Intra- and Intergeneric Similarities of *Pseudomonas* and *Xanthomonas* Ribosomal Ribonucleic Acid Cistrons

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We hybridized 23S 2-¹⁴C-labeled ribosomal ribonucleic acids (rRNAs) from type strains *Pseudomonas fluorescens* ATCC 13525, *Pseudomonas acidovorans* ATCC 15668, *Pseudomonas solanacearum* NCPPB 325, and *Xanthomonas campestris* NCPPB 528 with deoxyribonucleic acids (DNAs) from 65 *Pseudomonas* strains, 23 *Xanthomonas* strains, and 148 mostly gram-negative strains belonging to 43 genera and 93 species and subspecies including more than 60 type strains. Our findings confirm and extend the findings derived from ribonucleic acid hybridizations by the Berkeley group, but differed in some respects from the groupings of *Pseudomonas* in *Bergey's Manual of Determinative Bacteriology*, 8th ed. The genus *Pseudomonas* Migula 1894, 237 was divided into three large, distinct groups. The *Pseudomonas fluorescens* rRNA branch contains *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas chlororaphis*, *Pseudomonas aureofaciens*, *Pseudomonas syringae*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Pseudomonas mendocina*, *Pseudomonas cichorii*, *Pseudomonas alcaligenes*, and *Pseudomonas pseudoalcaligenes*. The *Pseudomonas acidovorans* rRNA branch contains *Pseudomonas acidovorans*, *Pseudomonas testosteroni*, *Pseudomonas delafieldii*, *Pseudomonas facilis*, *Pseudomonas palleronii*, *Pseudomonas saccharophila*, and *Pseudomonas flava*. The third rRNA branch contains *Pseudomonas solanacearum*, *Pseudomonas cepacia*, *Pseudomonas marginata*, *Pseudomonas caryophylli*, and *Pseudomonas lemoignei*. Each of these rRNA branches is as heterogeneous as a genus. The *Pseudomonas solanacearum* and *Pseudomonas acidovorans* rRNA branches are about as far removed from each other as they are from the genera *Janthinobacterium* and *Derrxia* and the authentic genus *Alcaligenes*. These branches are members of the third rRNA superfamily. The *Pseudomonas fluorescens* rRNA branch is quite different, as it is a member of the second rRNA superfamily, which also contains *Azotobacter*, *Azomonas*, *Xanthomonas*, and some other genera. Along with data from rRNA hybridizations involving many different gram-negative taxa, these results show clearly that the three *Pseudomonas* rRNA branches differ at least at the genus level. The genus *Xanthomonas* is separate in its own right. It constitutes a very tight cluster consisting of *Xanthomonas campestris*, *Xanthomonas fragariae*, *Xanthomonas axonopodis*, and *Xanthomonas albilineans* (*Xanthomonas campestris* covers older species names no longer in use). *Xanthomonas* (*Aplanobacter*) *populi* has rRNA cistrons that are indistinguishable from the rRNA cistrons of the xanthomonads mentioned above. There are a number of misnamed taxa. *Pseudomonas maltophilia* is a somewhat unusual member of *Xanthomonas*; likewise, *Pseudomonas diminuta* and *Pseudomonas vesicularis* are not members of the genus *Pseudomonas*, and *Xanthomonas ampelina* is definitely not a member of the genus *Xanthomonas*. The exact taxonomic positions of the latter three species are unknown. A quantitative comparison showed that fine differentiation of strains by means of DNA-DNA hybridization under stringent conditions at T_{OR} (temperature of optimal renaturation) was meaningful only in the top 7 to 8°C $T_{m(e)}$ (thermal elution temperature range, 73 to 81°C) of our DNA-rRNA similarity maps and dendrograms (a difference of 1°C in thermal elution temperature $T_{m(e)}$ from ribosomal DNA similarity corresponded to roughly 14% DNA homology).

The elucidation of relationships among bacteria at the generic and suprageneric levels is one of the main problems to be solved in modern

bacterial taxonomy. Previous papers from our laboratory on *Agrobacterium* (21), *Chromobacterium* and *Janthinobacterium* (17), *Acetobac-*

ter, *Gluconobacter* and *Zymomonas* (26), and various genera of free-living N₂-fixing bacteria (20) have shown that the deoxyribonucleic acid (DNA)-ribosomal ribonucleic acid (rRNA) hybridization technique of De Ley and De Smedt (15) is a fast, reliable, and relatively simple technique, which helps solve this problem. The theoretical aspects and practical implications of this approach have been set forth in the papers cited above and need not be repeated here.

The elucidation of the relationships among the different sections of the genus *Pseudomonas* and the relationships of these sections with other genera remains another formidable challenge. The pseudomonads constitute a very large and very varied conglomerate with great nutritional versatility; the members of this group range from innocent mineralizing saprophytes that are common in soil and water to economically important pathogens of plants, animals, and humans. Despite the valuable attempts of Rhodes (52), Lysenko (37), Stanier et al., (59) and Palleroni et al., (48), the taxonomy of the genus *Pseudomonas* is still incompletely known. In *Bergey's Manual of Determinative Bacteriology*, 8th ed., Doudoroff and Palleroni (22) retained only 29 species, which constituted less than 10% of the total number of *Pseudomonas* species ever isolated and named. Many taxonomically ill-defined species were listed in four addenda (22), and there were still others to be studied.

Using a competitive rRNA hybridization method, Palleroni et al. (48) detected five clusters in a group of 35 *Pseudomonas* and 3 *Xanthomonas* strains examined. Because almost no representatives of other bacteria were included, the positions of these five groups within the general framework of gram-negative taxa could not be established.

In this study we explored the intra- and inter-generic rRNA cistron similarities in and with the genus *Pseudomonas* and between *Pseudomonas* and *Xanthomonas* by using the DNA-rRNA hybridization method of De Ley and De Smedt (15). We examined a total of 236 strains, including 23 *Xanthomonas* strains and 65 *Pseudomonas* strains, which were representative of each of the four sections described in the 8th edition of *Bergey's Manual* (22). The remaining 148 strains, belonging to 43 genera and 93 species and subspecies, were included to detect the exact taxonomic locations of the *Pseudomonas* subgroups among the aerobic heterotrophic gram-negative bacteria and the location of *Xanthomonas* with respect to *Pseudomonas*.

MATERIALS AND METHODS

Bacterial strains and growth media. The strains used (Table 1) were checked by plating and by examining

living and Gram-stained cells. For mass cultures, cells were grown in Roux flasks on media solidified with 2 to 2.5% agar for 1 to 3 days at 28°C or at room temperature (*Flavobacterium* and *Aplanobacter* only). The compositions of the growth media used are listed in Table 2. In some cultures we discovered two different colony types, which we named t₁ and t₂; when these two types displayed different soluble protein electropherograms (K. Kersters, unpublished data), they were grown and hybridized separately. Otherwise, only one of the types was included.

Preparation of ¹⁴C-labeled rRNA. [¹⁴C]rRNAs were prepared from type strains *Pseudomonas fluorescens* ATCC 13525, *Pseudomonas acidovorans* ATCC 15668, *Pseudomonas solanacearum* NCPPB 325 (= ATCC 11696), and *Xanthomonas campestris* NCPPB 528 as described previously (15).

Preparation of high-molecular-weight DNA. DNA was prepared either by the method of Marmur (40) or by a combination of the methods of Marmur (40) and Kirby et al. (34, 35), as described by De Ley et al. (14). The final purification was carried out through a CsCl gradient (15). Several gram-positive and coryneform organisms lysed readily in the solvent described by Crombach (9).

Fixation of single-stranded high-molecular-weight DNA on membrane filters. We used the fixation procedure described by De Ley and Tytgat (18) and type SM 11309 Sartorius membrane filters. The filters were loaded with DNA and preserved at 4°C in vacuo (15).

Saturation-hybridization between ¹⁴C-labeled rRNA and filter-fixed DNA: thermal stability of the DNA-rRNA hybrids. The basic aspects of the hybridization conditions used, the effect of ribonuclease on hybridization, the effect of hybridization temperature on DNA leaching, and the conditions of saturation-hybridization, as well as other relevant aspects, have been described previously (15, 18).

Chemical determination of DNA on filters. After simulation of the hybridization step, as described by De Smedt and De Ley (15), each DNA was released from its filter by the method of Meys and Schilperoord (41) and was determined by the method of Burton (8).

