
<i>Pirella</i> ^d		
1189 ^T (= ATCC 27377 ^T)	Freshwater lake (45)	Enzyme A
SCH-1310 (= Schl-1)	Brackish water ^f	Detergent A
SCH-1313 (= Schl-106)	Brackish water ^f	Detergent A
SCH-1318 (= Schl-139)	Brackish water ^f	Detergent A
SCH-1319 (= Schl-140)	Brackish water ^f	Detergent A
SCH-1358 (= Schl-143)	Brackish water ^f	Cell mill A, 5
SCH-1441 (= Schl-181)	Hypersaline lake ^f	Cell mill A, 5
<i>Planctomyces</i>		
1008 (= Mü-290)	Freshwater (19)	Detergent B37
1190 ^T (= 534-30 ^T = ATCC 29201 ^T)	Seawater (2)	Detergent B60
SCH-1317 (= Schl-130)	Brackish water (19)	Detergent B60
SCH-1448 (= Schl-180)	Hypersaline lake ^f	Detergent B37

^a The strain designations given are the collection numbers of the Institut für Allgemeine Mikrobiologie, Kiel, Federal Republic of Germany. Designations in parentheses are designations used in previous publications. Genus T, genus D, and genus F have not been validly described and named yet. ATCC, American Type Culture Collection, Rockville, Md.; DSM, Deutsche Sammlung von Mikroorganismen, Göttingen, Federal Republic of Germany; VKM, All-Union Collection of Microorganisms, Moscow, USSR.

^b Details of the cell disintegration methods used are given in the text.

^c The number in parentheses are reference numbers.

^d Isolated by G.A. Zavarzin, Moscow, USSR.

^e Isolated by L. Wiczorek (Ph.D. thesis Universität Kiel, Kiel, Federal Republic of Germany, 1979).

^f Isolated by H. Schlesner, Kiel, Federal Republic of Germany.

^g See reference 36.

^h Isolated by J.A. Babinchak, Ann Arbor, Mich.

ⁱ Isolated by E. Dale and W. C. Ghiorse, Ithaca, N. Y.

^j Isolated by F.E.W. Eckhardt, Kiel, Federal Republic of Germany.

^k Isolated by P. Hirsch and B. Hoffmann, Kiel, Federal Republic of Germany.

^l See reference 42.

and *Pirella* sp. strain SCH-1319 were cultivated in PYGA, which contained (per liter) 0.15 g of peptone, 0.15 g of yeast extract, 1 g of glucose, 10 ml of vitamin solution, 20 ml of Hutner basal salts, 250 ml of ASW, 50 ml of Tris, and 0.25 g of (NH₄)₂SO₄; the final pH was 7.2. *Rhodomicrobium* sp. strain P-1093 was grown for 12 days anaerobically at 35°C in a mineral salts medium (8, 37, 38) illuminated by 450 foot-candles (4,844 lx) (8). Strains 1128 and 1300 were grown in batch cultures until sedimentation of the cells occurred. The incubation temperature used for strains 954, 958, and 1185 was 43°C; the incubation temperature used for strains SCH-1318 and SCH-1319 was 20°C. All other strains were grown at 30°C in the dark in aerated, stirred batch cultures.

Harvesting and storage. At the end of the exponential growth phase, all cultures were checked for purity by direct microscopic examination and by plating onto solidified growth media and nutrient agar (Difco). Cells were harvested by centrifugation at 16,000 × *g* for 20 min and washed twice with double-distilled water or 0.85% (wt/vol) NaCl. Cell pellets were stored at -20°C before use.

DNA preparation. Disintegration of the bacterial cell walls was achieved by various procedures (Table 1). The enzyme A method was a modification of a previously described procedure (10, 11); proteinase K (100 µg/ml; E. Merck AG, Darmstadt,) was used instead of pronase E. The enzyme B, enzyme C, and enzyme D procedures were modifications of the enzyme A method; the cell pellets were suspended not in Tris-MgCl₂-KCl buffer but in 0.05 M Tris (pH 8), 0.05 M Tris-0.05 M ethylenediaminetetraacetate (EDTA)-0.01 M NaCl (pH 8), and saline-EDTA (28), respectively. In the enzyme E method, the cells were suspended in 9.6 ml of Tris-MgCl₂-KCl buffer; then a solution containing 2 mg of sodium dodecyl sulfate per ml and 1 mg of proteinase K were added, and the mixture was incubated at 60 or 37°C for a

maximum of 45 min. Then the sodium dodecyl sulfate concentration was increased to 20 mg/ml for 5 to 15 min at 60 or 37°C.

The detergent A method was adopted from Schwinghamer (43) and consisted of a detergent treatment, osmotic shock, and lysozyme treatment. The suspension buffer of the detergent B procedure was saline-EDTA; cell lysis was achieved by adding 20 mg of sodium dodecyl sulfate per ml within 30 min at 37°C (detergent B37 method) or 60°C (detergent B60 method).

