Genome Size Determinations for 33 Strains of Budding Bacteria

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The genome sizes of 33 strains of budding bacteria were determined by deoxyribonucleic acid renaturation kinetics. The M_r values ranged from 1.67×10^9 to 4.43×10^9 for strains representing the following genera: *Hyphomicrobium* (seven strains; M_r , 2.08×10^9 to 2.73×10^9), *Hyphomonas* (six strains; M_r , 1.67×10^9 to 2.13×10^9), *Pedomicrobium* (five strains; M_r , 2.81×10^9 to 3.43×10^9), genus T (two strains; M_r , 2.32×10^9 to 2.46×10^9), genus D (one strain; M_r , 1.73×10^9), genus F (one strain; M_r , 2.03×10^9), *Pirella* (seven strains; M_r , 2.73×10^9 to 4.43×10^9), and *Planctomyces* (four strains; M_r , 2.67×10^9 to 3.65×10^9). The taxa designated genus T, genus D, and genus F represented new budding bacteria which will be formally described elsewhere. The genome size ranges of *Hyphomicrobium* spp., *Hyphomonas* spp., and *Pedomicrobium* spp. were relatively small compared with those of *Planctomyces* spp. and *Pirella* spp. The plotting of genome sizes against deoxyribonucleic acid base compositions demonstrated distinct cluster areas, some of which allowed clear separation of the genera studied.

The budding bacteria are a little known, heterogeneous group of procaryotes with unclear taxonomic relationships. Recently, a large number of new isolates has become available, and there is a need for clear separation of genera and a reliable method of identifying possible new taxa.

Genome size has been described as a characteristic property of bacterial genera or species (9, 14). High precision and easy protocols for deoxyribonucleic acid (DNA) renaturation kinetic experiments (3, 4, 8, 22, 24, 25) have allowed serial determination of the genome sizes of numerous bacteria (9, 21, 23). In the case of hyphal, budding bacteria, genome sizes have been determined previously for only two strains, *Hyphomicrobium* sp. strain B-522 (M_r , 3.1×10^9) (17) and *Rhodomicrobium vannieli* RM5 (M_r , 2.1×10^9) (20). In the present study renaturation experiments were carried out with 33 strains, most of which were classified as belonging to the genera *Hyphomicrobium*, *Hyphomonas*, *Pedomicrobium*, *Planctomyces*, and *Pirella*. Some other strains investigated in this study were morphologically and physiologically unlike any of the aforementioned genera and probably represent new taxa.

The data obtained for the 33 strains showed genome M_r values that ranged from 1.67×10^9 to 4.43×10^9 . There were distinct clusters of morphologically similar organisms, which indicated the potential of using genome size determinations for separation of taxa.

MATERIALS AND METHODS

Strains and cultivation. The bacterial strains which we studied are listed in Table 1. The sources of isolation and the culture conditions used are described in the accompanying paper (7).

Harvesting. Growth was followed by measuring turbidity at 670 nm. Cultures in the stationary growth phase (9, 22) were checked for purity by phase-contrast microscopy and by plating onto solidified growth media and nutrient agar (Difco Laboratories, Detroit, Mich.) supplemented with 10 g of glucose per liter. Cells were pelleted by centrifugation at $16,000 \times g$ for 20 min at 4°C and washed twice in double-distilled water or in a 0.85% NaCl solution. The final pellets were stored at –20°C.

DNA preparation. The methods used for cell wall disinte-

gration and DNA extraction are described in the accompanying paper (7). Cells of strain 1128 were pretreated five times (2 s each) in an Ultra Turrax homogenizer (Jahnke and Kunkel, Staufen, Federal Republic of Germany) and then disrupted mechanically.

All purified DNAs except strain 954 DNA exhibited ratios of absorbance at 260 nm to absorbance at 230 nm to absorbance at 280 nm of 1:0.470:0.520; strain 954 DNA exhibited a ratio of 1:0.490:0.530.