DNA base composition. The average guanine-plus-cytosine (G+C) content (moles percent) of each genome DNA was measured by the thermal denaturation method (19) and was calculated by the equation of Marmur and Doty (39). In a limited number of cases, the G+C content was calculated from the ratio of absorbance at 260 nm to absorbance at 280 nm, as described by De Ley (12). Some of the G+C content data were available in the literature (Table 1).

RESULTS

DNA base composition. The average G+C contents of the strains studied are shown in Table 1.

16S and 23S rRNA fractions. The 23S rRNA fraction can be prepared intact from many bacteria. Figure 1 shows the distribution of the 23S and 16S rRNA peaks from our reference rRNAs. Theoretically, the 23S peak should be twice as large as the 16S peak. This ratio was not always reached for the reference strains. A possible explanation for this is that the 23S rRNA was

TABLE 1. List of organisms studied, designations, strain DNA base compositions, taxonomic status on the Approved Lists, growth media used, and parameters of DNA-rRNA hybrids

Sequence no. ^a	Name as received	Strain	G+C content (mol%)	Type status (Approved Lists)	Growth medium ^b	¹⁴ C-labeled 23S rRNA from:							
						<i>Pseudomonas fluorescens</i> ATCC 13525 [†]		<i>Pseudomonas acidovorans</i> ATCC 15668 [†]		<i>Pseudomonas solanacearum</i> NCPPB 325 [†]		<i>Xanthomonas campestris</i> NCPPB 528 [†]	
						<i>T_m</i> (°C)	% rRNA binding	<i>T_m</i> (°C)	% rRNA binding	<i>T_m</i> (°C)	% rRNA binding	<i>T_m</i> (°C)	% rRNA binding
						<i>T_m</i> (°C)	% rRNA binding	<i>T_m</i> (°C)	% rRNA binding	<i>T_m</i> (°C)	% rRNA binding	<i>T_m</i> (°C)	% rRNA binding
Genus <i>Pseudomonas</i>													
1	<i>Pseudomonas fluorescens</i> biotype A	ATCC 13525	60.2 ^c	Type	Z5	81.0	0.14	61.0	0.10	62.5	0.09	69.0	0.08
2	<i>Pseudomonas fluorescens</i> biotype B	ATCC 17815	62.8 ^c		B	80.0	0.12	61.5	0.08				
3	<i>Pseudomonas fluorescens</i> biotype C	ATCC 17571			B	80.5	0.10	61.0	0.06				
4	<i>Pseudomonas chlororaphis</i>	CCEB 559	63.3 ^c	Type	B	77.0	0.10						
5	<i>Pseudomonas aureofaciens</i>	CCEB 518	62.8 ^c	Type	B	78.5	0.11						
6	<i>Pseudomonas syringae</i>	NCPPB 281	59.9 ^c	Type	Z3	78.0	0.14	59.0	0.08				
7	<i>Pseudomonas syringae</i>	NCPPB 1328	59.3 ^c		Z5	78.0	0.15						
8	<i>Pseudomonas aeruginosa</i>	CCEB 481	66.8 ^c	Type	B	76.5	0.12	59.5	0.07	61.5	0.06	66.5	0.08
9	<i>Pseudomonas putida</i> biotype A	ATCC 12633	62.3 ^c	Type	B	77.5	0.16	59.5	0.09			69.0	0.11
10	<i>Pseudomonas putida</i> biotype B	ATCC 17430	60.2 ^d		B	79.5	0.13	61.0	0.08			69.0	0.11
11	<i>Pseudomonas stutzeri</i>	ATCC 17588	64.5 ^c	Type	B	77.5	0.15						
12	<i>Pseudomonas stutzeri</i>	NCTC 10475	64.5 ^c		B	77.5	0.12	60.0	0.09			68.0	0.06
13	<i>Pseudomonas mendocina</i>	ATCC 25411	62.8 ^c	Type	B	77.5	0.11	61.5	0.07				
14	<i>Pseudomonas cichorii</i>	NCPPB 906	59.7 ^c		B	76.0	0.13						
15	<i>Pseudomonas cichorii</i>	NCPPB 1512	60.3 ^c		B	77.5	0.13	61.5	0.09				
16	<i>Pseudomonas alcaligenes</i>	ATCC 14909	66.3 ^d	Type	B	76.5	0.11	61.0	0.07				
17	<i>Pseudomonas pseudocalcali-genes</i>	ATCC 17440	62.5 ^c	Type	B	77.0	0.10	61.5	0.07				
18	<i>Pseudomonas cepacia</i>	ATCC 17759	67.4 ^c		B	64.5	0.07	71.0	0.07	76.0	0.09		
19	<i>Pseudomonas cepacia</i>	ATCC 25416	67.5 ^c		Z5	64.0	0.07	72.5	0.08	76.0	0.08		
20	<i>Pseudomonas cepacia</i>	NCTC 10661	66.9 ^c		Z5	62.0	0.04	72.0	0.08				
21	<i>Pseudomonas marginalis</i>	ATCC 10248	68.8 ^c	Type of <i>Pseudomonas gladioli</i>	Z5	63.5	0.06	69.5	0.06	76.0	0.07		
22	<i>Pseudomonas caryophylli</i>	NCPPB 2151	63.3 ^c	Type	Z5	62.0	0.06	70.5	0.06	75.5	0.09		
23	<i>Pseudomonas acidovorans</i>	ATCC 15668	66.6 ^c	Type	B	61.0	0.07	80.5	0.12	70.5	0.07	61.0	0.04
24	<i>Pseudomonas acidovorans</i>	ATCC 17476	68.4 ^c		Z5			81.0	0.10				

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25	<i>Pseudomonas acidovorans</i>	ATCC 17406	68.4 ^c	Type	Z5	61.0	0.06	79.5	0.12		
26	<i>Pseudomonas acidovorans</i>	ATCC 15005	68.5 ^c		B	61.0	0.06	81.0	0.10	72.5	0.09
27	<i>Pseudomonas acidovorans</i>	ATCC 9355 ₁	67.9 ^c		B			81.0	0.10		
28	<i>Pseudomonas testosteroni</i>	NCTC 10698	62.5 ^c		B	62.0	0.13	76.5	0.17	72.0	0.15
29	<i>Pseudomonas testosteroni</i>	ATCC 17407	64.5 ^c		B	61.0	0.17	77.5	0.17		
30	<i>Pseudomonas testosteroni</i>	ATCC 17409	63.0 ^c		B			77.5	0.15		
31	<i>Pseudomonas testosteroni</i>	ATCC 17510 ₁	62.8 ^c		B			76.0	0.13		
32	<i>Pseudomonas testosteroni</i>	ATCC 17510 ₂	63.2 ^c		B			76.5	0.13		
33	<i>Pseudomonas delafieldii</i>	ATCC 17506 ₂	63.8 ^c		Z5	62.5	0.07	78.0	0.10	72.0	0.09
34	<i>Pseudomonas facilis</i>	ATCC 17695 ₂	65.7 ^c		B			77.5	0.09		
35	<i>Pseudomonas facilis</i>	ATCC 17695 ₁	65.2 ^c	Type	B			77.0	0.09		
36	<i>Pseudomonas facilis</i>	ATCC 11228	64.7 ^c		Z9	60.0	0.07	77.0	0.09		
37	<i>Pseudomonas facilis</i>	ATCC 15376	63.7 ^c		Z9	61.0	0.07	76.5	0.09		
38	<i>Pseudomonas solanacearum</i> biotype 1 ^c	NCPPB 325	66.1 ^c	Type	Z5	63.0	0.05	72.0	0.07	81.5	0.08
39	<i>Pseudomonas solanacearum</i> biotype II	NCPPB 173			Z5					81.0	0.08
40	<i>Pseudomonas solanacearum</i> biotype I	NCPPB 215	66.9 ^f		Z5					81.0	0.10
41	<i>Pseudomonas solanacearum</i> biotype III	NCPPB 253	66.8 ^f		Z5			70.5	0.07	80.5	0.10
42	<i>Pseudomonas solanacearum</i> biotype II	NCPPB 339	67.4 ^f		Z5			72.0	0.04	81.0	0.07
43	<i>Pseudomonas solanacearum</i> biotype I	NCPPB 446	66.9 ^f		Z5					81.5	0.08
44	<i>Pseudomonas solanacearum</i> biotype II	NCPPB 613	68.1 ^c		B			69.5	0.07	80.0	0.08
45	<i>Pseudomonas solanacearum</i> biotype I	NCPPB 787	66.7 ^f		Z5					81.0	0.07
46	<i>Pseudomonas solanacearum</i> biotype I	NCPPB 789	66.9 ^f		Z5					81.0	0.09
47	<i>Pseudomonas solanacearum</i> biotype III	NCPPB 792	67.1 ^f		B					81.0	0.12
48	<i>Pseudomonas solanacearum</i> biotype II	NCPPB 282	67.4 ^c		B			71.0	0.06	80.5	0.07
49	<i>Pseudomonas solanacearum</i> biotype II	NCPPB 909	67.6 ^c		Z5					80.5	0.08
50	<i>Pseudomonas solanacearum</i> biotype II	NCPPB 1019	68.0 ^c		B					81.0	0.09
51	<i>Pseudomonas solanacearum</i> biotype I	NCPPB 1029	66.4 ^f		Z5					81.0	0.09
52	<i>Pseudomonas solanacearum</i> biotype I	3-S 107 (Kel- man)	67.7 ^c		Z5			71.0	0.04	80.5	0.07