For the cell mill A disintegration procedure, 1 to 2 g (wet weight) of bacterial pellets was suspended in 20 ml of saline-EDTA supplemented with 1 mg of proteinase K, and 50 g of glass beads (diameter, 0.1 mm) was added (31). The mixture was precooled on ice and then shaken in an MSK cell homogenizer (Braun, Melsungen, Federal Republic of Germany) for 5 or 10 s (Table 1). Cell lysis was completed by adding 20 mg of sodium dodecyl sulfate per ml to the suspension.

DNA was isolated from cells disrupted by an enzyme, detergent, or cell mill A treatment by a modification of the Marmur procedure (28), as follows: after NaClO₄ and chloroform-i-amylalcohol were added, the suspension of lysed cells was shaken for 30 min (enzyme and detergent treatments) or 15 min (cell mill treatment) at 200 rpm (enzyme and detergent treatments) or 100 rpm (cell mill treatment). Centrifugation at 27,000 × *g* (enzyme and detergent treatments) or 1,350 × *g* (cell mill treatment) for 20 min separated the emulsion into layers, from which the nucleic acids were precipitated and further purified by the method of Marmur. However, the length of the ribonuclease treatment was extended to 45 min, and this treatment was followed by proteinase K (200 µg/ml) treatment for 2 h at 37°C. Subsequently, 1 volume of phenol saturated with 1× SSC (0.15 M

NaCl plus 0.015 M trisodium citrate, pH 7) and 0.1 volume of chloroform-i-amylalcohol were added to the DNA solution, and the preparation was shaken for 10 min at 100 rpm. Centrifugation at $27,000 \times g$ for 20 min separated the emulsion into three layers. From the upper layer the DNA threads were precipitated with ethanol. The precipitate was drained by mild pressure and washed three times in $0.1 \times$ SSC containing ethanol (1:1) and once for 15 min in diethylether. The DNA was drained and kept in the air until no ether vapor was detectable. The precipitate was dissolved in $0.1 \times$ SSC, adjusted to $1 \times$ SSC, and finally deproteinized by adding chloroform-i-amylalcohol. The i-propanol step was omitted. The final DNA precipitate was dissolved in sterile $0.1 \times$ SSC to a concentration between 0.5 and 1.5 mg/ml. Such solutions were stored at -20°C in screw-capped tubes with 1 drop of chloroform in each cap. The concentrations and purities of DNA solutions were determined by spectrophotometry, using the following relationship: $1.0 \text{ U of optical density at } 260 \text{ nm} = 50 \mu\text{g of DNA per ml (5)}$.

DNA extraction from genus D strains required a different treatment. Lysis by the cell mill B procedure was achieved by suspending 1 g (wet weight) of bacterial cells in 20 ml of 1 M NaCl-0.1 M EDTA containing 1 mg of proteinase K and 1% (wt/vol) *N*-cetyl-*N,N,N*-trimethylammonium bromide (Merck), as well as 50 g of glass beads. The mixture was treated for 5 s in the cell homogenizer; this was followed by the addition of 1 volume of chloroform-i-amylalcohol and shaking at 100 rpm for 15 min. The resulting emulsion was centrifuged at $1,350 \times g$ for 20 min; the upper aqueous phase was removed and mixed with 1 volume of double-distilled water. Then 0.6 volume of i-propanol was added dropwise while the solution was stirred with a glass rod. Centrifugation at $1,350 \times g$ for 10 min yielded a nucleic acid pellet which was further purified as described above.

DNA analyses. At least six thermal denaturation profiles of each DNA species investigated were recorded at 260 nm with a Gilford model 250 spectrophotometer, as described previously (10). From the T_m value of these sigmoid curves the molar fraction of the DNA bases was calculated by using the following equation (26): $G+C \text{ content} = (T_m \text{ in } 0.1 \times \text{SSC}/50.2) - 0.990$. The melting curves were also used to determine the nucleotide distributions of the DNAs by the method of De Ley (6).

RESULTS

A large number of appendaged or prosthecate, budding bacteria have been isolated from various habitats all over the world and maintained at the Institut für Allgemeine Mikrobiologie collection. From this collection 65 representative strains were selected (Table 1), including all of the *Hyphomonas*, *Pedomicrobium*-like, genus T, genus F, and "Stella" strains.

