

Mini-Review Article

Prokaryotic Genome Size and SSU rDNA Copy Number: Estimation of Microbial Relative Abundance from a Mixed Population

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A B S T R A C T

Determination of the relative abundance of a specific prokaryote in an environmental sample is of major interest in applied and environmental microbiology. Relative abundance can be calculated using knowledge of SSU rDNA copy number, amount of SSU rDNA in the sample, and a weighted average estimate of the genome sizes for organisms in the original sample. By surveying the literature, we provide estimates of genome size and SSU rDNA copy number for 303 and 101 prokaryotes, respectively. This compilation can be used to make reasonable estimates for a wide range of organisms in the calculation of relative abundance. A statistical analysis suggests that no correlation exists between genome size and SSU rDNA copy number. A phylogenetic analysis is used to offer insights into the evolution of both genome size and SSU rDNA copy number.

In recent years, PCR amplification of prokaryotic small subunit ribosomal RNA genes (SSU rDNA) has generated a more complete understanding of prokaryotic ecology and evolution. PCR has been used to effectively determine the presence or absence of an organism in a sample. However, the relative abundance of organisms in a mixed population is not necessarily reflected in the amount of PCR products.

The use of an appropriate internal standard in competitive PCR will allow the determination of the amount of SSU rDNA for a specific taxon or group present in a complex sample. The amount of the SSU rDNA can then be used to determine the relative abundance of the specific taxon or group in the original complex. Such determinations require knowledge of genome sizes for the organisms in the sample and SSU rDNA copy number for the specific taxon or group of interest. Estimates for the genome size of 303 prokaryotic taxa and estimates for SSU rDNA copy number of 101 taxa collected from the literature are reviewed. This information,

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in combination with quantitative PCR, can be used to calculate the relative abundance of specific prokaryotic taxa or groups in a given sample. Such measurements should prove to be valuable in applied and environmental microbiology.

Competitive PCR

Molecular techniques provide a robust means for the identification of organisms independent of the ability to culture them in the laboratory [8, 9, 169, 170, 230]. PCR amplification [157] of SSU rDNA has dramatically improved the ability to characterize prokaryotes present in a sample. An appropriate choice of PCR primers allows for a wide variety of SSU rDNA sequences to be specifically amplified from a complex sample [33]. If universal primers are used, almost all SSU rDNA sequences from a mixed sample can theoretically be PCR amplified. However, if a set of specific PCR primers is chosen, only the SSU rDNA sequences of a small group or a single species of prokaryote will be PCR amplified. Although these methods are powerful and can determine the presence or absence of an organism, they do not always provide a measure of relative abundance of the organisms. There is no guarantee that all SSU rDNA sequences will be PCR amplified with equal efficiency.

Competitive PCR [34, 155, 177, 183], using specific primers and the amplification of an internal standard, can be used to determine the amount of target present in a sample. If the internal standard has an identical sequence to that of the target except for the modification of a unique restriction site (usually only 2–4 base pair differences), then during PCR amplification the internal standard will be amplified in exactly the same manner as the target [15, 34, 84, 152]. When a known amount of internal standard is mixed with a DNA sample, the ratio of target sequence to internal standard sequence can be determined by analyzing the PCR product after digestion with the restriction endonuclease unique to the internal standard. The ratio of internal standard to target in the PCR product is identical to the ratio of internal standard to target in the original sample. Knowing the amount of internal standard added to the sample, the amount of target in the original sample can easily be calculated. If the internal standard sequence and the target sequence differ by the modification of a unique restriction site in the target to another unique restriction site in the internal standard, then the PCR product may be analyzed with each of the unique restriction enzymes. This produces complementary information and significantly increases the accuracy of determining the ratio of internal standard to target in the PCR product [34].

Relative Abundance of Taxa

It is of great importance to determine the relative abundance of a specific organism in an original sample. In order to calculate relative abundance of a specific organism in an original sample, one must know the amount of target SSU rDNA in the original sample, the SSU rDNA copy number in the target organism, and the weighted average of the genome sizes for all organisms in the original sample.

The SSU rDNA copy number and the genome size for many prokaryotes are available in the literature. With recent advancements in DNA sequencing technology, this information is accumulating rapidly, as is the number of completely sequenced prokaryotic genomes. A variety of methods, including field inversion or pulse field gel electrophoresis and DNA hybridization C_0t curve analysis, have been used to estimate genome size. By surveying the literature we have gathered genome size estimates for 303 prokaryotes (Table 1). There are fewer estimates for SSU rRNA gene copy number to be found in the literature (101 are included in this survey). These estimates of SSU rDNA copy number are usually the result of restriction digestion, gel electrophoresis, and Southern hybridization using an SSU rDNA probe. In our survey of the literature, we found that estimates for both genome size and SSU rDNA copy number for 89 organisms are available (Table 1). In order to determine the relative abundance of a particular prokaryote in a population distribution, this information is a crucial resource.

Summary

This compilation of genome size and SSU rRNA gene copy number provides a guide for making a reasonable estimate of genome size and SSU rDNA copy number for a wide variety of organisms. Even if the SSU rDNA copy number for a specific taxon of interest is not given in the literature, a reasonable estimate can often be made from the copy numbers of closely related taxa. Similarly, although the exact composition of taxa in a sample is seldom known, a reasonable estimation for the average (weighted arithmetic mean) of the genome sizes can be determined from the genome sizes of taxa likely to occur in the sample. We have displayed taxa for which genome sizes and SSU rDNA copy number are available as a taxonomic tree to facilitate making these types of estimations. The data presented here allows one to estimate the relative abundance of a taxon in a sample based on the abundance of SSU rDNA present in the sample.

Table 1. Genome sizes ($N = 303$) and SSU rDNA copy numbers ($N = 89$) for bacterial taxa listed according to taxonomic ranking using the Ribosomal Database Project numbering system^a