All DNAs were dissolved in standard saline citrate (1× SSC, 0.15 M NaCl plus 0.015 M trisodium citrate [pH 7]) and sheared to an average double-stranded fragment M_r of 420,000 by passing them twice through a French pressure cell (Aminco, Silver Spring, Md.) at 165.5 MPa (6). Subsequently, DNA solutions were dialyzed three times for 10 h each time against 1× SSC and then stored at –20°C.

Renaturation kinetics. DNA renaturation was followed by measuring absorbance changes (3, 9) in hermetically sealed thermocuvettes (Gilford, Düsseldorf, West Germany) attached to a Gilford model 250 spectrophotometer which was equipped with a thermoprogrammer and a reference compensator. When this system was used, corrections for volume expansion or for evaporation of solvents were unnecessary (5). Optimal conditions for DNA renaturation were achieved at sodium concentrations of >0.39 M (9). Since most of the strains had high DNA base ratios, thermal denaturation of the DNAs had to be carried out in the presence of formamide, which reduced the melting temperature sufficiently. Therefore, we used 5× SSC buffer (Na^+ concentration, 0.975 M) which contained 25% (vol/vol) formamide (99% pure; Serva, Heidelberg, Federal Republic of Germany), a system appropriate for both denaturation and renaturation. Under our conditions, formamide lowered the midpoints of the DNA melting profiles in 5× SSC by 0.75°C for each 1 mol% guanine plus cytosine. However, renaturation reactions had to be followed at 270 nm instead of 260 nm (15). At this wavelength DNA absorption was still 80% of the absorption at 260 nm.

Before renaturation, the DNAs were denatured by heating the solutions for 10 min at 10°C above the midpoint of the DNA melting profile in 5× SSC containing 25% formamide. The cuvettes were then cooled (3 to 4 min) to the optimal renaturation temperature (25°C less than the midpoint of the DNA melting profile). This caused a rapid decrease in absorbancy (collapse hypochromicity), which resulted from

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TABLE 1. Genome sizes of appendaged or prosthecate, budding bacteria