Disintegration of the cell walls was particularly difficult with strains of *Hyphomicrobium*, *Pedomicrobium*, *Rhodomicrobium*, and genus D. In cases where detergent and enzyme treatments failed, the cells had to be disintegrated mechanically by using glass beads and the minimum time necessary for destroying approximately 50% of the cells. In this way, only one-half of the cell material was actually exploited; on the other hand, fragmentation of the genomes could be held to low levels, similar to the levels of DNA released by chemical disintegration procedures. Both methods, carefully used, yielded DNA fragments with M_r of $>3 \times 10^6$, which allowed us to record thermal denaturation profiles that were undisturbed by the fragment sizes of the molecules (4).

The purification of extracted DNAs by alternate chloroform and phenol treatments resulted in DNA preparations having ratios of absorbance at 260 nm to absorbance at 230 nm to absorbance at 280 nm below 1:0.490:0.520. DNA preparations from strains CO-582, SW-815, and VP-1384 gave absorbance ratios below 1:0.500:0.535.

The DNA base compositions and nucleotide distributions of the budding bacteria investigated are shown in Table 2. All strains but one had base compositions between 50 and 70 mol% G+C; strain SCH-1415 had a G+C content of 46.3 mol%. Within the genera themselves, the ratio varied within more or less narrow limits (e.g., *Hyphomicrobium* strains, 59.3 to 64.7 mol%); *Hyphomonas* and *Hyphomonas*-like bacteria, 57.5 to 60.4 mol%; *Pedomicrobium* [10] and *Pedomicrobium*-like strains, 62.8 to 65.7 mol%. The distributions of the DNA nucleotides of the budding bacteria varied considerably with respect to transition width (6.15 to 13.42 mol% G+C) and asymmetry (0.90 to 1.43). However, some genera had only narrow ranges of variation. For example, for *Hyphomonas* and similar strains the transition ranged from width 9.23 to 10.54 mol% G+C and the skewness ranged from 0.96 to 1.21, for genus T strains the transition width ranged from 7.69 to 8.53 mol% G+C and the skewness ranged from 0.96 to 0.99, and for "Stella" strains the transition width ranged from 8.42 to 8.85 mol% G+C and the skewness ranged from 0.98 to 1.15. Other genera were more heterogeneous in this respect.

DISCUSSION

The DNA base compositions of 21 strains were determined previously by CsCl buoyant density centrifugation (2, 25, 46). The buoyant densities (ρ) taken from the literature and the T_m values of the strains determined during this study could be correlated by regression analysis with a model 67 Hewlett-Packard calculator, yielding the following linear relationship: $T_m \text{ in } 0.1 \times \text{SSC} = 412.1(\rho - 1.526)$. Previous calculations, in which T_m and ρ values of various DNA species were also used, resulted in the following equations: $T_m \text{ in } 0.1 \times \text{SSC} = 428.5(\rho - 1.533)$ (10); $T_m \text{ in } 0.1 \times \text{SSC} = 489.4(\rho - 1.556)$ (26); and $T_m \text{ in } 1 \times \text{SSC} = 429.76(\rho - 1.5002)$ (7). Although these equations were based on data from a large number of DNA species which had been investigated in different laboratories, the relationships were similar. Therefore, we assume that a linear relationship between ρ and T_m values which is valid for all bacterial DNA species not containing unusual bases should be quite similar to the equations given above.

Since DNA nucleotide distributions and base compositions were determined by measurements which integrated the reactions of the 5×10^6 base pairs of an average bacterial chromosome, these characteristics must be rather conservative properties of bacteria. For example, total rearrangement (i.e., 100% transition from guanine · cytosine to adenine · thymine) of an average cistron (1,500 base pairs) would alter the base composition of the whole genome by only 0.03 mol% G+C. Additionally, many changes in the DNA sequence during divergent evolution cannot be discovered by T_m analyses, because at least some of the transitions from guanine · cytosine to adenine · thymine are most likely neutralized by transitions from adenine · thymine to guanine · cytosine (or vice versa) which take place at different sites on the chromosome. However, changes in the DNA nucleotide distribution depend on the sites where base pair transitions occur, thus providing taxonomists with additional genotypic characters which change independently of compositional alterations. In our hands, width and skewness

TABLE 2. DNA base compositions and nucleotide distributions of budding bacteria^a