RDP	Taxon	Identifier	Genome size (kb)	Ref.	SSU rDNA copy number	Ref.
1	Archaeobacteria					
1.1	Euryarchaeota					
1.1.1	<i>Methanococcus jannaschii</i>	Mjan (17-2)	1664974†	35	2	35
1.1.1	<i>Methanococcus thermolithotrophicus</i>		1100	114		201
1.1.1	<i>Methanococcus voltae</i>	Mvol (18-1)	1840 ± 57	114,201	1	83,204
1.1.2	<i>Methanobacterium thermoautotrophicum</i>	Mthe (18-2)	1751377†	204	2	208
1.1.2	<i>Methanobacterium wolfei</i>	Mwol (17-2)	1729	208	2	208
1.1.2	<i>Methanobrevibacter arboriphilicus</i>		1800	114		
1.1.3.2.1	<i>Methanosarcina barkeri</i>		1100	114		
1.1.3.3	<i>Halobacterium halobium</i>	Hhal (22-1)	2200	24	1	162
1.1.3.3	<i>Halobacterium salinarium</i>	Hsal (35-1)*	3500 ± 866	105,161,222	1	105
1.1.3.3	<i>Halobacterium sp. GRB</i>	Hgrb (25-1)	2470	206	1	206
1.1.3.3	<i>Halobacterium volcanii</i>		4000	44		
1.1.3.3	<i>Haloferax (Halobacterium) mediterranei</i>	Hmed (29-2)	2900	142,143	2	142,143
1.1.3.3	<i>Haloferax volcanii</i>	Hvol (38-2)	3820 ± 613	45,56,105,143	2	45,56,105
1.1.3.4	<i>Thermoplasma acidophilum</i>	Taci (17-2)	1700	222	2	226
1.1.3.5	<i>Archaeoglobus fulgidus</i>	Aful (22-1)	2178400†	115	1	115
1.1.4	<i>Pyrococcus furiosus</i>		2100	161,222		
1.1.?	<i>Pyrococcus horikoshii</i>		2000	161		
1.1.?	<i>Pyrococcus shinkaj</i>		2000	222		
1.1.4	<i>Thermococcus celer</i>	Tcel (19-1)	1890	164	1	162
	1.1 Average		2197 ± 816			
1.2	Crenarchaeota					
1.2.?	<i>Crenarchaeum symbiosum</i>		2500	161		
1.2.?	<i>Desulfurococcus Hvv3</i>				1	162
1.2.?	<i>Desulfurococcus mucosus</i>				1	162
1.2.1	<i>Pyrobaculum aerophilum</i>		2224	69,222		
1.2.1	<i>Sulfolobus acidocaldarius</i>	Saci (31-1)	3050	237	1	162
1.2.1	<i>Sulfolobus solfatricus</i>		3025 ± 35	161,199,222		
1.2.1	<i>Thermoproteus tenax</i>				1	162
1.2.2	<i>Thermofilum pendens</i>				1	162
	1.2 Average		2700 ± 406			
	1.0 Average		2449 ± 357			
2	Bacteria					
2.1	Thermophilic Oxygen Reducers					
2.?	<i>Aquifex aeolicus</i>	Aaeo (16-2)*	1551328†	69,222	2	69
2.1	<i>Aquifex pyrophilus</i>	Apyr (16-6)	1620	200	6	200
	2.1 Average		1586 ± 49			
2.2	Thermotogales					
2.2	<i>Thermotoga maritima</i>		1800	222		
2.4	Green Non-sulfur Bacteria and Relatives					
2.4.2	<i>Deinococcus radiodurans</i>		3118 ± 167	95,222		
2.4.2.1	<i>Thermus thermophilus</i>	Tthe (19-2)	1905 ± 233	27,216	2	27,91,93
	2.4 Average		2512 ± 858			
2.7	Flexibacter–Cytophaga–Bacteroides Phylum					
2.7.1.1.2	<i>Porphyromonas gingivalis</i>		2200	161,222		
2.7.1.2.?	<i>Flavobacterium balustinum</i>		4838	95		
2.7.1.2.?	<i>Flavobacterium breve</i>		5040 ± 217	95		
2.7.1.2.5	<i>Flavobacterium</i> group IIb		5291	95		
2.7.1.2.5	<i>Flavobacterium</i> group IIc		2832	95		
2.7.1.2.5	<i>Flavobacterium</i> group IIIc		4061	95		
2.7.1.2.?	<i>Flavobacterium meningosepticum</i>		4434	95		

Table 1. Continued

RDP	Taxon	Identifier	Genome size (kb)	Ref.	SSU rDNA copy number	Ref.
2.7.1.2.?	<i>Flavobacterium odoratum</i> type 1		4790 ± 861	95		
2.7.2.8	<i>Rhodothermus marinus</i>	Rmar (35-1)	3450	156	1	5
	2.7 Average		4104 ± 1065			
2.8	Green Sulfur Bacteria					
2.8	<i>Chlorobium tepidum</i>		2100	160		
2.10	Planctomyces and Relatives					
2.10.1	<i>Pirellula marina</i>				2	132
2.10.1	<i>Planctomyces limnophilus</i>	Plim (52-2)	5200	231	2	231
2.10.2	<i>Chlamydia psittaci</i>		1227 ± 316	78,95		
2.10.2	<i>Chlamydia trachomatis</i>	Ctra (11-2)	1048 ± 328	21,78,95,222	2	21
	2.10 Average		2492 ± 2347			
2.11	Cyanobacteria					
2.11.1.1	<i>Oscillatoria</i> sp.		5672 ± 264	95,96		
2.11.1.2	<i>Dermocarpa</i> sp.		5844 ± 272	95,96		
2.11.1.2	<i>Dermocarpella</i> sp.		5217 ± 243	95,96		
2.11.1.2	<i>Gloeocapsa</i> sp.		5010 ± 238	95,96		
2.11.1.2	<i>Gloeotheca</i> sp.		8022 ± 373	95,96		
2.11.1.2	<i>Myxosarcina</i> sp.		5530 ± 257	95,96		
2.11.1.2	<i>Pleurocapsa</i> sp.		5139 ± 238	95,96		
2.11.1.2	<i>Prochloron</i> sp.		5809	95,96		
2.11.1.2	<i>Spirulina</i> sp.		3964 ± 185	95,96		
2.11.1.2	<i>Synechocystis</i> sp. strain PCC 6803	Syns (36-2)	3572470†	108	2	108
2.11.1.3	<i>Anabaena</i> sp.	Aspe (58-2)*	5793 ± 458	6,95,96	2	6
2.11.1.3	<i>Calothrix</i> sp.		8966 ± 537	95,96		
2.11.1.3	<i>Chlorogloeopsis</i> sp.		7396 ± 344	95,96		
2.11.1.3	<i>Chroococciopsis</i> sp.		6101 ± 1083	95,96		
2.11.1.3	<i>Cylindrospermum</i> sp.		9290 ± 431	95,96		
2.11.1.3	<i>Fischerella</i> sp.		6639 ± 314	95,96		
2.11.1.3	<i>Nodularia</i> sp.		5233 ± 243	95,96		
2.11.1.3	<i>Nostoc</i> sp.		7973 ± 373	95,96		
2.11.1.3	<i>Scytonema</i> sp.		11593 ± 539	95,96		
2.11.1.4	<i>Chamaesiphon</i> sp.		5793 ± 275	95,96		
2.11.1.5	<i>Synechococcus</i> sp.	Sysp (36-2)	3547 ± 936	49,95,96,107	2	49
2.11.1.?	<i>Xenococcus</i> sp.		6251 ± 291	95,96		
2.11.2.3	<i>Pseudanabaena</i> sp.		4778 ± 222	95,96		
2.11.3	<i>Gloeobacter violaceus</i>		4353	95		
	2.11 Average		6149 ± 1915			
2.13	Spiriochaetes and Relatives					
2.13.1	<i>Serpulina hydroysenteriae</i>	Shyd (32-1)	3200	207,244	1	245
2.13.2.2	<i>Treponema denticola</i>		3000	223		
2.13.2.2	<i>Treponema pallidum</i> subsp. pallidum	Tpal (11-2)	1137961†	223	2	218
2.13.2.4	<i>Borrelia afzelli</i>	Bafz (10-1)	948	166	1	160
2.13.2.4	<i>Borrelia burgdorferi</i>	Bbur (15-1)	1463725†	76	1	76,82,168,197,241
2.13.2.4	<i>Borrelia garinii</i>	Bgar (10-1)	953	166	1	166
2.13.3.1	<i>Leptospira borgpetersenii</i>		4900	243		
2.13.3.1	<i>Leptospira interrogans</i>	Lint (46-2)	4590 ± 119	7,243,244	1 or 2	7,243
	2.13 Average		2524 ± 1630			
2.14	Purple					
2.14.1	Alpha Subdivision					
2.14.1.1.4	<i>Acetobacter acetii</i>		4061	95		
2.14.1.1.?	<i>Acetobacter rancens</i>		2929	95		
2.14.1.1.4	<i>Acetobacter xylinum</i>		2200	61		
2.14.1.1.4	<i>Gluconobacter oxydans</i>		2540	95		