Taxon	Strain (IFAM no.) ^a	t _{0.5} (min) ^b	c ₀ t _{0.5} value (mol of nucleotides × s per liter) ^c	Ratio of c ₀ t _{0.5} value of strain to c ₀ t _{0.5} value of <i>E. coli</i>	Genome M _r (×10 ⁹)
<i>Hyphomicrobium</i> spp.	MC-750	61.4 ± 2.1	0.95 ± 0.03	0.79	21.3 ± 0.07
	B-522	67.4 ± 1.7	1.05 ± 0.03	0.87	2.35 ± 0.06
	KB-677	70.0 ± 2.6	1.09 ± 0.04	0.90	2.43 ± 0.09
	NQ-521gr	75.2 ± 2.4	1.17 ± 0.04	0.97	2.62 ± 0.08
	ZV-580	78.8 ± 3.1	1.22 ± 0.05	1.01	2.73 ± 0.11
<i>Hyphomicrobium</i> -like	SW-808	60.0 ± 1.9	0.93 ± 0.03	0.77	2.08 ± 0.07
	T-854	72.5 ± 2.2	1.13 ± 0.03	0.93	2.51 ± 0.08
<i>Hyphomonas polymorpha</i>	PS-728 ^T	61.5 ± 1.7	0.96 ± 0.03	0.79	2.13 ± 0.06
<i>Hyphomonas neptunium</i>	LE-670 ^T	47.9 ± 2.3	0.75 ± 0.04	0.62	1.67 ± 0.08
<i>Hyphomonas</i> -like	VP-1383	50.1 ± 1.0	0.78 ± 0.02	0.65	1.76 ± 0.05
	VP-1382	56.5 ± 2.6	0.88 ± 0.04	0.73	1.97 ± 0.09
<i>Pedomicrobium manganicum</i>	H-1354	57.0 ± 1.3	0.89 ± 0.02	0.74	2.00 ± 0.05
	SCH-1325	57.8 ± 1.6	0.90 ± 0.03	0.74	2.00 ± 0.07
	E-1129 ^T	81.1 ± 2.5	1.26 ± 0.04	1.04	2.81 ± 0.09
	S-1290 ^T	99.3 ± 2.8	1.54 ± 0.04	1.27	3.43 ± 0.10
	ST-1306	85.8 ± 2.8	1.33 ± 0.04	1.10	2.97 ± 0.09
<i>Pedomicrobium</i> -like	G-1381	86.2 ± 3.6	1.34 ± 0.06	1.11	3.00 ± 0.13
	WD-1355	86.3 ± 3.5	1.34 ± 0.05	1.11	3.00 ± 0.11
	ST-1307	67.0 ± 2.6	1.05 ± 0.04	0.86	2.32 ± 0.09
Genus T	1128	71.7 ± 2.1	1.10 ± 0.03	0.91	2.46 ± 0.07
Genus D	954	49.6 ± 1.7	0.77 ± 0.03	0.64	1.73 ± 0.06
Genus F	SCH-1315	57.8 ± 1.1	0.90 ± 0.02	0.75	2.03 ± 0.04
<i>Pirella staley</i>	1189 ^T	78.5 ± 1.8	1.22 ± 0.03	1.01	2.73 ± 0.07
<i>Pirella</i> spp.	SCH-1310	95.6 ± 1.5	1.49 ± 0.02	1.23	3.32 ± 0.05
	SCH-1318	101.6 ± 1.1	1.58 ± 0.02	1.31	3.54 ± 0.05
	SCH-1441	107.5 ± 1.3	1.67 ± 0.02	1.38	3.73 ± 0.05
	SCH-1313	108.1 ± 3.2	1.68 ± 0.05	1.39	3.75 ± 0.11
	SCH-1358	127.9 ± 2.1	1.99 ± 0.03	1.64	4.43 ± 0.07
<i>Pirella</i> -like	SCH-1319	110.9 ± 3.6	1.72 ± 0.06	1.42	3.83 ± 0.13
<i>Planctomyces</i> spp.	1008	77.0 ± 1.6	1.20 ± 0.03	0.99	2.67 ± 0.07
	SCH-1448	80.9 ± 1.1	1.26 ± 0.02	1.04	2.81 ± 0.05
<i>Planctomyces maris</i>	1190 ^T	104.3 ± 4.8	1.62 ± 0.08	1.34	3.62 ± 0.18
<i>Planctomyces</i> sp.	SCH-1317	105.0 ± 2.2	1.63 ± 0.03	1.35	3.65 ± 0.07
<i>E. coli</i>	931	78.1 ± 2.2	1.21 ± 0.03	1.00	2.70 ^d

^a IFAM, Institut für Allgemeine Mikrobiologie.^b Average of at least six determinations.^c The initial DNA concentration was 2.59 × 10⁻⁴ mol of nucleotides per liter.^d Data from reference 11.

nonspecific base pairing within single-stranded DNA fragments (3). This collapse hypochromicity was excluded from the calculation of the 50% DNA renaturation time. Generally, we considered true renaturation to begin 5 min after the onset of cooling.

The DNAs were again denatured after each renaturation. The difference between the melting point of the denaturation mixture before renaturation and that after renaturation indicated the accuracy of the reaction, since 1% mismatched bases generally lowered the midpoint of the DNA melting profile of a renatured DNA by 1°C (1). The time of 50% renaturation (t_{0.5}) was determined from the absorbancy profiles recorded during the reaction; by multiplication with the initial DNA concentration (c₀), the c₀t_{0.5} value was calculated (4). The average molecular weight of a nucleotide was taken to be 309 (19). Bacterial DNAs exhibit an inverse proportionality of molecular complexity and renaturation velocity (4). Therefore, the genome size of a test DNA (x) was calculated by using the following relationship: c₀t_{0.5}(x)/c₀t_{0.5}(*Escherichia coli*) = M_r(x)/M_r(*E. coli*). The c₀t_{0.5} values were not corrected for differences between the DNA base compositions of the test strains and *E. coli* (8, 24). The molecular weight of *E. coli* K-12 reference DNA was taken to be 2.7 × 10⁹ (11).