TABLE 1—Continued

Sequence no. ^a	Name as received	Strain	G+C content (mol%)	Type status (Approved Lists)	Growth medium ^b	¹⁴ C-labeled 23S rRNA from:									
						<i>Pseudomonas fluorescens</i> ATCC 13525 [†]	<i>Pseudomonas acidovorans</i> ATCC 15668 [†]	<i>Pseudomonas solanacearum</i> NCPPB 325 [†]	<i>Xanthomonas campestris</i> NCPPB 528 [†]						
						<i>T_m</i> (°C)	% rRNA binding	<i>T_m</i> (°C)	% rRNA binding	<i>T_m</i> (°C)	% rRNA binding	<i>T_m</i> (°C)	% rRNA binding		
53	<i>Pseudomonas solanacearum</i> biotype I	25-K 60 (Kel-man)	66.3 ^c		Z5					80.5	0.08				
54	<i>Pseudomonas solanacearum</i> biotype II	81-S 207 (Kel-man)	67.7 ^f		Z5			71.0	0.06	80.5	0.09				
55	<i>Pseudomonas saccharophila</i>	ATCC 15946	67.4 ^c	Type	7			75.5	0.03	71.0	0.05				
56	" <i>Pseudomonas ruhlandii</i> "	ATCC 15749 ₁	68.1 ^c	Type of Alkali-genes ruhlandii	Z5	63.5	0.05	70.5	0.06	73.0	0.06				
57	<i>Pseudomonas palleronii</i>	ATCC 17724 ₁	65.7 ^c	Type	Z5	62.5	0.04	76.0	0.05	72.5	0.04				
58	<i>Pseudomonas flava</i>	DSM 619	66.7 ^c	Type	Z5	58.0	0.02	75.5	0.02	69.0	0.04				
58a	<i>Pseudomonas lemoignei</i>	ATCC 17989	67.2 ^c	Type	Z14			71.0	0.05	74.5	0.04				
59	<i>Pseudomonas maltophilia</i>	ATCC 13637	65.5 ^c	Type	B	67.5	0.09	58.5	0.07	63.0	0.08			76.5	0.10
60	<i>Pseudomonas maltophilia</i>	ATCC 17448	66.8 ^d		Z5	66.0	0.10							78.0	0.10
61	<i>Pseudomonas maltophilia</i>	CIP 5960			Z5									78.0	0.13
62	<i>Pseudomonas maltophilia</i>	ATCC 17806	67.5 ^c		Z5	67.0	0.09			61.0	0.05			77.5	0.13
63	<i>Pseudomonas diminuta</i>	CCEB 513	67.3 ^c	Type	Z5	61.0	0.05	59.0	0.06		0.05			60.5	0.05
64	<i>Pseudomonas vesicularis</i>	ATCC 11426	65.8 ^c	Type	Z5	61.5	0.06	56.0	0.06						
Other gram-negative bacteria															
65	<i>Xanthomonas campestris</i>	NCPPB 528	65.2 ^f	Type	X	69.0	0.07			63.0	0.06			81.0	0.09
66	<i>Xanthomonas campestris</i>	ICPB A121	67.3 ^c		X									81.0	0.10
67	<i>Xanthomonas campestris</i>	ICPB C110	65.9 ^c		X									81.0	0.07
68	<i>Xanthomonas campestris</i>	ICPB C144	69.2 ^c		X									80.5 ^f	0.07
69	<i>Xanthomonas campestris</i>	ICPB C5	66.6 ^c		X									81.0	0.08
70	<i>Xanthomonas campestris</i>	ICPB G1	63.5 ^c		X	66.5	0.04	61.0	0.04					81.5	0.07
71	<i>Xanthomonas campestris</i>	ICPB H110	68.5 ^c		X	66.5	0.05	60.5	0.04					80.5	0.05
72	<i>Xanthomonas campestris</i>	ICPB M16	67.7 ^c		X									81.0	0.08
73	<i>Xanthomonas campestris</i>	ICPB P121	66.5 ^c		X	68.5	0.06	60.5	0.04					81.0	0.06
74	<i>Xanthomonas campestris</i>	ICPB P137	66.0 ^c		X									81.0	0.07
75	<i>Xanthomonas campestris</i>	ICPB P10	66.8 ^c		X									81.0	0.06
76	<i>Xanthomonas campestris</i>	ICPB T11	64.3 ^c		X	67.5	0.06	60.0	0.04					81.0	0.08
77	<i>Xanthomonas campestris</i>	ICPB L1	66.6 ^c		X									80.0	0.07
78	<i>Xanthomonas campestris</i>	ICPB V136	66.4 ^c		X									80.0	0.06

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79	<i>Xanthomonas fragariae</i>	NCPPB 1822	63.3 ^c	Type	X	67.5	0.07	60.5	0.05	63.5	0.05	81.0	0.08
80	<i>Xanthomonas albinopodis</i>	NCPPB 2503	64.5 ^c	Type	Z5	69.0	0.05	60.0	0.05	63.0	0.04	80.0	0.07
81	<i>Xanthomonas axonopodis</i>	NCPPB 457	65.0 ^c		Z3			59.0	0.03			81.0	0.07
82	<i>Xanthomonas ampelina</i>	NCPPB 2217	70.8 ^c		Z3							61.5	0.03
83	<i>Xanthomonas ampelina</i>	P5 (Ridé)	68.1 ^c		Z3							61.0	0.09
84	<i>Xanthomonas ampelina</i>	C13 (Ridé)	68.2 ^c		Z3							63.0	0.09
85	<i>Xanthomonas ampelina</i>	C2' (Ridé)	68.5 ^c		X							63.0	0.09
86	<i>Xanthomonas ampelina</i>	P7 (Ridé)			X							61.0	0.07
87	<i>Xanthomonas ampelina</i>	P6 (Ridé)	68.5 ^c		Z3							63.0	0.07
88	" <i>Aplanobacter populi</i> "	Bt ₃ (Ridé)	65.0 ^c		Z3							81.0	0.06
89	" <i>Aplanobacter populi</i> "	Spm11 (Ridé)	65.2 ^c		Z3				0.05			81.0	0.06
90	" <i>Aplanobacter populi</i> "	Mlj (Ridé)	63.2 ^c		Z3				0.04			81.0	0.09
91	" <i>Aplanobacter populi</i> "	PC3 (Ridé)	64.3 ^c		Z3							80.5	0.07
92	" <i>Aplanobacter populi</i> "	S8 (Ridé)	64.9 ^c		Z3							80.5	0.06
93	" <i>Aplanobacter populi</i> "	175 (Ridé)	65.2 ^c		Z3							80.5	0.06
94	" <i>Aplanobacter populi</i> "	45.51 (Ridé)	63.5 ^c		Z3							81.0	0.08
95	" <i>Aplanobacter populi</i> "	Sma ₄ (Ridé)	65.2 ^f		Z3							81.0	0.08
96	" <i>Aplanobacter populi</i> "	BII (Ridé)	62.0 ^c		Z3							80.5	0.09
97	" <i>Aplanobacter populi</i> "	NCPPB 2432			Z3							80.5	0.08
98	<i>Azotobacter chroococcum</i>	DSM 281	66.3 ^c		E	75.0	0.20					66.0	0.12
99	<i>Azotobacter chroococcum</i>	DSM 369	66.1 ^f		E							67.0	0.13
100	<i>Azotobacter beijerinckii</i>	DSM 367	66.2 ^c		E	75.0	0.21					67.5	0.10
101	<i>Azotobacter vinelandii</i>	NCIB 8660	65.0 ^c		E	75.0	0.10					66.5	0.08
102	<i>Azotobacter vinelandii</i>	DSM 86	66.3 ^f		E	75.5	0.12						
103	<i>Azotobacter paspali</i>	15B (Döbereiner)	63.3 ^c		E								
104	<i>Azotobacter paspali</i>	22B (Döbereiner)	63.7 ^c		E	76.0	0.19						
105	<i>Azotobacter miscellum</i>	ATCC 17962	65.6 ^c		E								
106	<i>Azomonas agilis</i>	DSM 89	52.0 ^g		E								
107	<i>Azomonas agilis</i>	NCIB 8638	53.2 ^f		E	75.0	0.09						
108	<i>Azomonas agilis</i>	NCIB 8637	53.2 ^c		E	75.5	0.10						
109	<i>Azomonas agilis</i>	NCIB 8636	52.6 ^c		E	76.0	0.10						
110	<i>Azomonas agilis</i>	SS4 (Becking)	52.8 ^c		H	76.0	0.10						
111	<i>Azomonas macrocytogenes</i>	NCIB 8700	59.0 ^f	Type of Azoto- bacter macro- cyto- genes	K	76.5	0.13						
112	<i>Azomonas macrocytogenes</i>	NCIB 8702	59.0 ^f		E	76.0	0.14						
113	<i>Azomonas macrocytogenes</i>	NCIB 9128	58.6 ^c		Z12	76.0	0.11						
114	<i>Beijerinckia fluminensis</i>	Hilger	56.2 ^c		Z16	59.5	0.09						
115	<i>Beijerinckia indica</i>	Hilger	57.4 ^c		Z16	60.5	0.07						
116	<i>Derxia gummosa</i>	D	71.4 ^c		Z13	63.0	0.09	69.0	0.05	71.0	0.06	66.0	0.05