Table 1. Continued

RDP	Taxon	Identifier	Genome size (kb)	Ref.	SSU rDNA copy number	Ref.
2.14.1.2.4	<i>Rickettsia bellii</i>		1660	193		
2.14.1.2.4	<i>Rickettsia helvetica</i>		1397	193		
2.14.1.2.4	<i>Rickettsia massiliae</i>		1370	193		
2.14.1.2.?	<i>Rickettsia melolonthae</i>		1720	78		
2.14.1.2.4	<i>Rickettsia prowazekii</i>	Rpro (13-1)	1329 ± 391	67,95,222	1	171,172
2.14.1.2.?	<i>Rickettsia rickettsia</i>		1634	95		
2.14.1.2.4	<i>Rickettsia typhi</i>		1441 ± 435	67,95		
2.14.1.2.?	<i>Rickettsiella grylli</i>		2100	78		
2.14.1.2.7	<i>Wolbachia pipientis</i>				1	16
2.14.1.2.7	Endosymbiont of <i>Sitophilus oryzae</i>		3000	46		
2.14.1.3	<i>Paracoccus denitrificans</i>		4369	95		
2.14.1.3	<i>Rhodobacter capsulatus</i>	Rcap (37-4)	3715	71,72	4	71,72
2.14.1.3	<i>Rhodobacter sphaeroides</i>	Rsph (42-3)	4180 ± 311	72,210,211	3	210
2.14.1.6	<i>Zymomonas mobilis</i>		2023	95		
2.14.1.8	<i>Caulobacter crescentus</i>		3911 ± 130	6,66,95,222		
2.14.1.9.1	<i>Bradyrhizobium japonicum</i>	Bjap (87-1)*	8700	120,121	1	120
2.14.1.9.5	<i>Hyphomicrobium</i> sp.		4045	95		
2.14.1.9.5	<i>Rhodomicrobium vannielii</i>		3398	95		
2.14.1.9.?	<i>Thiobacillus acidophilus</i>		3400	64		
2.14.1.9.?	<i>Thiobacillus cuprinus</i>		3800	149		
2.14.1.9.6	<i>Thiobacillus novellus</i>		3500	64		
2.14.1.9.8	<i>Agrobacterium</i> sp.		5502	95		
2.14.1.9.8	<i>Rhizobium galegae</i>	Rgal (59-3)	5892	103	3	103
2.14.1.9.?	<i>Rhizobium trifolii</i>		5081	95		
2.14.1.9.8	<i>Sinorhizobium meliloti</i>	Smel (59-3)	5928 ± 809	95,102	3	102
2.14.1.9.9	<i>Bartonella bacilliformis</i>	Bbac (16-2)	1565 ± 31	118,119	2	118
2.14.1.9.10	<i>Brucella abortus</i>		2600	2		
2.14.1.9.10	<i>Brucella melitensis</i>	Bmel (29-3)	2925 ± 460	2,152	3	2
	2.14.1. Average		3288 ± 1694			
2.14.2	Beta Subdivision					
2.14.2.1	<i>Chromobacterium violaceum</i>		7598 ± 354	95,96		
2.14.2.?	<i>Neisseria catarrhalis</i>		1576	96		
2.14.1.?	<i>Neisseria crassa</i>		2710 ± 126	95,96		
2.14.2.1	<i>Neisseria gonorrhoeae</i>		2001 ± 197	62,95,96,203,222		
2.14.2.1	<i>Neisseria meningitidis</i>	Nmen (22-4)*	2188 ± 212	60,79,95,222	4	60
2.14.2.1	<i>Neisseria sicca</i>		2346	95		
2.14.2.?	<i>Neisseria subflava</i>		2346	95		
2.14.2.?	<i>Alcaligenes eutrophus</i>		7443	95		
2.14.2.2.3	<i>Burkholderia cepacia</i>		8125 ± 35	104,187		
2.14.2.2.5	<i>Alcaligenes faecalis (odorans)</i>		3285	95		
2.14.2.2.5	<i>Bordetella pertussis</i>		3700	209		
2.14.2.2.5	<i>Taylorella equigenitalis</i>		1682	150		
2.14.2.2.6	<i>Variovorax (Alcaligenes) paradoxus</i>		7686	95		
2.14.2.6	<i>Nitrosococcus</i> sp.		3317	95		
2.14.2.6	<i>Nitrosomonas</i> sp.		2265	95		
	2.14.2 Average		3885 ± 2463			
2.14.3	Gamma Subdivision					
2.14.3.?	<i>Xanthomonas axonopodis</i>		4500	89		
2.14.3.4	<i>Xanthomonas campestris</i>	Xcam (47-2)	4725	53	2	133,134
2.14.3.?	<i>Xanthomonas pelargonii</i>		4790	95		
2.14.3.7.1	<i>Coxiella burnetii</i>	Cbur (17-1)	1683	95	1	1
2.14.3.7.2	<i>Legionella pneumophila</i>		4073 ± 39	95,222		