RESULTS

The genome sizes of 33 strains selected from eight genera of budding bacteria are listed in Table 1. The genome molecular weights of these strains ranged from 1.67 × 10⁹ to 4.43 × 10⁹. Our data were not corrected for non-complementary base pairings, since the level of mismatching was less than 1% in all experiments. The position of each strain within the genome size range of its genus is shown in Fig. 1; strains having similar morphology were included in the same genus. Evidently, some genera were characterized by the sizes of their genomes and could be distinguished from each other because their genome size ranges did not overlap, as in the case of *Hyphomonas* and *Pedomicrobium*. On the other hand, the genome size ranges of *Hyphomonas* and *Hyphomicrobium*, as well as those of *Planctomyces* and *Pirella*, overlapped (Fig. 1), which correlated with the phenotypic similarities of these taxa (12).

DISCUSSION

Only a few genome sizes of budding bacteria have been reported previously. The *Hyphomicrobium* sp. strain B-522 genome was found by Moore and Hirsch (17) to have an M_r of 3.1 × 10⁹. These authors obtained a ratio of strain B-522

FIG. 1. Genome size ranges of budding bacteria. The taxa designated genus D, genus F, and genus T represent new budding bacteria which have not been described thus far.

$c_{0.5}$ value to *E. coli* $c_{0.5}$ value of 0.83; our ratio of 0.87 agrees well with this value. However, our genome size value differs significantly from the value reported by Moore and Hirsch (M_r of 2.35×10^9 compared with M_r of 3.1×10^9) (17). This difference can be explained as follows: (i) Moore and Hirsch assumed that the average molecular weight of a nucleotide pair was 660, whereas we used a value of 618 (19). By multiplying the Cairn value of 4.5×10^6 nucleotide pairs for *E. coli* DNA by 660, Moore and Hirsch based their calculation of strain B-522 genome size on an M_r value of 2.97×10^9 for the *E. coli* chromosome, whereas we used an M_r value of 2.70×10^9 , a value which appeared to be more probable (11). (ii) A larger difference in the data resulted from the $c_{0.5}$ value for *E. coli* DNA used by Moore and Hirsch (17); considering the "10% difference in base composition of the two DNAs," these authors corrected the $c_{0.5}$ value of *E. coli* DNA (2.90) "by a factor of 0.8 as indicated by the data of Wetmur and Davidson (25)." This reduced the *E. coli* $c_{0.5}$ value to 2.32, which resulted in a higher value for the *Hyphomicrobium* sp. strain B-522 genome size. More recently, Wetmur (24) and others (8)

refrained from using correction factors for differences in DNA base composition. Since all discussions concerning this problem did not offer strong arguments for the necessity of such a correction, we avoided it. A new calculation of the data of Moore and Hirsch, in which an M_r value of 2.70×10^9 was used for the *E. coli* genome and no guanine-plus-cytosine correction was used, gave an M_r value of 2.24×10^9 for the strain B-522 genome, which is close to our estimate (M_r , 2.35×10^9).

The genome size of *R. vannielii* RM5 was determined previously by Potts et al. (20). These authors reported an M_r value of 2.1×10^9 for the DNA of this hyphal, budding bacterium. However, a recalculation of the $c_{0.5}$ ratios of these authors (compared with *E. coli* DNA) gave M_r values of 1.81×10^9 to 1.98×10^9 .

The genome sizes of other budding bacteria are not known at this time. Our values could be compared with those determined for other groups of bacteria. The genome molecular weights of 18 strains of *E. coli* were found to vary from 2.29×10^9 to 2.97×10^9 (2), and those of 13 *Klebsiella pneumoniae* strains ranged from 2.2×10^9 to 3.0×10^9 (21).