TABLE 1—Continued

Sequence no. ^a	Name as received	Strain	G+C content (mol%)	Type status (Approved Lists)	Growth medium ^b	¹⁴ C-labeled 23S rRNA from:							
						<i>Pseudomonas fluorescens</i> ATCC 13525 ^T		<i>Pseudomonas acidovorans</i> ATCC 15668 ^T		<i>Pseudomonas solanacearum</i> NCPPB 325 ^T		<i>Xanthomonas campestris</i> NCPPB 528 ^T	
						<i>T_m</i> (°C)	% rRNA binding	<i>T_m</i> (°C)	% rRNA binding	<i>T_m</i> (°C)	% rRNA binding	<i>T_m</i> (°C)	% rRNA binding
117	<i>Derxia gummosa</i>	D12	72.6 ^c		Z13	62.0	0.07	69.0	0.04	72.5	0.08	65.5	0.04
118	<i>Aeromonas hydrophila</i>	AB833 (Lau-trop)	58.6 ^c		F	67.5	0.17						
119	<i>Aeromonas punctata</i> subsp. <i>caviae</i>	NCIB 9232	59.1 ^c		F	67.0	0.17						
120	<i>Aeromonas salmonicida</i>	NCMB 833	58.3 ^h		F	70.0	0.16						
121	<i>Aeromonas hydrophila</i>	NCIB 9233	58.9 ^c		F	70.0	0.16						
122	<i>Plesiomonas shigelloides</i>	NCTC 10360	52.0 ^c	Type	Z5	66.0	0.16					66.0	0.15
123	<i>Photobacterium phosphoreum</i>	NCMB 1282	41.1 ^c	Type	T	67.0	0.21					67.0	0.28
124	<i>Photobacterium mandapamensis</i>	NCMB 391	40.7 ^c		T	67.0	0.21						
125	<i>Photobacterium mandapamensis</i>	NCMB 1198	41.6 ^c		T	64.5	0.22					67.5	0.27
126	<i>Lucibacterium harveyi</i>	NCMB 1	45.0 ⁱ		T	66.0	0.13					65.5	0.09
127	<i>Lucibacterium harveyi</i>	NCMB 24	46.5 ⁱ		T	64.5	0.12					67.0	0.10
128	<i>Lucibacterium harveyi</i>	NCMB 1280	45.0 ^c	Type	T	65.5	0.15					66.0	0.10
129	<i>Vibrio albensis</i>	NCMB 41	48.1 ^c	Type	Z10	67.0	0.13						
130	<i>Vibrio</i> sp. (not <i>Vibrio cholerae</i>)	E509 (Colwell)	49.0 ^c		Z5	66.5	0.16	59.5	0.13				
131	<i>Vibrio parahaemolyticus</i>	FC1011 (Colwell)	47.9 ^c		Z5							65.0	0.17
132	<i>Vibrio fischeri</i>	NCMB 1281	38.4 ^c	Type	T	64.5	0.20					65.0	0.16
133	<i>Vibrio fischeri</i>	NCMB 1274	45.5 ⁱ		T	67.0	0.22						
134	<i>Vibrio fischeri</i>	NCMB 25	38.6 ^c		T	66.5	0.19						
135	<i>Vibrio anguillarum</i>	ATCC 19264	45.4 ^c	Type	Z11	66.5	0.14	59.5	0.12				
136	<i>Beneckea nereida</i>	ATCC 25917	47.8 ^c		Z11	67.5	0.18					66.5	0.15
137	<i>Beneckea campbellii</i>	ATCC 25920	50.3 ^c	Type	Z11	68.0	0.10					66.5	0.19
138	<i>Beneckea natriegens</i>	ATCC 14048	46.4 ^j	Type	Z11	66.5	0.11						
139	<i>Beneckea pelagia</i>	ATCC 25916	46.6 ^c	Type	Z11	69.0	0.23						
140	<i>Beneckea nigrapulchrituda</i>	ATCC 27043	45.9 ^c	Type	Z11	67.0	0.10						
142	<i>Alteromonas haloplanktis</i>	ATCC 14393	41.5 ^c	Type	Z11	62.5	0.13						
143	<i>Alteromonas haloplanktis</i>	ATCC 19855	42.1 ^c		Z11	66.0	0.15						
144	<i>Alteromonas communis</i>	ATCC 27118	46.7 ^c	Type	T	72.0	0.19						
145	<i>Alteromonas vaga</i>	ATCC 27119	47.9 ^c	Type	T	71.5	0.16						
146	<i>Alteromonas macleodii</i>	ATCC 27126	46.4 ^c	Type	T	67.0	0.09	62.0	0.10				