Table 1. Continued

RDP	Taxon	Identifier	Genome size (kb)	Ref.	SSU rDNA copy number	Ref.
2.14.3.10.1	<i>Acinetobacter calcoaceticus</i>		2654	95		
2.14.3.10.1	<i>Moraxella (Branhamella) catarrhalis</i>		1886 ± 262	68,95		
2.14.3.10.1	<i>Moraxella osloensis</i>		2346	95		
2.14.3.10.?	<i>Azomonas macrocytogenes</i>	Amac (24-6/9)*	2350	145	6 to 9	145
2.14.3.10.?	<i>Azotobacter agilis</i>		2832	95		
2.14.3.10.?	<i>Azotobacter chroococcum</i>	Achr (26-6/9)*	2642 ± 878	95,145	6 to 9	145
2.14.3.10.?	<i>Azotobacter paspali</i>	Apas (42-6/9)*	4180	145	6 to 9	145
2.14.3.10.?	<i>Azotobacter vinelandii</i>	Avin (42-6.9)*	4237 ± 500	145	6 to 9	145
2.14.3.10.3	<i>Pseudomonas aeruginosa</i>	Paer (36-4)	3639 ± 1637	13,54,88,96,182, 188,189,196,223	4	92,196
2.14.3.10.?	<i>Pseudomonas cepacia</i>		7000	50		
2.14.3.10.?	<i>Pseudomonas facilis</i>		4531	95		
2.14.3.10.?	<i>Pseudomonas flava</i>		5016	95		
2.14.3.10.3	<i>Pseudomonas fluorescens</i>		4921 ± 1453	54,95,96,100		
2.14.3.10.?	<i>Pseudomonas oleovorans</i>		6121	96		
2.14.3.10.?	<i>Pseudomonas palleronii</i>		4531	95		
2.14.3.10.?	<i>Pseudomonas piscicida</i>		6375	95		
2.14.3.10.?	<i>Pseudomonas pseudoflava</i>		6553	95		
2.14.3.10.3	<i>Pseudomonas putida</i>		4114 ± 801	95,99		
2.14.3.10.?	<i>Pseudomonas putrefaciens</i>		5453	95		
2.14.3.10.?	<i>Pseudomonas rubescens</i>		5210	95		
2.14.3.10.?	<i>Pseudomonas saccharophila</i>		5663	95		
2.14.3.10.?	<i>Pseudomonas solanacearum</i>		5242 ± 433	95,99		
2.14.3.10.3	<i>Pseudomonas stutzeri</i>	Pstu (41-4)	4137 ± 769	54,85,95,96,181	4	85
2.14.3.10.?	<i>Pseudomonas trifolii</i>		6100	95		
2.14.3.12	<i>Shewanella putrefaciens</i>		4500	223		
2.14.3.13.1	<i>Salinivibrio (Vibrio) costicola</i>		2382	151		
2.14.3.13.2	<i>Vibrio cholerae</i>	Vcho (28-7)	2843 ± 321	52,147,222	7	147
2.14.3.13.2	<i>Vibrio harveyi</i>				1	124
2.14.3.13.3	<i>Vibrio metschnikovii</i>		3540 ± 165	95,96		
2.14.3.15.1	<i>Buchnera aphidicola</i>				1	158,190
2.14.3.15.2	<i>Escherichia coli</i> K-12	Ecol (46-7)	4639221†	22	7	22
2.14.3.15.?	<i>Salmonella enteritidis</i>	Sent (48-7)	4746	137	7	137
2.14.3.15.2	<i>Salmonella paratyphi</i>	Spar (47-7)*	4718 ± 96	137,138,141	7	137,141
2.14.3.15.2	<i>Salmonella pullorum</i>		4442 ± 217	95,96		
2.14.3.15.2	<i>Salmonella typhi</i>	Styi (47-7)*	4727 ± 254	137,195,222	7	137
2.14.3.15.2	<i>Salmonella typhimurium</i>	Styp (46-7)*	4605 ± 319	137,139,140, 203,236	7	137,236
2.14.3.15.?	<i>Citrobacter amalonaticus</i>		4595	95		
2.14.3.15.?	<i>Citrobacter diversus</i>		4693	95		
2.14.3.15.3	<i>Citrobacter freundii</i>		4288	95		
2.14.3.15.3	<i>Erwinia herbicola</i>		4935	95		
2.14.3.15.3	<i>Erwinia uredovora</i>		5275	95		
2.14.3.15.3	<i>Serratia marcescens</i>		7490 ± 997	95,96		
2.14.3.15.5	<i>Yersinia pseudotuberculosis</i>		5875 ± 273	95,96		
2.14.3.15.?	<i>Klebsiella ozaenae</i>		3698 ± 172	95,96		
2.14.3.15.?	<i>Klebsiella pneumoniae</i>		4142	95		
2.14.3.15.?	<i>Klebsiella rubiaceurum</i>		4660	95		
2.14.3.15.?	<i>Shigella boydii</i>		3722	95		
2.14.3.15.?	<i>Shigella dysenteriae</i>		4466	95		
2.14.3.15.?	<i>Shigella flexneri</i>		4142	95		
2.14.3.15.?	<i>Shigella sonnei</i>		3275 ± 152	95,96		
2.14.3.15.?	<i>Enterobacter aerogenes</i>		4207	95		
2.14.3.15.?	<i>Enterobacter cloacae</i>		4207	95		

Table 1. Continued

RDP	Taxon	Identifier	Genome size (kb)	Ref.	SSU rDNA copy number	Ref.
2.14.3.15.?	<i>Proteus morganii</i>		3061	96		
2.14.3.15.6	<i>Proteus vulgaris</i>		3275 ± 152	95,96		
2.14.3.16.1	<i>Haemophilus parainfluenzae</i>		2340	109		
2.14.3.16.?	<i>Haemophilus (Actinobacillus) actinomycetemcomitans</i>		2200	161,222		
2.14.3.16.2	<i>Haemophilus aegyptius</i>		1833 ± 85	95,96		
2.14.3.16.2	<i>Haemophilus ducreyi</i>		1785	128		
2.14.3.16.2	<i>Haemophilus influenzae</i> Rd KW20	Hinf (18-6)	1830137†	70	6	70
2.14.3.16.3	<i>Pasteurella multocida</i>		1770 ± 82	95,96		
	2.14.3 Average		4137 ± 1365			
2.14.4	Delta Subdivision					
2.14.4.1	<i>Desulfovibrio desulfuricans</i>		2600 ± 707	63,233		
2.14.4.1	<i>Desulfovibrio gigas</i>		1764	95		
2.14.4.1	<i>Desulfovibrio vulgaris</i>		2723 ± 1241	63,95		
2.14.4.?	<i>Bdellovibrio bacteriovorans</i>		2092 ± 130	32,95		
2.14.4.?	<i>Bdellovibrio starrii</i>		2663 ± 125	95		
2.14.4.?	<i>Bdellovibrio stolpii</i>		2341 ± 100	32,95		
2.14.4.4	<i>Bdellovibrio W</i>		2136	90		
2.14.4.5	<i>Desulfobulbus propionicus</i>		3700	63		
2.14.4.6	<i>Myxococcus xanthus</i>		9452 ± 2	47,48,94		
	2.14.4. Average		3275 ± 2380			
2.14.5	Epsilon Subdivision					
2.14.5.1	<i>Helicobacter mustelae</i>		1700	219		
2.14.5.1	<i>Helicobacter pylori</i> 26695	Hpyl (17-2)	1667867†	225	2	225
2.14.5.2	<i>Campylobacter coli</i>	Ccol (19-3)	1926 ± 585	43,95,220,238	3	220
2.14.5.2	<i>Campylobacter fetus</i>	Cfet (18-3)	1785 ± 992	43,95,194	3	194
2.14.5.2	<i>Campylobacter helveticus</i>				2	136
2.14.5.2	<i>Campylobacter jejuni</i>	Cjej (21-3)	2067 ± 765	43,95,113,163,165	3	113,163
2.14.5.2	<i>Campylobacter laridis</i>		1451	43		
2.14.5.2	<i>Campylobacter upsaliensis</i>	Cups (20-3)	2000	31	3	206
	2.14.5 Subdivision Average		1800 ± 215			
	2.14 Average		3277 ± 907			
2.16	Gram-positive					
2.16.1	High G+C Subdivision (39)					
2.16.1.4	<i>Streptomyces ambofaciens</i>				6	20
2.16.1.4	<i>Streptomyces coelicolor</i>	Scoe (98-6)	9801 ± 2547	95,110	6	14,228
2.16.1.4	<i>Streptomyces griseus</i>	Sgri (78-6)	7800	131	6	111
2.16.1.4	<i>Streptomyces lividans</i>	Sliv (80-6)	8000	95	6	127,213
2.16.1.4	<i>Streptomyces rimosus</i>		10599	95		
2.16.1.6.2	<i>Bifidobacterium breve</i>	Bbre (21-3)	2100	30	3	30
2.16.1.7.?	<i>Micrococcus flavus</i>		4061	96		
2.16.1.7.3	<i>Micrococcus luteus</i>		4466	95		
2.16.1.7.?	<i>Micrococcus lysodeikticus</i>		4273	96		
2.16.1.7.3	<i>Micrococcus</i> sp Y-1		4061	173		
2.16.1.12.1.1	<i>Nocardia asteroides</i>		4531	95		
2.16.1.12.1.?	<i>Nocardia caviae</i>		5178	95		
2.16.1.12.1.1	<i>Nocardia corynebacteroides</i>		3560	95		
2.16.1.12.1.?	<i>Brevibacterium ammoniagenes</i>		3000	96		
2.16.1.12.1.?	<i>Corynebacterium (Brevebacterium) ammoniagenes</i>		3204	95		
2.16.1.12.1.2	<i>Corynebacterium diphtheriae</i>		1942	95		
2.16.1.12.1.2	<i>Corynebacterium glutamicum</i>		2998 ± 120	10,95		
2.16.1.12.1.?	<i>Corynebacterium (Brevebacterium) liquefaciens</i>		2913	95		