FIG. 2. Similarity of budding bacteria based on genome sizes and DNA base compositions. Solid lines encircle strains of the same genus. The numbers indicate the positions of strains, as follows: 1, strain MC-750; 2, strain B-522; 3, strain KB-677; 4, strain NQ-521gr; 5, strain ZV-580; 6, strain SW-808; 7, strain T-854; 8, strain PS-728^T; 9, strain LE-670^T; 10, strain VP-1383; 11, strain VP-1382; 12, strain H-1354; 13, strain SCH-1325; 14, strain E-1129^T; 15, strain S-1290^T; 16, strain ST-1306; 17, strain G-1381; 18, strain WD-1355; 19, strain ST-1307; 20, strain 1128; 21, strain 954; 22, strain SCH-1315; 23, strain 1189^T; 24, strain SCH-1310; 25, strain SCH-1318; 26, strain SCH-1341; 27, strain SCH-1313; 28, strain SCH-1319; 29, strain SCH-1358; 30, strain 1008; 31, strain SCH-1448; 32, strain 1190^T; 33, strain SCH-1317; 34, strain 931. G+C, Guanine-plus-cytosine content.

These large ranges of variation within individual species were similar to the genome size differences which we found within genera (*Hyphomicrobium* M_r , 2.08×10^9 to 2.73×10^9 ; *Hyphomonas* M_r , 1.67×10^9 to 2.13×10^9 ; *Pedomicrobium* M_r , 2.81×10^9 to 3.43×10^9). The genome sizes of these budding bacteria may be more conserved characters than the genome sizes of members of the *Enterobacteriaceae*.

The usefulness of genome size values for taxonomic separation of budding bacteria became particularly evident when the data were plotted against the corresponding DNA base compositions (7) (Fig. 2).

The two-dimensional combination of genome sizes and DNA base ratios results in a clustering of related strains in distinct areas of a similarity map. *Hyphomicrobium*-like strains SW-808 and T-854 did not cluster with the other hyphomicrobia since their DNA base compositions differed significantly from those of all hyphomicrobia investigated so far. The similarity map allows taxonomists to deduce, from the distant positions of a pair of strains, their possibly distant relationship to each other; however, close positions may not necessarily reflect a close relationship. For example, strain LE-670^T (T = type strain) was originally named "*Hyphomicrobium neptunium*" (13). On the map this strain was located at a considerable distance from the *Hyphomicrobium* region, which indicated only a faint relationship. The results of previous DNA-DNA hybridization studies (16), detailed comparisons with 82 hyphomicrobia (10), and recent DNA-DNA hybridizations (6a) suggested that strain LE-670^T would have to be transferred to the genus *Hyphomonas* (18). This was in good agreement with the position of this strain close to the *Hyphomonas* area (Fig. 2). On the other hand, morphological and physiological properties of genus T strains indicated little relationship with *Hyphomicrobium* spp., which could not be deduced from the data presented in Fig. 2.