Taxon	IFAM strain no. ^b	T _m in 0.1× SSC (°C)	Base composition (mol% G+C) ^c	Nucleotide distribution ^d		
				Width (σ ₁ + σ _r ; mol% G+C)	Skewness (σ _r /σ _r)	
<i>Hyphomicrobium</i>	Wi-926	79.46 ± 0.27	59.29 ± 0.54	8.41 ± 0.53	1.26 ± 0.15	
	B-522	79.49 ± 0.09 ^e	59.34 ± 0.19 ^e	8.84 ± 0.10	1.21 ± 0.07	
	I-551	79.52 ± 0.17	59.40 ± 0.33	8.97 ± 0.47	1.11 ± 0.04	
	H-526	79.58 ± 0.17	59.53 ± 0.33	8.87 ± 0.17	1.10 ± 0.06	
	CO-558	79.71 ± 0.15	59.78 ± 0.30	8.75 ± 0.28	1.09 ± 0.04	
	F-550	79.77 ± 0.17	59.91 ± 0.33	9.17 ± 0.19	1.07 ± 0.11	
	CO-559	79.83 ± 0.23	60.02 ± 0.46	9.69 ± 0.25	1.05 ± 0.03	
	CO-582	80.09 ± 0.28	60.54 ± 0.57	9.46 ± 0.21	1.03 ± 0.04	
	MC-750	80.51 ± 0.17	61.38 ± 0.34	8.67 ± 0.08	1.09 ± 0.05	
	ZV-580	80.71 ± 0.19	61.77 ± 0.37	8.82 ± 0.26	1.20 ± 0.02	
	KB-677	81.03 ± 0.20	62.41 ± 0.40	9.80 ± 0.14	1.15 ± 0.04	
	MC-651	81.28 ± 0.10	62.91 ± 0.20	9.85 ± 0.39	1.21 ± 0.05	
	WH-563	81.37 ± 0.32	63.09 ± 0.64	10.53 ± 0.24	1.25 ± 0.10	
	EA-617	81.56 ± 0.39	63.46 ± 0.77	10.76 ± 0.23	1.18 ± 0.06	
	NQ-521gr	81.88 ± 0.11	64.11 ± 0.22	10.63 ± 0.15	1.24 ± 0.04	
<i>Hyphomicrobium</i> -like	MEV-533gr	82.17 ± 0.13	64.69 ± 0.26	10.42 ± 0.29	1.21 ± 0.12	
	SCH-1415	72.96 ± 0.66	46.34 ± 1.32	9.16 ± 0.12	1.11 ± 0.05	
	SW-808	77.38 ± 0.11	55.15 ± 0.21	9.72 ± 0.35	1.40 ± 0.06	
	T-854	78.75 ± 0.04	57.88 ± 0.15	9.02 ± 0.15	1.15 ± 0.04	
	SW-814	79.37 ± 0.24	59.11 ± 0.48	10.11 ± 0.21	1.13 ± 0.06	
	SX-821	79.65 ± 0.35	59.66 ± 0.70	9.56 ± 0.10	1.12 ± 0.05	
	SW-815	79.93 ± 0.13	60.23 ± 0.26	9.12 ± 0.13	1.06 ± 0.06	
	PC-1356	82.18 ± 0.13	64.71 ± 0.26	8.41 ± 0.35	1.06 ± 0.07	
	<i>Hyphomonas</i>	PR-727	79.84 ± 0.23 ^e	60.05 ± 0.47 ^e	10.13 ± 0.50	1.15 ± 0.07
		PS-728 ^T	79.85 ± 0.09 ^e	60.07 ± 0.17 ^e	10.31 ± 0.41	1.03 ± 0.07
LE-670 ^T		80.02 ± 0.40 ^e	60.40 ± 0.80 ^e	9.53 ± 0.12	1.08 ± 0.05	
<i>Hyphomonas</i> -like	VP-1386	78.56 ± 0.30 ^e	57.49 ± 0.60 ^e	9.43 ± 0.17	0.96 ± 0.04	
	SCH-1416	78.84 ± 0.11	58.06 ± 0.22	9.98 ± 0.13	1.10 ± 0.07	
	SCH-1325	79.31 ± 0.24 ^e	58.98 ± 0.47 ^e	10.54 ± 0.27	1.21 ± 0.07	
	H-1354	79.53 ± 0.21 ^e	59.42 ± 0.42 ^e	9.88 ± 0.35	1.11 ± 0.04	
	VP-1384	79.74 ± 0.10 ^e	59.84 ± 0.20 ^e	9.76 ± 0.26	1.10 ± 0.08	
	VP-1385	79.83 ± 0.09 ^e	60.02 ± 0.18 ^e	9.56 ± 0.42	1.07 ± 0.04	
	SCH-1417	79.84 ± 0.12	60.05 ± 0.23	9.79 ± 0.33	1.08 ± 0.05	
	VP-1382	79.91 ± 0.22 ^e	60.18 ± 0.44 ^e	9.57 ± 0.61	1.08 ± 0.05	
	VP-1383	79.92 ± 0.15 ^e	60.20 ± 0.30 ^e	9.23 ± 0.42	1.09 ± 0.05	
	<i>Pedomicrobium</i>	S-1290 ^{Tf}	82.46 ± 0.19	65.27 ± 0.38	9.20 ± 0.30	1.21 ± 0.08
<i>Pedomicrobium</i> -like	WD-1355	81.23 ± 0.34	62.80 ± 0.68	9.63 ± 0.20	1.29 ± 0.08	
	G-1381	81.80 ± 0.12	63.97 ± 0.24	8.54 ± 0.25	1.09 ± 0.07	
	869	82.03 ± 0.16	64.41 ± 0.33	7.96 ± 0.22	1.02 ± 0.09	
	868	82.19 ± 0.13	64.72 ± 0.25	8.08 ± 0.24	1.09 ± 0.12	
	ST-1306	82.33 ± 0.19	65.00 ± 0.38	8.86 ± 0.13	1.16 ± 0.04	
	<i>Rhodomicrobium</i>	P-1093	80.00 ± 0.35	60.36 ± 0.71	13.42 ± 0.39	1.43 ± 0.05
	Genus T	1128	80.62 ± 0.06	61.60 ± 0.11	7.69 ± 0.19	0.99 ± 0.02
		ST-1307	80.77 ± 0.11 ^e	61.91 ± 0.22 ^e	8.28 ± 0.19	0.96 ± 0.04
	Genus D	1300	81.23 ± 0.22	62.82 ± 0.43	8.53 ± 0.26	0.96 ± 0.02
		1185	81.18 ± 0.10	62.71 ± 0.20	8.85 ± 0.14	0.98 ± 0.02
958		81.26 ± 0.14	62.86 ± 0.28	8.58 ± 0.40	1.00 ± 0.11	
	954	81.61 ± 0.27	63.58 ± 0.54	8.56 ± 0.14	1.10 ± 0.02	
	Genus F	SCH-1315	80.58 ± 0.27	61.15 ± 0.54	8.24 ± 0.30	1.10 ± 0.07
<i>Prosthecomicrobium</i>	SCH-1314	81.92 ± 0.06	64.18 ± 0.13	9.62 ± 0.07	1.24 ± 0.05	
	SCH-1316	82.20 ± 0.28	64.75 ± 0.56	8.84 ± 0.23	0.90 ± 0.04	
	1187 ^T	82.28 ± 0.30	64.90 ± 0.60	8.78 ± 0.41	0.93 ± 0.04	
	1188 ^{Tf}	83.84 ± 0.14	68.01 ± 0.27	10.09 ± 0.24	1.00 ± 0.07	
<i>Stella</i>	SCH-1320	83.55 ± 0.26	67.43 ± 0.52	8.85 ± 0.41	0.98 ± 0.12	
	1203	83.96 ± 0.22	68.25 ± 0.44	8.51 ± 0.21	1.15 ± 0.09	
	SCH-1312	84.19 ± 0.12	68.71 ± 0.25	8.42 ± 0.18	1.05 ± 0.03	
<i>Pirella</i>	SCH-1441	76.69 ± 0.15	53.77 ± 0.30	9.29 ± 0.33	1.02 ± 0.07	
	1189 ^T	78.02 ± 0.20	56.42 ± 0.40	8.23 ± 0.82	1.36 ± 0.11	
	SCH-1310	78.04 ± 0.11	56.45 ± 0.22	6.15 ± 0.08	1.04 ± 0.05	
	SCH-1318	78.19 ± 0.07	56.75 ± 0.14	9.73 ± 0.12	1.02 ± 0.03	
	SCH-1358	78.19 ± 0.42	56.76 ± 0.83	7.81 ± 0.16	0.98 ± 0.09	
	SCH-1313	78.56 ± 0.13	57.49 ± 0.26	9.12 ± 0.11	1.28 ± 0.06	
	<i>Pirella</i> -like	SCH-1319	78.64 ± 0.18	57.65 ± 0.36	7.74 ± 0.23	1.06 ± 0.02
<i>Planctomyces</i>	1190 ^T	75.21 ± 0.16	50.81 ± 0.32	10.93 ± 0.06	1.11 ± 0.04	