Table 1. Continued

RDP	Taxon	Identifier	Genome size (kb)	Ref.	SSU rDNA copy number	Ref.
2.16.1.12.1.?	<i>Corynebacterium minutissimum</i>		2265	95		
2.16.1.12.1.2	<i>Corynebacterium renale</i>		1942	95		
2.16.1.12.1.?	<i>Corynebacterium (Brevebacterium) vitarumen</i>		1952	95		
2.16.1.12.2	<i>Mycobacterium avium</i>		5838 ± 988	95,223		
2.16.1.12.2	<i>Mycobacterium bovis</i>	Mbov (51-2)	5065	95	2	214
2.16.1.12.2	<i>Mycobacterium chelonae</i>		4045	95		
2.16.1.12.2	<i>Mycobacterium farcinogenes</i>		7023	95		
2.16.1.12.2	<i>Mycobacterium fortuitum</i>		5000	112		
2.16.1.12.2	<i>Mycobacterium gastri</i>		6796	95		
2.16.1.12.2	<i>Mycobacterium gordonae</i>		7395	95,112		
2.16.1.12.2	<i>Mycobacterium intracellulare</i>	Mint (50-1)	5016	112	1	19
2.16.1.12.2	<i>Mycobacterium kansasii</i>		6197	95		
2.16.1.12.2	<i>Mycobacterium leprae</i>		2104	95,176		
2.16.1.12.2	<i>Mycobacterium lepraemurium</i>	Mlep (29-1)*	2913	95	1	212
2.16.1.12.2	<i>Mycobacterium marinum</i>		5825	95		
2.16.1.12.2	<i>Mycobacterium phlei</i>	Mphl (63-2)	6311	95	2	19
2.16.1.12.2	<i>Mycobacterium scrofulaceum</i>		5639	95		
2.16.1.12.2	<i>Mycobacterium smegmatis</i>	Msme (72-2)	7168	95	2	19
2.16.1.12.2	<i>Mycobacterium stercooides</i>		6149	95		
2.16.1.12.2	<i>Mycobacterium tuberculosis</i>	Mtub (43-1)	4323 ± 153	95,112,174,176,223	1	19
2.16.1.12.2	<i>Mycobacterium vaccae</i>		4045	95		
2.16.1.12.2	<i>Mycobacterium xenopi</i>		5324	95		
	2.16.1 Average		6734 ± 3276			
2.16.2	Clostridia and Relatives					
2.16.2.1.1	<i>Clostridium stercorarium</i>		3000	240		
2.16.2.1.1	<i>Clostridium thermocellum</i>		3500	240		
2.16.2.2	<i>Caldocellum saccharolyticum</i>		2780	26		
2.16.2.4.1	<i>Clostridium botulinum</i>		4039	135		
2.16.2.4.2	<i>Clostridium pasteurianum</i>		3900	240		
2.16.2.4.2	<i>Clostridium tyrobutyricum</i>		2500	240		
2.16.2.4.6	<i>Clostridium acetobutylicum</i>		4800 ± 1476	223,234,240		
2.16.2.4.7	<i>Clostridium perfringens</i>	Cper (35-10)	3505 ± 182	36,37,240	9 or 10	36,80
2.16.2.5	<i>Clostridium difficile</i>		3200	240		
	2.16.2 Average		3469 ± 706			
2.16.4	Mycoplasmas and Relatives					
2.16.4.1	<i>Mycoplasma arginini</i>				2	3
2.16.4.1	<i>Mycoplasma flocculare</i>		890	186		
2.16.4.1	<i>Mycoplasma hominis</i>	Mhom (7-2)	733 ± 27	123,186	2	123
2.16.4.1	<i>Mycoplasma hyopneumoniae</i>	Mhyo (11-1)	1105 ± 50	179,186	1	218
2.16.4.1	<i>Mycoplasma mobile</i>		780	11		
2.16.4.?	<i>Mycoplasma sturni</i>		870	73		
2.16.4.1	<i>Mycoplasma synoviae</i>		900	179		
2.16.4.2	<i>Mycoplasma gallisepticum</i>	Mgal (11-2)	1052 ± 3	87,179	2	3
2.16.4.2	<i>Mycoplasma genitalium</i> G-37	Mgen (6-1)	580070†	77	1	77
2.16.4.2	<i>Mycoplasma iowae</i>		1280	179		
2.16.4.2	<i>Mycoplasma penetrans</i>		1358	185		
2.16.4.2	<i>Mycoplasma pneumoniae</i> M129	Mpne (8-1)	816397†	98	1	98
2.16.4.2	<i>Ureaplasma urealyticum</i>	Uure (9-2)	858 ± 99	19,55,86,184,222	2	3,55
2.16.4.3	<i>Mycoplasma capricolum</i>	Mcap (9-2)	940 ± 305	148,154	1 or 2	3,4
2.16.4.3	<i>Mycoplasma mycoides</i>	Mmyc (13-2)	1261 ± 56	179,180,186	2	180
2.16.4.3	<i>Mycoplasma</i> sp. PG-50	Mspe (10-2)	1040	180	2	180
2.16.4.3	<i>Spiroplasma citri</i>	Scit (17-1)	1707 ± 103	95,239	1	239
2.16.4.?	<i>Spiroplasma platyhelix</i>		780	235		
2.16.4.?	<i>Spiroplasma velocicrescens</i>		1480	117		
2.16.4.4	<i>Acholeplasma axanthum</i>				2	3