Continued on next page

TABLE 1—Continued

Sequence no. ^a	Name as received	Strain	G+C content (mol%)	Type status (Approved Lists)	Growth medium ^b	¹⁴ C-labeled 23S rRNA from:							
						<i>Pseudomonas fluorescens</i> ATCC 13525 ^T	<i>Pseudomonas acidovorans</i> ATCC 15668 ^T	<i>Pseudomonas solanacearum</i> NCPPB 325 ^T	<i>Xanthomonas campestris</i> NCPPB 528 ^T				
						<i>T_m(°C)</i>	<i>T_m(°C)</i>	<i>T_m(°C)</i>	<i>T_m(°C)</i>	% rRNA binding	% rRNA binding	% rRNA binding	% rRNA binding
190	<i>Janthinobacterium lividum</i>	HD (Sneath)	66.0 ^c		H	64.5				0.11			
191	<i>Chromobacterium violaceum</i>	NCTC 9757	67.2 ^c	Type	H	64.5		72.0	64.0	0.11	0.18		0.14
192	<i>Chromobacterium violaceum</i>	NCTC 9371	66.1 ^c		H								
193	<i>Chromobacterium violaceum</i>	NCTC 9370	66.4 ^c		H		70.0				0.15		
194	<i>Chromobacterium violaceum</i>	NCTC 8683	65.2 ^c		H		70.0				0.15		
195	<i>Chromobacterium violaceum</i>	NCTC 9695	65.2 ^c		H		67.5				0.15		
196	<i>Chromobacterium violaceum</i>	NCTC 9374	65.9 ^c		H		67.0	70.5			0.15		
199	<i>Acetobacter aceti</i>	Ch31	59.5 ^c		N	58.5	58.5	60.0	58.5	0.06	0.04		0.05
200	<i>Acetobacter aceti</i> subsp. <i>aceti</i>	NCIB 8621t ₁	58.7 ^c	Type	N	57.0				0.04			
201	<i>Acetobacter aceti</i> subsp. <i>xylinum</i>	NCIB 8623	55.1 ^f		N				60.0				0.05
202	<i>Acetobacter pasteurianus</i> subsp. <i>estunensis</i>	E	62.2 ^c		N	59.0	58.5			0.05	0.04		
203	<i>Acetobacter rancens</i>	23k1 ⁺	55.4 ^c		Z2	58.5				0.11			
204	" <i>Acetobacter aurantius</i> "	IFO 3248	58.4 ^c		N	57.5			61.0	0.10		0.08	
205	" <i>Acetobacter aurantius</i> "	IFO 3246t ₁	56.3 ^c		N	57.0			61.0	0.08		0.09	
206	<i>Gluconobacter oxydans</i> subsp. <i>suboxydans</i>	NCIB 7069	62.9 ^f		N				57.5			0.08	
207	<i>Gluconobacter oxydans</i> subsp. <i>suboxydans</i>	NCIB 9108	56.0 ^c		N	57.5	56.5			0.07	0.06		
208	<i>Gluconobacter oxydans</i> subsp. <i>suboxydans</i>	NCIB 9013	57.9 ^c	Type	N	58.5	59.5			0.07	0.09		
209	<i>Gluconobacter oxydans</i> subsp. <i>oxydans</i>	NCIB 9099	60.7 ^c		N				60.5		0.06		
210	<i>Gluconobacter oxydans</i> subsp. <i>industrius</i>	NCIB 8086	60.6 ^c		N	58.0				0.07			
211	<i>Gluconobacter oxydans</i> subsp. <i>melanogenes</i>				N								
212	<i>Frateuria aurantia</i>	IFO 3247	63.6 ^c		N	66.5			71.5	0.12		0.15	
213	<i>Frateuria aurantia</i>	IFO 13333	62.2 ^c		N				71.0			0.13	
214	<i>Frateuria aurantia</i>	IFO 3249	63.4 ^c		N	66.0			72.0	0.10		0.11	
215	<i>Frateuria aurantia</i>	IFO 13330	63.1 ^c		N	65.0			72.5	0.13		0.14	
216	<i>Rhizobium leguminosarum</i>	4.1	62.5 ^c	C			56.0				0.05		
217	<i>Agrobacterium tumefaciens</i>	ATCC 11156	60.8 ^c	A		60.0				0.07			
218	<i>Agrobacterium tumefaciens</i>	ICPB T111	60.6 ^c	A		59.0	56.0		59.5	0.07	0.04		0.06
	<i>Agrobacterium tumefaciens</i>	CIP 67.1	60.2 ^c	T									

Continued on next page

219	<i>Agrobacterium rhizogenes</i>	ICPB TR7	61.4 ^c	A	59.5	0.05		
226	<i>Aquaspirillum iersonii</i> subsp. <i>vulgatum</i>	NCIB 9071	62.3 ^c	Z7			59.0	0.10
227	<i>Aquaspirillum polymorphum</i>	NCIB 9072	63.7 ^c	Z7	Type		59.0	0.09
229	<i>Rhodopseudomonas sphaeroides</i>	NCIB 8253	68.4 ^c	Z4	Type	0.06	57.5	0.05
230	<i>Rhodopseudomonas capsulata</i>	NCIB 8254	65.2 ^c	Z4	Type	0.07	56.0	0.05
231	<i>Rhodopseudomonas palustris</i>	NCIB 8252	67.2 ^c	Z4	Type	0.04	57.0	0.04
237	<i>Campylobacter jejuni</i>	JJ91	29.8 ^c			0.07		
238	<i>Campylobacter fetus</i> subsp. <i>jejuni</i>	M ₂	34.0 ^c			0.10		
239	<i>Zoogloea ramigera</i>	NCTC 10482	64.1 ^c	Z5	Type	0.07	56.0	0.04
240	<i>Agaracterium alginicum</i>	NCMB 886	53.2 ^c	B		0.10	60.0	0.08
241	<i>Zymomonas mobilis</i> subsp. <i>mobilis</i>	Z ₁	48.8 ^c	Z15		0.07		
242	<i>Paracoccus denitrificans</i>	ATCC 19367	67.4 ^c	Z5		0.03	59.0	0.03
243	<i>Flavobacterium meningosepticum</i>	NCTC 10588	36.1 ^m			0.07		
Gram-positive bacteria								
244	" <i>Flavobacterium flavescens</i> "	NCIB 8187	67.2 ^c	Z5		0.04		
245	" <i>Flavobacterium esteroaromaticum</i> "	NCIB 8186	65.1 ^c	B	Type	0.04		
246	" <i>Flavobacterium suaveolens</i> "	NCIB 8188	67.1 ^c	Z5		0.02		
247	<i>Bacillus subtilis</i>	SB556	44.7 ^c	B		0.10		
248	<i>Bacillus megatherium</i>	899 thy ⁻	39.9 ^c	B		0.11		
249	<i>Arthrobacter oxydans</i>	CBRI 21010	62.4 ⁿ	B		0.04		
250	<i>Corynebacterium insidiosum</i>	Joubert A		B		0.05		

^a Our sequence numbers are not strain numbers.^b See Table 2.^c G+C content was determined from the thermal denaturation temperature of the genome DNA, as described in the text. Some of these data have been published previously; other data are either new values or averages of previous values and values determined in this study.^d From reference 38.^e See reference 28.^f G+C content was calculated from the ratio of optical density at 260 nm to optical density at 280 nm (12).^g From reference 60.^h From reference 56.ⁱ From reference 29.^j From reference 7.^k From reference 43.^l From reference 6.^m From reference 44.ⁿ From reference 57.

TABLE 2. Compositions of the growth media used for the strains of bacteria from which DNAs were isolated^a

Component	% (wt/vol) in the following media: ^b							
	Z9	Z10	Z11	Z12	Z13	Z14	Z15	Z16
Glucose				1	2		2	1
Starch	0.2							
Yeast extract (Difco)	0.1		0.3	0.05			0.5	
Meat extract (Oxoid)		1						
Peptone (Oxoid)		1						
Proteose peptone (Oxoid)			1					
Tryptose (Oxoid)	0.5							
KH ₂ PO ₄	0.02			0.02		0.208		0.49
K ₂ HPO ₄				0.08	0.1	0.256		
KCl			0.07					
Na ₂ HPO ₄								0.07
NaCl			2.4					
Na ₂ MoO ₄ · 2H ₂ O				Trace				0.00025
FeCl ₃ · 6H ₂ O				Trace				
FeSO ₄ · 7H ₂ O					0.0005			
Fe ₂ (SO ₄) ₃ (aq.)								0.00025
CaCl ₂ · 2H ₂ O						0.0005		
CaSO ₄ · 2H ₂ O				0.01	0.02			
MgSO ₄ · 7H ₂ O	0.01		0.7	0.02	0.02	0.05		0.0125
MgCl ₂ · 6H ₂ O			0.53					
MnSO ₄ · H ₂ O								0.00025
NH ₄ Cl						0.01		
Ammonium acetate					0.1			
Sodium acetate · 3H ₂ O	0.03							
Sodium citrate · 2H ₂ O	0.05							
Ferric ammonium citrate						0.005		
Sodium glutamate · H ₂ O	0.1							
Sodium succinate · 7H ₂ O	0.2							
Succinic acid						0.178		

^a The compositions of media A to Z8 have been given previously (21, 26).

^b All media except Z10 and Z15 were made with distilled water. Medium Z10 was made with 25% distilled water and 75% artificial seawater; medium Z15 was made with aged filtered seawater. The pH values of some of the media were as follows: Z10, 7.3; Z11, 7 to 7.2; Z12, 7.2; Z14, 6.8; Z16, 6.0.

partially nicked and the 16S peak was contaminated with fragmented 23S rRNA, as was the case with the 16S peaks of *Agrobacterium tumefaciens* ICPB TT111 and *Agrobacterium rhizogenes* ICPB TR7 (21). Most hybridizations were carried out with 23S rRNA, and there was no evidence that this rRNA fraction was contaminated. There was no noticeable difference in thermostability between 23S rRNA-DNA and 16S rRNA-DNA hybrids (21).

Comparisons of the rRNA hybrids. All of our results are shown in Table 1. rRNA similarities are expressed by the following two parameters: (i) the midpoint in degrees Celsius of the thermal denaturation curve [$T_{m(e)}$]; and (ii) the percent rRNA binding, which was calculated from the amount of rRNA (in micrograms) duplexed to 100 μ g of DNA fixed on a membrane filter. Both parameters were calculated from the thermal denaturation curves of the DNA-rRNA hybrids. A few examples of such curves are shown in Fig. 2. For each reference rRNA, the $T_{m(e)}$ value for each organism examined was plotted versus its

percent rRNA binding; we call the resulting plots rRNA similarity maps (17, 20, 21, 26). Our data are summarized in the rRNA similarity maps shown in Fig. 3 to 6.