Continued on following page

TABLE 2—Continued

Taxon	IFAM strain no. ^b	T_m in 0.1× SSC (°C)	Base composition (mol% G+C) ^c	Nucleotide distribution ^d	
				Width ($\sigma_1 + \sigma_r$; mol% G+C)	Skewness (σ_1/σ_r)
<i>Escherichia</i>	SCH-1317	76.26 ± 0.10	52.90 ± 0.21	11.16 ± 0.19	1.35 ± 0.05
	1008	76.43 ± 0.30	53.24 ± 0.59	8.11 ± 0.57	1.01 ± 0.06
	SCH-1448	77.85 ± 0.16	56.08 ± 0.33	8.00 ± 0.50	0.94 ± 0.08
	1308 ^f	76.04 ± 0.10	52.47 ± 0.30	12.71 ± 0.24	1.57 ± 0.09
	<i>Micrococcus</i> ATCC 381 ^f	84.41 ± 0.01	69.15 ± 0.02	9.60 ± 0.11	1.03 ± 0.04

^a The data are means ± standard errors of at least five determinations.^b IFAM, Institut für Allgemeine Mikrobiologie, Kiel, Federal Republic of Germany.^c Base compositions were calculated by using the following equation: G+C content = (T_m in 0.1× SSC/50.2) - 0.990 (26).^d Nucleotide distributions were calculated by the method of De Ley (6).^e Data taken from reference 11a.^f Data taken in part from reference 10.

of DNA melting profiles are valuable tools for the characterization and identification of morphologically similar bacteria with limited biochemical capabilities (e.g., hyphal, budding bacteria).