Table 1. Continued

RDP	Taxon	Identifier	Genome size (kb)		Ref.	SSU rDNA copy number	Ref.
2.16.4.?	<i>Acholeplasma granularum</i>					2	3
2.16.4.4	<i>Acholeplasma laidlawii</i>		1649 ± 57	186			
2.16.4.4	<i>Acholeplasma oculi</i>		1633	224			
	2.16.4 Average		1086 ± 335				
2.16.5	Bacillus–Lactobacillus–Streptococcus Subdivision						
2.16.5.?	<i>Lactococcus cremoris</i>		2600	29			
2.16.5.1	<i>Lactococcus lactis</i>	Llac (32-6)	3183 ± 1481	28,29,59,95,125,217,227		6	126
2.16.5.1	<i>Streptococcus agalactiae</i>	Saga (27-6)	2689 ± 1232	95,96		6	51
2.16.5.1	<i>Streptococcus bovis</i>		5631	95			
2.16.5.1	<i>Streptococcus dysgalactiae</i>	Sdys (39-6)	3883	95		6	18
2.16.5.?	<i>Streptococcus faecium</i>		8091	88			
2.16.5.1	<i>Streptococcus gordonii</i>	Sgor (21-4)	2120	74,75		4	75
2.16.5.1	<i>Streptococcus mutans</i>		2145 ± 28	90,167			
2.16.5.1	<i>Streptococcus pneumoniae</i>	Spne (23-6)	2267 ± 74	81,95,222		6	81
2.16.5.1	<i>Streptococcus pyogenes</i>		1986 ± 66	95,96,222			
2.16.5.?	<i>Streptococcus raffinolactis</i>		4126	95			
2.15.5.1	<i>Streptococcus sanguis</i>		2300	29			
2.16.5.1	<i>Streptococcus thermophilus</i>	Sthe (19-6)	1943 ± 440	29,192		6	18
2.16.5.?	<i>Streptococcus uberis</i>		3398	95			
2.16.5.2.1	<i>Leuconostoc oenos</i>		1684 ± 316	58,178,221			
2.16.5.2.2	<i>Lactobacillus acidophilus</i>	Laci (19-4)	1850	191		4	191
2.16.5.2.2	<i>Lactobacillus delbrueckii</i>		2300	129			
2.16.5.2.2	<i>Lactobacillus gasseri</i>		2020	186			
2.16.5.2.2	<i>Lactobacillus helveticus</i>		1925	144			
2.16.5.2.3	<i>Lactobacillus casei</i>		2071	95			
2.16.5.2.?	<i>Lactobacillus cremoris</i>		2600	29			
2.16.5.2.?	<i>Lactobacillus lactis</i>		2460 ± 89	29			
2.16.5.2.3	<i>Lactobacillus plantarum</i>		2259 ± 769	57,95			
2.16.5.2.3	<i>Pediococcus acidilactici</i>		1560	57			
2.16.5.2.3	<i>Pediococcus pentosaceus</i>		1200	57			
2.16.5.3.1	<i>Enterococcus faecalis</i>	Efae (29-4)	2875 ± 555	29,95,96,222		4	159
2.16.5.3.1	<i>Enterococcus hirae</i>					6	198,242
2.16.5.4	<i>Carnobacterium divergens</i>		3200	57			
2.16.5.6	<i>Listeria monocytogenes</i>	Lmon (32-6)	3150	153		6	153
2.16.5.8.1	<i>Caryophanon latum</i>		1861	95			
2.16.5.8.1	<i>Caryophanon tenue</i>		1570	95			
2.16.5.9	<i>Bacillus anthracis</i>		4355 ± 203	95,96			
2.16.5.9	<i>Bacillus cereus</i>	Bcer (50-10/12)	4959 ± 1213	38,42,95,106,116		10 to 12	100
2.16.5.?	<i>Bacillus polymyxa</i>		4309 ± 200	95,130			
2.16.5.9	<i>Bacillus thuringiensis</i>		5533 ± 1305	39,40,41			
2.16.5.10.?	<i>Staphylococcus albus</i>		1697	96			
2.16.5.10.2	<i>Staphylococcus aureus</i>		2583 ± 365	25,95,96,174,175,232			
2.16.5.11	<i>Bacillus subtilis</i>	Bsub (42-10)	4214810†	122		10	122
2.16.5.12	<i>Bacillus megaterium</i>		4584 ± 121	95,229			
	2.16.5 Average		2978 ± 1432				
	2.16 Average		3567 ± 2348				

^a Genome sizes are given in terms of kilobase pairs (kb). For species with more than one genome size estimate, an average and standard deviation are shown. Identifiers are used on the branch tips in Fig. 1. An asterisk (*) denotes taxa without a complete SSU rRNA sequence in RDP (see Fig. 1). Complete genomes are denoted with a (†) symbol and are given in exact base pairs.

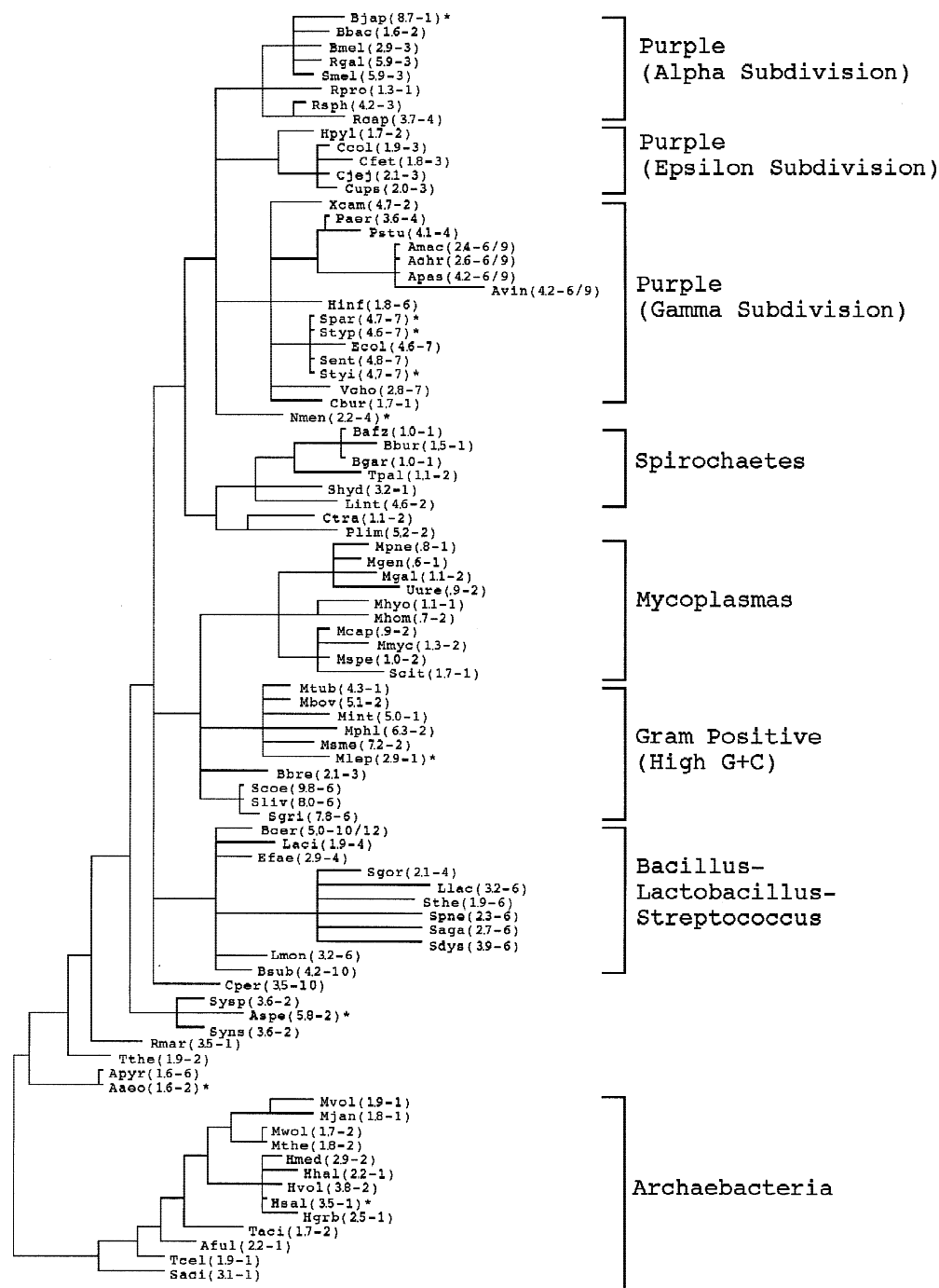


Fig. 1. User-derived tree reflective of the generalized groupings in the RDP Prokaryotic Taxonomic List for the 89 taxa having values for both genome size and SSU rRNA copy number in Table 1. See Table 1 for definitions of species identifiers. Numbers in parentheses give genome size (Mb) and SSU rRNA copy number.