DISCUSSION

The value and importance of $T_{m(e)}$ and percent rRNA binding have been discussed and illustrated previously (15, 17, 20, 21, 26) and need not be described here. Experience has shown that $T_{m(e)}$ is the most important parameter and seems to be correlated directly with the overall phenotypic similarities among the organisms concerned. Taxonomically, this parameter reveals similarities at the generic and suprageneric levels and has helped us to detect misnamed strains and, in many cases, their taxonomic locations (17, 20, 21, 26). Figures 7 and 8 show the essence of our findings as expressed in $T_{m(e)}$ dendrograms.

Genus *Pseudomonas*. Each phenotypically and genotypically well-described and reliable genus occupies a well-delineated area around the refer-

FIG. 1. Fractionation of [^{14}C]rRNAs from *Pseudomonas fluorescens* ATCC 13525^T, *Pseudomonas acidovorans* ATCC 15668^T, *Pseudomonas solanacearum* NCPPB 325^T, and *Xanthomonas campestris* NCPPB 528^T on a 15 to 30% sucrose gradient. The method used has been described previously (15).

ence strain on the rRNA similarity map (17, 20, 21, 26). When the DNAs of many strains are included, the size of this area is a measure of the heterogeneity of the genus. The most heterogeneous genus examined so far, *Acetobacter* (26), has a $T_{m(e)}$ range of about 5°C and a percent rRNA binding range of 0.2%. The hybridization data (Table 1) and the similarity maps (Fig. 3 to 6) show that *Pseudomonas* occurs all over the maps. Nevertheless, we detected five discrete groups. The simplified $T_{m(e)}$ dendrogram in Fig. 7 summarizes the relationships. The first group lies in the vicinity of *Pseudomonas fluorescens* type strain ATCC 13525. We call this group the *Pseudomonas fluorescens* rRNA branch, and it consists of the named strains (Table 1) and the type strains of *Pseudomonas fluorescens*, *Pseudomonas chlororaphis*, *Pseudomonas aureofaciens*, *Pseudomonas syringae*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Pseudomonas mendocina*, *Pseudomonas cichorii*, *Pseudomonas alcaligenes*, and *Pseudomonas pseudoalcaligenes*. The $T_{m(e)}$ values of this group range from 76.0 to 81.0°C, and its rRNA binding values range from 0.10 to 0.16%; thus, this group is a rather tight cluster and is about the size of a genus. In this group we find all of the *Pseudomonas* species from *Pseudomonas* section I in the 8th edition of *Bergey's Manual* (22) and also from rRNA group I of Palleroni et al. (48). The only confusing species in this group is *Pseudomonas pseudoalcaligenes*. Both by the competition method of Palleroni et al. (48) and by DNA-DNA hybridizations (46, 51) this species was classified in the *Pseudomonas fluorescens* group. However, in the 8th edition of *Bergey's Manual* (22) *Pseudo-*

monas pseudoalcaligenes was classified in *Pseudomonas* section II, although only some of its strains accumulate poly- β -hydroxybutyrate (51, 59). Later, Palleroni (45) moved this species back to the *Pseudomonas fluorescens* rRNA homology group. According to our data *Pseudomonas pseudoalcaligenes* belongs indeed in the *Pseudomonas fluorescens* rRNA branch.

Our rRNA method is usually not able to detect differences among the species within a genus. Here too it allowed definite differentiation neither between the fluorescent species and the nonfluorescent species nor between the plant-pathogenic species and the saprophytic species. Nevertheless, when we compared the $T_{m(e)}$ values of the strains in our *Pseudomonas fluorescens* rRNA branch (Table 1) with the DNA competition values at $T_m - 25^\circ\text{C}$ (T_m : midpoint in degrees Celsius of the thermal denaturation of native DNA) from the DNA-DNA hybridization studies of Palleroni et al. (46), there was a quite reasonable correlation; e.g., *Pseudomonas fluorescens* biotypes B, C, and E (now *Pseudomonas aureofaciens*) show both high $T_{m(e)}$ and high DNA similarities compared with *Pseudomonas fluorescens* biotype A, whereas *Pseudomonas aeruginosa*, *Pseudomonas alcaligenes*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas cichorii*, and *Pseudomonas mendocina* gave lower values with both techniques. From the correlation between both methods, we estimated that 1°C of $T_{m(e)}$ corresponds to roughly 14% DNA homology or that a fine differentiation of strains by means of DNA-DNA hybridization under stringent conditions at T_{OR} (27) is meaningful only in the top 7 to 8°C [at $T_{m(e)}$ values of

FIG. 2. Examples of denaturation curves between 23S [^{14}C]rRNA from *Pseudomonas fluorescens* ATCC 13525^T and filter-fixed DNAs from various bacteria. The $T_{m(e)}$ values are indicated by arrows. Σcp50m , Sum of the radioactive rRNA released (in counts per 50 min) at any given temperature.

FIG. 3. Similarity map of the DNA-rRNA hybrids between the 23S [^{14}C]rRNA fraction of *Pseudomonas fluorescens* ATCC 13525^T and DNAs from a variety of bacteria. $T_{m(e)}$ and percent rRNA binding are defined in the text. To simplify the drawing, each strain is represented by a sequence number (see Table 1). Since this figure contains a very large number of data, the sequence numbers are replaced by dots below 72°C for clarity. The area of all strains belonging to the same phenotypic taxa is indicated by a solid line. These areas locate the taxa on the map. Not all DNAs were hybridized with all reference rRNAs, because reciprocal hybridizations revealed identical $T_{m(e)}$ values. Abbreviations: *Alteromonas comm./vag.*, *Alteromonas communis*-*Alteromonas vaga*; *Enterobact.*, *Enterobacteriaceae*; marine "Alc.", marine *Alcaligenes*; *Aplanob.*, "*Aplanobacter*"; *Xanthom.*, *Xanthomonas*; *P. solan. rRNA branch*, *Pseudomonas solanacearum* rRNA branch; "Alc." eutroph., *Alcaligenes eutrophus*; "Alc." parad., *Alcaligenes paradoxus*; "P." ruhl., "*Pseudomonas ruhlandii*"; *Bordet.*, *Bordetella*; *Janthinobact.-Chromobact.*, *Janthinobacterium*-*Chromobacterium*; "P." dimin.-vesic., *Pseudomonas diminuta*-*Pseudomonas vesicularis*; *Rhodops.*, *Rhodopseudomonas*; *Agrobact.*, *Agrobacterium*; *P. acid. rRNA branch*, *Pseudomonas acidovorans* rRNA branch; *Zymom.*, *Zymomonas*; *Acetic acid bact.*, acetic acid bacteria; *Corynebact.*, *Corynebacterium*; *Fl. mening.*, *Flavobacterium meningosepticum*; *Campyl.*, *Campylobacter*; *Arthrob.*, *Arthrobacter*; *Gram + bact.*, gram-positive bacteria.

FIG. 4. Similarity map of the DNA-rRNA hybrids between the 23S [^{14}C]rRNA fraction of *Pseudomonas acidovorans* ATCC 15668^T and DNAs from a variety of bacteria. For further details, see the legend to Fig. 3. Abbreviations: P. acid, *Pseudomonas acidovorans*; P. delaf., *Pseudomonas delafieldii*; P. fac., *Pseudomonas facilis*; P. pall., *Pseudomonas palleronii*; "Alc." parad., *Alcaligenes paradoxus*; P. sacch., *Pseudomonas saccharophila*; P. test., *Pseudomonas testosteroni*; "P." ruhl., "*Pseudomonas ruhlandii*"; "Alc." eutrophus, *Alcaligenes eutrophus*; P. fluor. rRNA branch, *Pseudomonas fluorescens* rRNA branch; PARACOC., *Paracoccus*; APLANOB., "*Aplanobacter*"; marine "Alc.," marine *Alcaligenes*; AQUASPIR., *Aquaspirillum*; Acetic acid bact., acetic acid bacteria; "P." diminuta-vesicularis, *Pseudomonas diminuta*-*Pseudomonas vesicularis*; RHIZOB.-AGROBACT., *Rhizobium*-*Agrobacterium*.