ACKNOWLEDGMENTS

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

LITERATURE CITED

- Bautz, E. K. F., and F. A. Bautz. 1964. The influence of non-complementary bases on the stability of ordered polynucleotides. *Biochemistry* 52:1476-1481.
- Brenner, D. J., G. R. Fanning, F. J. Skerman, and S. Falkow. 1972. Polynucleotide sequence divergence among strains of *Escherichia coli* and closely related organisms. *J. Bacteriol.* 109:953-965.
- Britten, R. J., D. E. Graham, and B. R. Neufeld. 1974. Analysis of repeating DNA sequences by reassociation. *Methods Enzymol.* 29:363-405.
- Britten, R. J., and D. E. Kohne. 1968. Repeated sequences in DNA. *Science* 161:529-540.
- Gebbers, R., M. Mandel, and P. Hirsch. 1981. Deoxyribonucleic acid base composition and nucleotide distribution of *Pedomicrobium* spp. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe C* 2:332-338.
- Gebbers, R., R. L. Moore, and P. Hirsch. 1981. DNA-DNA reassociation studies on the genus *Pedomicrobium*. *FEMS Microbiol. Lett.* 11:283-286.
- Gebbers, R., R. L. Moore, and P. Hirsch. 1984. Physiological properties and DNA-DNA homologies of *Hyphomonas polymorpha* and *Hyphomonas neptunium*. *Syst. Appl. Microbiol.* 5:510-517.
- Gebbers, R., U. Wehmeyer, T. Roggentin, H. Schlesner, J. Köbel-Boelke, and P. Hirsch. 1985. Deoxyribonucleic acid base compositions and nucleotide distributions of 65 strains of budding bacteria. *Int. J. Syst. Bacteriol.* 35:260-269.
- Gillis, M., and J. De Ley. 1975. Determination of the molecular complexity of double-stranded phage genome DNA from initial renaturation rates. The effect of DNA base composition. *J. Mol. Biol.* 98:447-464.
- Gillis, M., J. De Ley, and M. De Cleene. 1970. The determination of molecular weight of bacterial genome DNA from renaturation rates. *Eur. J. Biochem.* 12:143-153.
- Hirsch, P. 1974. Genus *Hyphomicrobium* Stutzer et Hartleb, 1898, p. 148-150. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
- Klotz, L. C., and B. H. Zimm. 1972. Size of DNA determined by viscoelastic measurements: results on bacteriophages, *Bacillus subtilis* and *Escherichia coli*. *J. Mol. Biol.* 72:779-800.
- König, E., H. Schlesner, and P. Hirsch. 1984. Cell wall studies on budding bacteria of the *Planctomyces/Pasteuria* group and on a *Prosthecomicrobium* sp. *Arch. Microbiol.* 138:200-205.
- Leifson, E. 1964. *Hyphomicrobium neptunium* sp. n. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 30:249-256.
- Mandel, M. 1969. New approaches to bacterial taxonomy: perspective and prospects. *Annu. Rev. Microbiol.* 23:239-274.
- McConaughy, B. L., C. D. Laird, and B. J. McCarthy. 1969. Nucleic acid renaturation in formamide. *Biochemistry* 8:3289-3295.
- Moore, R. L., and P. Hirsch. 1972. Deoxyribonucleic acid base sequence homologies of some budding and prosthecate bacteria. *J. Bacteriol.* 110:256-261.
- Moore, R. L., and P. Hirsch. 1973. Nuclear apparatus of *Hyphomicrobium*. *J. Bacteriol.* 116:1447-1455.
- Moore, R. L., R. M. Weiner, and R. Gebbers. 1984. Genus *Hyphomonas* Pongratz 1957 nom. rev. emend., *Hyphomonas polymorpha* Pongratz 1957 nom. rev. emend., and *Hyphomonas neptunium* (Leifson 1964) comb. nov. emend. (*Hyphomicrobium neptunium*). *Int. J. Syst. Bacteriol.* 34:71-73.
- Park, I. W., and J. De Ley. 1967. Ancestral remnants in deoxyribonucleic acid from *Pseudomonas* and *Xanthomonas*. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 33:1-16.
- Potts, L. E., C. S. Dow, and R. J. Avery. 1980. The genome of *Rhodospirillum rubrum*, a polymorphic prosthecate bacterium. *J. Gen. Microbiol.* 117:501-507.
- Seidler, R. J., M. D. Knittel, and C. Brown. 1975. Potential pathogens in the environment: cultural reaction and nucleic acid studies on *Klebsiella pneumoniae* from clinical and environmental sources. *Appl. Microbiol.* 29:819-825.
- Seidler, R. J., and M. Mandel. 1971. Quantitative aspects of deoxyribonucleic acid renaturation: base composition, state of chromosome replication and polynucleotide homologies. *J. Bacteriol.* 106:608-614.
- Seidler, R. J., M. Mandel, and J. N. Baptist. 1972. Molecular heterogeneity of the bdellovibrios: evidence of two new species. *J. Bacteriol.* 109:209-217.
- Wetmur, J. G. 1976. Hybridization and renaturation kinetics of nucleic acids. *Annu. Rev. Biophys. Bioeng.* 5:337-361.
- Wetmur, J. G., and N. Davidson. 1969. Kinetics of renaturation of DNA. *J. Mol. Biol.* 31:349-370.