Phylogenetic distances could not be derived from the melting profiles, since convergent evolution of the physicochemical DNA characters had obviously occurred (Table 3). However, intragenetic heterogeneity of strains and species and differences between closely related genera could be evaluated easily by plotting DNA base compositions against the widths of the melting transitions (Fig. 1). Strains of the same genus occupied distinct areas on our dissimilarity map. In some cases the generic areas overlapped, which did not necessarily indicate a close relationship between the genera, as in the case of *Pedomicrobium* and *Prosthecomicrobium*. However, overlapping of the *Hyphomicrobium* and *Hyphomonas* areas could indeed reflect a close relationship.

The *Hyphomicrobium* strains formed clusters based on their DNA base compositions and nucleotide distributions (Fig. 1 and Table 4). Cluster I consisted of five isolates from mixed soil samples (strains B-522, I-551, H-526, CO-558, and F-550) and one strain from a freshwater pond (strain Wi-926). The latter strain appeared (Fig. 1) at a small distance from the others, and its DNA melting transition revealed the highest asymmetry of these strains (Table 2). However, these deviations were not significant enough to justify a separate entity.

The soil isolates of cluster I were very similar to each other and to the strains of cluster II. The cluster II strains were also isolated from soil but were isolated in the presence of carbon monoxide (25).

Strains MC-750 (cluster IIIa) and ZV-580 (IIIb) had similar DNA base compositions (T_m values) and melting transition widths. However, the asymmetry of the melting transitions (Table 4) and the ρ values of their DNAs (25) were different. These strains were isolated from different habitats (Table 1) and are clearly distinguished by morphology and growth behavior. Therefore, it seemed likely that strains MC-750 and ZV-580 are only distantly related to each other.

The similarity of the cluster IV strains, strains KB-677 and MC-651, although these strains were isolated from different environments, was demonstrated by a previous investigation (25).

Cluster V, which contained the strains with the highest DNA base ratios (strains WH-563, EA-617, NQ-521gr, and MEV-553gr), was established because of the broad DNA melting transitions of these strains (Table 2). All of the

cluster V strains came from brackish water habitats (25); strains EA-617, NQ-521gr, and MEV-553gr were subcultures of the original strain B of Mevius (30).

The results of DNA-DNA homology studies (33) correlated well with the results presented in this paper and suggested a grouping of hyphomicrobia (Table 4) similar to the clusters discussed above. Even serological relationships of *Hyphomicrobium* strains (41) correlated to some extent with our results. Strains belonging to cluster I (strains I-551, H-526, and CO-558) and cluster II (strain CO-582) were serologically related to each other, whereas strain B-522 was not related to any of these strains. Strains MC-750 and ZV-580 did not show any serological relationship to one another, which confirmed the divergence of cluster III. Strains NQ-521gr and MEV-553gr of cluster V, although they were derived from one original culture, were serologically unrelated to one another or to any other strain.

Previous numerical taxonomy studies (P. Hirsch and R. R. Colwell, unpublished data) on 84 *Hyphomicrobium* strains led to cluster formations similar to those shown in Table 4; however, the results of the previous studies suggested that cluster I should be split into two groups (strains B-522 and H-526 in one group and strains I-551 and CO-558 in the other). Strain CO-582 (cluster II) was typical of a third group. Again, the cluster III strains, strains MC-750 and ZV-580, were different. The strains of cluster IV (strains KB-677 and MC-651), like the strains of cluster V, formed a group when they were studied by numerical taxonomy.

The distinction between *Hyphomicrobium*-like bacteria and *Hyphomonas*-like bacteria is still uncertain. Most of the *Hyphomicrobium* strains described in the literature metabo-

TABLE 3. Convergence of DNA base compositions and nucleotide distributions^a

Examples for:	Genus	DNA base composition (mol% G+C)	Nucleotide distribution	
			$\sigma_1 + \sigma_r$ (mol% G+C)	σ_1/σ_r
Convergent base composition	<i>Corynebacterium</i>	54-55	8.50	1.13
	<i>Erwinia</i>	54-57	10.20	1.33
Convergent nucleotide distribution	<i>Cytophaga</i>	33-40	8.00	1.00
	<i>Nocardia</i>	70	8.00	1.00

^a Data from reference 6.