Literature Survey

Taxa Names

A list of organisms identified by genus and species was developed from the literature. These organisms were arranged taxonomically according to the RDP SSU rRNA. Prokaryotic Taxonomic List [146] (Table 1, Fig. 1). Organisms not explicitly found in the RDP database were grouped with the most appropriate taxa [32]. These taxa are identified by a “?”

in the last digit of their RDP taxonomic number. Genus and species names were cross-checked to ensure appropriate placement [101,202]. Synonymous names were placed in parentheses in Table 1.

Genome and SSU rDNA Estimates

For species with multiple estimates of genome size in the literature, an average and standard deviation was recorded

(Table 1). In the event that the complete genome sequence for an organism was known, the exact number of base pairs was listed instead of any previous estimates. SSU rDNA copy number estimates were collected from the literature and organized in a spreadsheet (Microsoft Excel 5.0). In cases where estimates of SSU rDNA copy number varied for a particular genus (an infrequent situation), the most commonly cited and/or most recent estimates was used for Table 1. If SSU rDNA copy number varied from strain to strain for a particular species, the range was included in Table 1.

Phylogenetic Analysis

The majority of SSU rRNA sequences were downloaded from the RDP database in a prealigned format. Sequences downloaded from GenBank Release 104.0 [17] were aligned with the RDP database. This set of aligned SSU rRNA sequences was used to create a user-derived tree for the set of 89 organisms having estimates for both genome size and SSU rDNA copy number using PAUP 3.1.1 [215]. The “user-specified tree” was derived using the RDP grouping numbers as a guide (Fig. 1). Species identifiers at the tips of each branch are listed in Table 1. The genome size (Mb) and SSU rDNA copy number for each organism is provided as a part of the species identifier for ease of comparison on the tree.

Genome Size Analysis

Genome size estimates for 303 prokaryotes following the RDP taxonomy are given in Table 1, including mean and standard deviation where a range of values appeared in the literature. The variation in genome size estimates for a number of taxa, including several *Pseudomonas* species, *Desulfovibrio vulgaris*, *Mycobacterium avium*, *Clostridium acetobutylicum*, *Lactococcus lactis*, *Streptococcus agalactiae*, and *Bacillus cereus*, was quite large. For *Mycoplasma* species and related taxa of RDP grouping 2.16.4, the standard deviation of reported genome size in the literature was very low. A histogram of genome size over all taxonomic groupings is displayed in Fig. 2. The distribution spans roughly one order of magnitude with a mean and standard deviation of 3,604 kb \pm 1,997 kb. The genome of *Mycoplasma genitalium* G-37 is completely sequenced and is the smallest genome reported with only 580,070 bp [77]. Cyanobacterial taxa form a tail of large genome organisms, with the genome of *Scytonema* sp. estimated to be the largest at 11,593 kb [95]. One-half of the genomes are between 2.0 and 4.8 kb, with 25% of the genomes under 2.0 kb and 25% over 4.8 kb.

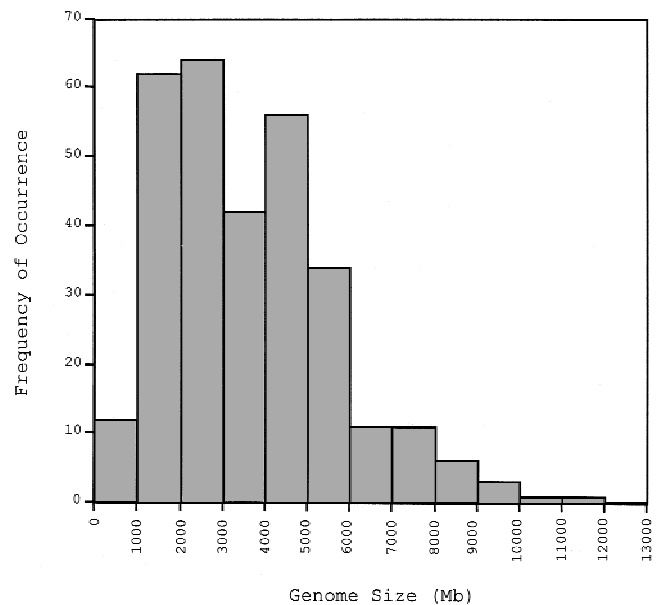


Fig. 2. Histogram of known and estimated genome sizes for prokaryotic organisms found in the literature ($N = 303$) with a mean of 3,604 kb and standard deviation of 1,996 kb. The data do not follow a normal distribution ($\chi^2 = 25.19$).

Relationship of Genome Size and SSU rDNA Copy Number

The relation between genome size and SSU rDNA copy number for 101 organisms is shown in Fig. 3. The genome size and SSU rDNA copy number might be expected to be positively correlated; however, little correlation is apparent ($y = 2267.5 \times x$; $r^2 = 0.128$). The organisms with 10 to 12 SSU rRNA genes (the largest number observed) have genome sizes less than 5 Mb, whereas organisms with the largest observed genomes (10 to 11.6 MB) have only 6 SSU rRNA genes.

SSU rRNA Copy Number Analysis

A histogram of SSU rDNA copy number for 101 taxa is given in Fig. 4. The mean SSU rDNA copy number is 3.8 with a standard deviation of 2.9. SSU rDNA copy number ranges from 1 (over 10% of the taxa) to 12 in some strains of *Bacillus cereus* [106]. The relationships between the 89 taxa with a value for both genome size and SSU rDNA copy number are displayed in a user-derived tree (Fig. 1). The tree was constructed following RDP taxonomic classification. In most cases, genome sizes and copy numbers were very similar within the major taxonomic groupings. For instance, all Archaea have similar genome sizes (2449 ± 357 kb) and have two or fewer SSU rRNA genes.

Genome sizes for a total of 303 prokaryotes are presented in this review. It is clear that the methods used to determine each estimate have varying degrees of accuracy. In particular, it appears that older citations generally have a higher variance in genome size estimation, perhaps as a result of methods used (data not shown). For ease of calculation, we treated all citations as having equal merit in making estimates of genome size averages (Table 1).

Over 40% of the organisms surveyed have 1 or 2 SSU rRNA genes. Curiously, no organisms with a copy number of 5 were found in this survey. This anomaly could be the result of a small sample size; however, the abundance of organisms with 6 and 7 SSU rRNA genes suggests that organisms with 5 SSU rRNA genes might be precluded by some type of selection pressure on SSU rRNA gene copy number. We also note that some *Azomonas* and *Bacillus* species have strain-dependent differences in SSU rRNA gene copy number. A bias in the data set might be the result of the higher growth rates for cultured prokaryotes relative to the native population. A positive correlation between growth rate and the number of rRNA genes is expected. Therefore, our survey may reflect somewhat of an artificial selection for high rRNA gene copy number.