73 to 81°C] of our DNA-rRNA similarity maps. All other pseudomonads examined are more removed from *Pseudomonas fluorescens*, with $\Delta T_{m(e)}$ values of $\geq 13.5^\circ\text{C}$ (Fig. 7).

The second *Pseudomonas* group is located around *Pseudomonas acidovorans* type strain

ATCC 15668 (Table 1 and Fig. 4). The following taxa occur in the $T_{m(e)}$ range from 75.5 to 78.0°C $T_{m(e)}$ but have quite different percent rRNA binding values: *Pseudomonas acidovorans*, *Pseudomonas testosteroni*, *Pseudomonas delafieldii*, *Pseudomonas facilis*, *Pseudomonas pal-*

FIG. 5. Similarity map of the DNA-rRNA hybrids between the 23S [^{14}C]rRNA fraction of *Pseudomonas solanacearum* NCPPB 325^T and DNAs from a variety of bacteria. For further details, see the legend to Fig. 3. Abbreviations: *P. solan.*, *Pseudomonas solanacearum*; "Alc." eutrophus, *Alcaligenes eutrophus*; *P. margin.*, *Pseudomonas marginata*; *P. caryoph.*, *Pseudomonas caryophylli*; "P." ruhl., "*Pseudomonas ruhlandii*"; "Alc." parad., *Alcaligenes paradoxus*; *P. acid. rRNA branch*, *Pseudomonas acidovorans* rRNA branch; *P. fluor. rRNA branch*, *Pseudomonas fluorescens* rRNA branch.

FIG. 6. Similarity map of the DNA-rRNA hybrids between the 23S [^{14}C]rRNA fraction of *Xanthomonas campestris* NCPPB 528^T and DNAs from a variety of bacteria. For further details, see the legend to Fig. 3. Abbreviations: P. FLUOR. rRNA branch, *Pseudomonas fluorescens* rRNA branch; P. ACID. & P. SOLAN. rRNA branches, *Pseudomonas acidovorans* and *Pseudomonas solanacearum* rRNA branches.

leronii, *Pseudomonas saccharophila*, and *Pseudomonas flava*. The five *Pseudomonas acidovorans* strains, the five *Pseudomonas testosteroni* strains, and the four *Pseudomonas facilis* strains examined each form a small tight cluster quite separate from all of the other clusters. Each cluster is probably a real species. The seven species mentioned above are only part of *Pseudomonas* section III in the 8th edition of *Bergey's Manual* (22). We call this group the *Pseudomonas acidovorans* rRNA branch. *Pseudomonas lemoignei* and *Pseudomonas solanacearum* are also included in *Pseudomonas* section III in *Bergey's Manual*, but these taxa belong elsewhere (see below) as does

"*Pseudomonas ruhlandii*," which was misnamed and is now an authentic member of the genus *Alcaligenes* (2; K. Kersters, P. Segers, and J. De Ley, manuscript in preparation).

The total $T_{m(e)}$ range of the *Pseudomonas acidovorans* branch (5.5°C) is comparable to the range of the *Pseudomonas fluorescens* rRNA branch (see above). Four of the species in this branch (*Pseudomonas palleronii*, *Pseudomonas facilis*, *Pseudomonas flava*, and *Pseudomonas saccharophila*) are able to grow autotrophically with hydrogen. Based on $T_{m(e)}$ values, these species cannot be differentiated from *Pseudomonas testosteroni* and *Pseudomonas delafieldii*, which are not able to grow under these

FIG. 7. rRNA cistron similarities [expressed as $T_{m(e)}$, in degrees Centigrade] within and between the genera *Pseudomonas* and *Xanthomonas*. The solid bars indicate the extents of the individual rRNA groups. The branching levels are average values and were calculated by the average unweighted pair group method (58).

conditions. Based on percent rRNA binding, 0.095% is a border separating the H_2 oxidizers from the nonoxidizers. The $T_{m(e)}$ range (5.5°C) suggests that measurable DNA homologies

among these species can be expected. Indeed, there is high DNA homology (83%) between *Pseudomonas delafeldii* and *Pseudomonas facilis*; the DNA homology between *Pseudomonas*

FIG. 8. Relationships among rRNA cistrons of various taxa of gram-negative bacteria, expressed as $T_{m(e)}$ values (in degrees Centigrade). The solid bars indicate the extents of the individual rRNA branches. Details of some branches (dotted lines) will be described in future papers. All branching levels were calculated from the results presented in this paper and previous papers (17, 20, 21, 26) and from unpublished data of J. De Ley, J. De Smedt, R. Tytgat, and P. De Vos, P. Segers and J. De Ley, M. Gillis and J. De Ley, M. Bauwens and J. De Ley, A. Van Landschoot and J. De Ley, and D. C. Jordan (personal communication).

acidovorans and *Pseudomonas testosteroni* is 33% (50), and the DNA homology between *Pseudomonas palleronii* and *Pseudomonas flava* is about 30% (3). Additional DNA hybridizations among the members of this group might yield interesting results.

A third group of organisms is located in the vicinity of *Pseudomonas solanacearum* (Table 1 and Fig. 5). We included 17 strains of *Pseudomonas solanacearum* in our study. Hayward (28) proposed four biotypes for this species, which could be distinguished from each other by denitrification and acid formation from carbohydrates. Eight of our strains belonged to biotype I, seven belonged to biotype II, and two belonged to biotype III. We expected that the rRNA hybridization method would not differentiate among these biotypes, and indeed all strains formed a tight cluster on the rRNA similarity map (Fig. 5) within a $T_{m(e)}$ range of 1.5°C and an rRNA binding range of 0.05%; these organisms are quite separate from all of the other taxa studied. Our results agree with the results of Palleroni and Doudoroff (47), who found that the phenotypic and genotypic features of the members of this species are very similar; these authors showed that the DNA homology among strains from the four biotypes is at least 54% (average 75%) and that the phenotypic similarities are also high (simple matching coefficient, 85 to 100%) (47). The closest relatives are three other phytopathogenic species (*Pseudomonas cepacia*, *Pseudomonas marginata*, and *Pseudomonas caryophylli*) at a $T_{m(e)}$ of 76.0°C and an rRNA binding value of 0.07 to 0.09%. The DNA-DNA similarities among *Pseudomonas cepacia* (= *Pseudomonas multivorans*), *Pseudomonas marginata*, and *Pseudomonas caryophylli* are at least 24% (5). The DNA similarity between *Pseudomonas solanacearum* and any one of the three species mentioned above is zero or, at most, very low (47). *Pseudomonas lemoignei* is at the lower end of the complex. We call this entire group the *Pseudomonas solanacearum* rRNA branch. The *Pseudomonas acidovorans* and *Pseudomonas solanacearum* rRNA branches are linked at a $T_{m(e)}$ of about 71°C.

Our fourth group consists of *Pseudomonas maltophilia*, *Xanthomonas* spp., and "*Aplanobacter populi*" (not on the Approved Lists).

(i) **Genus *Xanthomonas*.** We used the type strains and other strains of the five *Xanthomonas* species mentioned in the 8th edition of *Bergey's Manual* (23) (i.e., *Xanthomonas campestris*, *Xanthomonas fragariae*, *Xanthomonas axonopodis*, *Xanthomonas albilineans*, and *Xanthomonas ampelina*). Furthermore, a number of our strains which are now placed in the species *Xanthomonas campestris* have in the

past been placed in other species, the names of which are no longer in use, such as "*Xanthomonas alfalfae*," "*Xanthomonas cassava*," "*Xanthomonas celebensis*," "*Xanthomonas corylina*," "*Xanthomonas geranii*," "*Xanthomonas hyacinthi*," "*Xanthomonas lespedezae*," "*Xanthomonas maculifoliigardeniae*," "*Xanthomonas pelargonii*," "*Xanthomonas poinsettiaeicola*," "*Xanthomonas pruni*," "*Xanthomonas taraxaci*" and "*Xanthomonas vesicatoria*." All of the species studied except one (see below) formed an extremely tight cluster within a $T_{m(e)}$ range of 1°C and an rRNA binding range of 0.05%. By using the standards described above, we predicted that all of our strains should have DNA homology values of 80 to 100% under stringent conditions. Why segmental homology data among some *Xanthomonas* species are lower (42) remains to be investigated. Previously (16), De Ley et al. proposed that all *Xanthomonas* species should be included in the genus *Pseudomonas*. Here we formally withdraw this proposal; *Xanthomonas* is a quite separate genus in its own right and is removed from the closest *Pseudomonas* rRNA branch at a $T_{m(e)}$ of at least 14°C.