FIG. 1. Dissimilarity of budding bacteria based on their DNA base compositions and nucleotide distributions. The solid lines are generic border lines; the dashed lines indicate areas where border lines are uncertain. The numbers indicate the positions of strains, as follows: 1, strain Wi-926; 2, strain B-522; 3, strain I-551; 4, strain H-526; 5, strain CO-558; 6, strain F-550; 7, strain CO-559; 8, strain CO-582; 9, strain MC-750; 10, strain ZV-580; 11, strain KB-677; 12, strain MC-651; 13, strain WH-563; 14, strain EA-617; 15, strain NQ-521gr; 16, strain MEV-533gr; 17, strain SCH-1415; 18, strain SW-808; 19, strain T-854; 20, strain SW-814; 21, strain SX-821; 22, strain SW-815; 23, strain PC-1356; 24, strain PR-727; 25, strain PS-728^T; 26, strain VP-1386; 27, strain SCH-1416; 28, strain SCH-1325; 29, strain H-1354; 30, strain VP-1384; 31, strain VP-1385; 32, strain SCH-1417; 33, strain VP-1382; 34, strain VP-1383; 35, strain LE-670^T; 36, strain S-1290^T (10); 37, strain P-1196 (10); 38, strain Q-1197 (10); 39, strain R-1198 (10); 40, strain T-1130 (10); 41, strain F-1225 (10); 42, strain E-1129^T (10); 43, strain WD-1355; 44, strain G-1381; 45, strain 869; 46, strain 868; 47, strain ST-1306; 48, strain P-1093; 49, strain 1128; 50, strain ST-1307; 51, strain 1300; 52, strain 1185; 53, strain 958; 54, strain 954; 55, strain SCH-1315; 56, strain SCH-1314; 57, strain SCH-1316; 58, strain 1187^T; 59, strain 1188^T; 60, strain SCH-1320; 61, strain 1203; 62, strain SCH-1312; 63, strain 1189^T; 64, strain SCH-1441; 65, strain SCH-1310; 66, strain SCH-1318; 67, strain SCH-1358; 68, strain SCH-1313; 69, strain SCH-1319; 70, strain 1190^T; 71, strain SCH-1317; 72, strain 1008; 73, strain SCH-1448; 74, strain 1308; 75, strain ATCC 381.

TABLE 4. Clustering of *Hyphomicrobium* strains according to DNA base composition and nucleotide distribution

Cluster	Strain	Range of DNA base composition (mol% G+C) ^a	Range of DNA melting transition		Clustering according to:	
			Width (mol% G+C) ^a	Skewness ^a	DNA base composition ^b	DNA-DNA homology ^c
I	Wi-926	59.3-59.9	8.4-9.2	1.07-1.26	NT ^d	NT
	B-522				Group I	Group III
	I-551				Group I	NT
	H-526				Group I	Group III
	CO-558				Group I	NT
	F-550				Group I	NT
II	CO-559	60.0-60.6	9.4-9.7	1.03-1.05	Group I	NT
	CO-582				Group I	NT
III ^a	MC-750	61.3	8.6	1.09	Group I	NT
III ^b	ZV-580	61.8	8.9	1.20	Group II	Group II
IV	KB-677	62.4-63.0	9.8-9.9	1.15-1.21	Group II	Group II
	MC-651				Group II	NT
V	WH-563	63.0-64.7	10.4-10.8	1.18-1.25	Group III	Group I
	EA-617				Group III	Group I
	NQ-521gr				Group III	Group I
	MEV-533gr				Group III	Group I

^a Means of at least five determinations (see Table 2); limits of error were not taken into account.^b See reference 25.^c See reference 33.^d NT, Not tested.

lize a limited spectrum of carbon compounds; one-carbon substrates in particular enable good growth, whereas peptides or amino acids generally allow only slow growth (1, 12, 18, 22, 29). On the other hand, utilization of amino acids appears to be characteristic for *Hyphomonas* spp. (13, 39). Some of our strains (strains SW-814, SW-815, and SX-821) grew better on peptone- and yeast extract-containing media than on medium 337+1/2 with methylamine as the sole carbon source. These bacteria were located close to the *Hyphomicrobium-Hyphomonas* overlapping area (Fig. 1). Further investigations must show to which genus these strains belong or whether they represent intermediate types.

The *Hyphomicrobium*-like strains were not incorporated into the *Hyphomicrobium* area (Fig. 1), because of their obvious heterogeneity. Strains SW-808, T-854, PC-1356, and SCH-1415 had similar DNA nucleotide distributions, but their DNA base ratios differed 3.4, 3.5, 6.5, and 15.0 mol% G+C, respectively, from with the mean base composition of 61.35 mol% G+C for 16 *Hyphomicrobium* strains. Furthermore, strain SW-808 DNA revealed a highly asymmetrical melting transition (skewness, 1.40).