Our user-derived tree is based on RDP taxonomy and general relationships of groups. This type of tree may offer insights into the evolution of both genome size and SSU

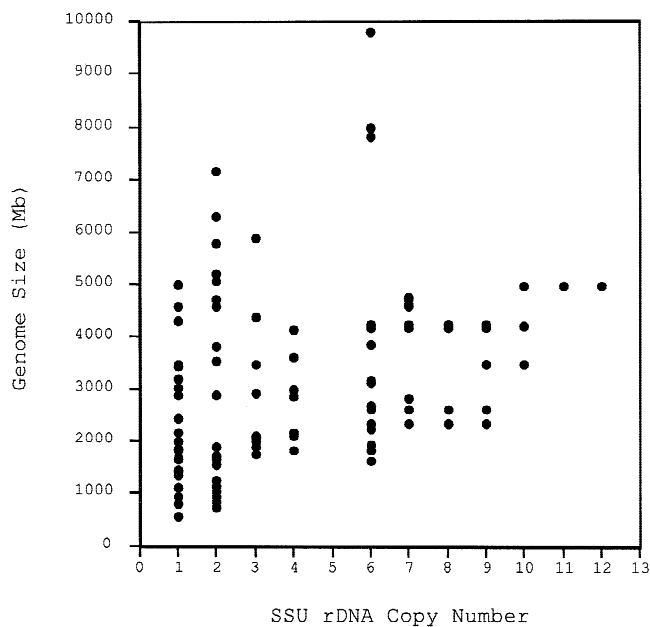


Fig. 3. Distribution of prokaryotic genome size versus SSU rRNA copy number ($N = 101$). Increased genome size shows only a slight correlation with increased copy number ($y = 2268 \times x$; $r^2 = 0.128$).

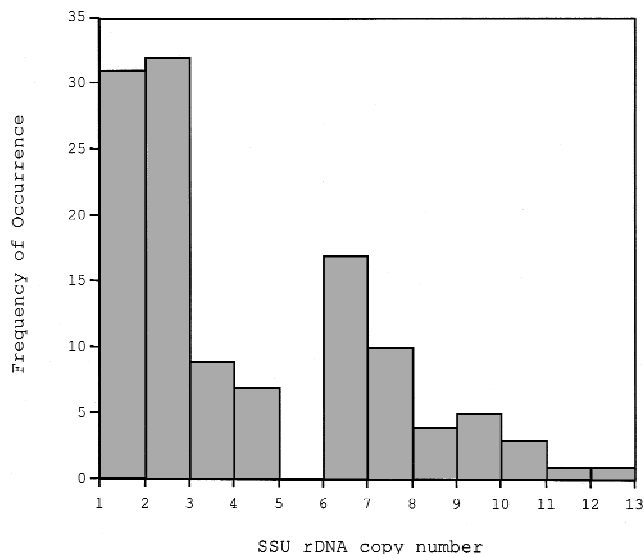


Fig. 4. Histogram of SSU rRNA copy numbers for the set of 101 taxa with estimated copy number in Table 1. The mean rRNA copy number is 3.8 with a standard deviation of 2.9. The data are not normally distributed.

rDNA copy number. The tree is also useful for choosing neighboring values for the estimation of copy number or genome size where the values for a specific organism are not available in the literature. A few potential anomalies can be seen in the tree relative to rDNA copy number estimation for the 89 taxa available in the literature. For instance, *Aquifex pyrophilus* is reported to contain six copies of SSU rDNA [200]; however, all neighboring taxa to *A. pyrophilus* have only two SSU rDNA copies.

An Estimation Method

It is of major interest to determine the relative abundance of a target organism in an environmental or complex laboratory sample. The amount of target SSU rDNA in a sample can be determined by quantitative PCR. To calculate the abundance of a target organism, we must also know the number of SSU rRNA genes in the target organism and the weighted average of the genome sizes for the other organisms in the sample.

Using quantitative PCR with specific primers and an internal standard, it is possible to measure the amount of SSU rDNA for a specific target in a given sample [15, 34, 84]. In cases where SSU rDNA copy number and/or genome size are unknown, estimates can be made using averages from neighboring organisms or groups based on the RDP taxonomy shown in Fig. 1. The closest relatives are likely to have similar

genome sizes and SSU rDNA copy numbers to the taxa of interest.

As an example, to determine the amount of *Bacillus subtilis* present in an environmental sample, a known amount of internal standard DNA is added to a known amount of total genomic DNA and the mixture is PCR amplified with a set of primers specific for *B. subtilis* SSU rDNA. From the amount of internal standard sequence in the PCR product, the amount of *B. subtilis* rDNA present in the original sample can be calculated. Assume that our measurement indicates that 20 pg of *B. subtilis* rDNA is present in a total of 100 ng of genomic DNA. From the literature we know that *B. subtilis* has 10 SSU rRNA genes. From a general knowledge of the organisms present in the sample, we estimate the weighted average of the genome sizes in the sample to be 3.6×10^6 bp. From this weighted average it can be calculated that 100 ng of genomic DNA represents 2.5×10^7 organisms. The *B. subtilis* specific SSU rDNA PCR product is about 1200 bp in length; thus, 20 pg of this product corresponds to 1.5×10^7 SSU rRNA genes. Because each *B. subtilis* has 10 SSU rRNA genes, 20 pg of this product corresponds to 1.5×10^6 *B. subtilis*. In this case, the sample of 100 ng of genomic DNA represents 2.5×10^7 organisms, of which 1.5×10^6 are *B. subtilis*. Thus, *B. subtilis* represent 6% of the organisms in the sample.

Summary

An estimation of the *in situ* relative abundance of a specific prokaryotic taxon or group from a sample is of extreme value in microbial ecology. This is especially true in terms of monitoring temporal variance of bacterial population distributions from environmental or medical samples. For instance, a governmental agency might be interested in tracking the relative abundance of a particular organism in a bay or harbor believed to be polluted. Competitive PCR amplification provides a measure of the number of taxa or group-specific SSU rRNA genes present in a sample. The relative abundance of a specific organism present in the sample can be estimated with knowledge of genome sizes and SSU rRNA copy numbers such as those that are presented in this survey.

With future advances in genome sequencing, estimates of genome size and SSU rDNA copy number will become more numerous and more precise. It would be interesting to couple this survey with estimates of prokaryotic growth rates in order to determine a correlation between rRNA gene copy number and generation time. Such data could be used to test the hypothesis that there is a bias in the number of known

rRNA gene copy numbers for relatively fast growing prokaryotes already in pure culture.

New estimates of genome size and SSU rDNA copy number will offer even more data from which to make measurements of prokaryotic relative abundance. These measurements can be made in situations where it was previously only possible to identify the presence or absence of prokaryotic taxa. In terms of microbial ecology, presence or absence of a taxon at a given time does not provide a detailed understanding of the population dynamics. Future advances in microbial ecology will require rapid determination of both the temporal and spatial variation of organisms and their abundance in a mixed population. Together, the data and methods presented in this review offer a novel approach that can be used for the quantitative measure of microbial populations in natural environments.

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