Most of the *Hyphomonas*-like strains had very similar DNA base compositions and nucleotide distributions (Table 2), which resulted in a dense cluster close to the *Hyphomicrobium* area (Fig. 1). Strains VP-1386, SCH-1416, and SCH-1325 had lower base ratios, deviating 2.5, 2.0 and 1.0 mol% G+C, respectively, from the average (60.02 mol% G+C) of the seven remaining strains. However, these differences were too small to justify exclusion of these bacteria from the *Hyphomonas* area; it seemed more likely that the three strains represent different species.

Previously investigated *Pedomicrobium* spp. (10) formed a dense cluster with narrow limits (Fig. 1). The *Pedomicrobium*-like strains which we studied deviated more or less from this main cluster. The low DNA base composition of strain WD-1355 caused its positioning close to *Hyphomicrobium* strains KB-677 and MC-651. Since there was no evidence of any phenotypic similarity between strain WD-1355 and these *hyphomicrobia*, a close relationship was doubtful. Since all of the *Pedomicrobium*-like strains exhib-

ited morphological characters and deposition of heavy metal oxides typical of *Pedomicrobium* (9), these strains probably represent new species of this genus.

The remaining genera of hyphal, budding bacteria, *Rhodomicrobium*, genus T, genus D, and genus F, were represented in this study by only one or three strains each. The respective strains of genus T and genus D revealed high levels of similarity in their DNA properties (Table 2 and Fig. 1), which confirmed the preliminary classification of these taxa as new genera.

The *Prosthecomicrobium* DNAs showed considerable heterogeneity (Table 2). *Prosthecomicrobium enhydrium* 1187^T and *Prosthecomicrobium pneumaticum* 1188^T were scattered on the dissimilarity map (Fig. 1), suggesting that they are distantly related. This was in agreement with previous investigations on DNA base compositions (46) and DNA-DNA homologies (35) in which the authors emphasized the diversity of *Prosthecomicrobium* and suggested creation of new species. Since strain SCH-1314 differed from the other *prosthecomicrobia* in all DNA properties (Table 2), it also may represent a new species; detailed taxonomic studies are indicated. Strain SCH-1316, on the other hand, was very similar in all respects to *Prosthecomicrobium enhydrium* 1187^T (Table 2 and Fig. 1).

"*Stella humosa*" 1203 and two additional strains, strains SCH-1320 and SCH-1312, were similar to each other, confirming the preliminary classification of this genus based on morphological characters.

Strains of *Pirella* revealed considerable diversity based on their DNA nucleotide distributions (Table 2). However, their DNA base compositions varied only within 1.3 mol% G+C. The heterogeneity of this group of budding bacteria is presently being investigated.

The four strains collected as *Planctomyces* spp., including *Planctomyces maris* 1190^T, differed widely in many respects (Table 2). Considerable distances between the positions of these strains (Fig. 1) suggested that each of them represents a different *Planctomyces* species or even a separate genus.

It should be emphasized that all strains of genus T, strain 1185 of genus D, and *Hyphomonas* sp. strain VP-1386, as

well as *Prosthecomicrobium* spp. strains SCH-1316 and 1187^T, "*Stella*" sp. strain SCH-1320, and *Planctomyces* sp. strain SCH-1448, were distinguished by melting transition asymmetry ratios below 1.0 (Table 2). All other DNA species investigated in this study had symmetrical melting profiles (σ_1/σ_r , 1.0) or had asymmetry ratios higher than 1.0. According to the investigations of De Ley (6) on 2,500 different strains, the asymmetry ratios of bacterial DNAs range from 1.0 to 1.6, with an average of 1.33. The average ratio of our 65 prosthecate, budding bacterial strains was 1.11; the range of variation was 0.90 to 1.43. Melting profiles skewed to the left (σ_1/σ_r , <1.0) have not been reported previously.

The excellent correlation of the two-dimensional combination of physicochemical properties of bacterial DNAs with DNA-DNA homologies and with numerical taxonomy underlined the suitability of this procedure for bacterial taxonomy. One further advantage of this method was the availability of three different DNA characters (i.e., melting point and width and skewness of melting transition) from only one experiment (i.e., thermally controlled denaturation).

ACKNOWLEDGMENTS

We gratefully acknowledge the donation of strains by F. E. W. Eckhardt, Kiel, Federal Republic of Germany; W. C. Ghiorse, Ithaca, N.Y.; R. L. Moore, Calgary, Alberta, Canada; J. S. Poindexter, New York, N.Y.; P. Roggentin, Kiel, Federal Republic of Germany; and J. T. Staley, Seattle, Wash.

Part of this work was supported by a grant from the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg, Federal Republic of Germany, to P.H.

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