(i) ***Xanthomonas ampelina*.** *Xanthomonas ampelina* (49) is a special case. This organism is the cause of a serious grapevine disease which is called "tsilik marasi" in Greece (49) and "vlamsiekte" in South Africa (24) and may also be the cause of similar vine diseases called "mal nero," "gommose bacillaire," and "maladie d'Oleron" in various European countries. This organism was classified in the genus *Xanthomonas* because it is a phytopathogenic, aerobic, nonsporing, gram-negative, rod-shaped bacterium which has one polar flagellum, produces a water-insoluble yellow pigment, and metabolizes carbohydrates oxidatively (49). However, our results show that this taxon is ca. 19° $T_{m(e)}$ removed from the authentic xanthomonads and from the *Pseudomonas fluorescens* rRNA branch. Thus, it is quite clear that *Xanthomonas ampelina* is not a member of either *Xanthomonas* or *Pseudomonas* section I. This is supported by the findings (49) that *Xanthomonas ampelina* has a number of characteristics that do not occur in authentic xanthomonads, including very slow growth, maximum growth temperature of 30°C, strong urease production, utilization of tartrate, no utilization of glucose, mannose, fructose, sucrose, or propionic acid, and no hydrolysis of either gelatin or esculin. The exact taxonomic position of *Xanthomonas ampelina* is unknown.

(ii) "***Aplanobacter populi***." "*Aplanobacter populi*" (not on the Approved Lists) includes a group of bacteria that cause bacterial canker in poplars in France (53), Belgium (55), Britain

(63), and the Netherlands (11). These bacteria were discovered by Ridé (53) in 1958. We examined 10 strains at the request of M. Ridé; these strains were provided by M. Ridé. All of these strains lay within the *Xanthomonas* area at $T_{m(e)}$ values of 80.5 to 81.0°C and rRNA binding values of 0.06 to 0.09%. Likewise, the G+C contents (62.0 to 65.2 mol%) were within the range reported for this genus (62 to 69 mol%). We could not differentiate these bacteria from authentic *Xanthomonas* strains. On the basis of an extensive phenotypic analysis and our genotypic results, Ridé and Ridé (54, 55) renamed this taxon "*Xanthomonas populi*." At the present time this name does not have official status, as it has not been placed on the Approved Lists.

(iii) *Pseudomonas maltophilia*. Because of its need for growth factors, *Pseudomonas maltophilia* was classified in *Pseudomonas* section IV in the 8th edition of *Bergey's Manual* (22). Three of the *Pseudomonas maltophilia* strains which we used were isolated from clinical materials (the source of many *Pseudomonas maltophilia* strains), and strain ATCC 17806, which originally was the type strain of "*Pseudomonas melanogena*" (32) (not on the Approved Lists), was isolated from Japanese rice paddies (32). Komagata et al. (36) have shown that "*Pseudomonas melanogena*" is a later subjective synonym of *Pseudomonas maltophilia*. The latter name was not on the Approved Lists, but has been revived by Hugh (30), with strain ATCC 13637 as the type strain (31). Our four strains formed a tight cluster (Fig. 6), supporting the conclusion of Komagata et al. (36). The *Pseudomonas maltophilia* cluster is distinctly different from the three *Pseudomonas* rRNA groups discussed above; it is removed from them by a considerable distance [$\Delta T_{m(e)}$, 10 to 16°C]. Our hybridization data also showed (Fig. 3 through 5) that *Pseudomonas maltophilia* is always located close to *Xanthomonas*. Hybridizations with rRNA from *Xanthomonas capestris* type strain NCPPB 528 confirmed that the *Pseudomonas maltophilia* cluster is quite close to the genus *Xanthomonas* and is removed from it at a $T_{m(e)}$ of only 3°C. The transfer of *Pseudomonas maltophilia* Hugh 1981 to the genus *Xanthomonas* as *Xanthomonas maltophilia* (Hugh 1981) comb. nov. has recently been proposed (61).

The fifth group includes the remaining pseudomonads from *Pseudomonas* section IV in the 8th edition of *Bergey's Manual* (22). This section consists of *Pseudomonas maltophilia*, *Pseudomonas vesicularis*, and *Pseudomonas diminuta*. The type strains of *Pseudomonas vesicularis* and *Pseudomonas diminuta* are removed from the four reference strains used at a $T_{m(e)}$ of about 20°C. We do not know the taxonomic affiliation of either of these species, but it is quite clear that

both of them should be removed from the genus *Pseudomonas*. Thus, section IV of *Pseudomonas* in the 8th edition of *Bergey's Manual* (22) disappears completely.

Relationships of *Pseudomonas* and *Xanthomonas* with other genera. When only *Pseudomonas* and *Xanthomonas* strains are compared by DNA-rRNA hybridization, it is possible to show the degree of heterogeneity within each genus, the mutual relationships of the strains, and whether species have been misnamed. However, in this study our second and main target was to establish the relationships of the three *Pseudomonas* groups and *Xanthomonas* with a great variety of other gram-negative bacteria. Therefore, we performed DNA-rRNA hybridizations by using DNAs from 148 strains belonging to 43 genera and 93 species and subspecies, most of them gram negative, and labeled rRNAs from our reference strains. The results are represented in Table 1 and in the rRNA similarity maps (Fig. 3 to 6). In addition, we have included the results of many hundreds of hybridizations with labeled rRNAs from *Agrobacterium* (21), *Chromobacterium* and *Janthinobacterium* (17), the acetic acid bacteria and *Zymomonas* (26), the free-living, N₂-fixing bacteria (20), and *Frateriuria* (62), as well as data on other genera currently being investigated in our laboratory. Only that part of this information which is useful in the present discussion is summarized in Fig. 8. As far as rRNA and phenotypic similarities are concerned, we distinguished five rRNA superfamilies as defined by De Ley (13). (i) The first rRNA superfamily consists of all of the genera of the *Enterobacteriaceae* and the *Vibrionaceae*, *Aeromonas*, *Plesiomonas*, and several other taxa to be discussed elsewhere (A. Van Landschoot and J. De Ley, unpublished data; J. De Ley, R. Tytgat, J. De Smedt, and P. De Vos, unpublished data). Most of these organisms are fermentative and share a number of phenotypic features. (ii) The second rRNA superfamily consists of *Azotobacter*, *Azomonas*, the *Pseudomonas fluorescens* rRNA branch, *Alteromonas communis*, and *Alteromonas vaga* (this paper), as well as the misnamed marine "*Alcaligenes*" (K. Kersters, P. Segers and J. De Ley, unpublished data), *Xanthomonas*, "*Aplanobacter*," *Xanthomonas maltophilia*, and *Frateriuria*. (iii) The third rRNA superfamily consists of the *Pseudomonas solanacearum* rRNA branch, *Alcaligenes eutrophus*, the *Pseudomonas acidovorans* rRNA branch, *Alcaligenes paradoxus*, *Alcaligenes*, *Bordetella bronchiseptica* (Kersters et al., unpublished data), *Derxia*, *Janthinobacterium*, and *Chromobacterium*. (iv) The fourth rRNA superfamily consists of *Agrobacterium*, *Rhizobium*, *Bradyrhizobium* (33), *Acetobacter*, *Gluconobacter*, *Zymomonas*, *Bei-*

jerinckia, some spirilla, *Rhodopseudomonas*, and *Paracoccus* (M. Gillis and J. De Ley, manuscript in preparation); the misnamed organisms *Pseudomonas diminuta* and *Pseudomonas vesicularis* may also belong in this superfamily. (v) The fifth rRNA superfamily consists of the authentic flavobacteria and *Cytophaga* (M. Bauwens and J. De Ley, unpublished data). Together with the genera *Campylobacter* and *Zoogloea*, the gram-positive organisms tested are the organisms that are least related to the pseudomonads and are completely outside these five rRNA superfamilies. Our major groupings correspond quite well to the groupings revealed by 16S rRNA oligonucleotide catalogs (25).

The dendrogram in Fig. 8 and the present and previously published rRNA similarity maps (17, 20, 21, 26) show that all strains belonging to a well-known genus or all genera belonging to a well-known family (*Enterobacteriaceae* and *Vibrionaceae*) occur close together in a rather tight cluster on the similarity maps or in a separate rRNA branch. Exceptions are *Pseudomonas* and *Alcaligenes*, strains of which are distributed over the second and third rRNA superfamilies, and *Flavobacterium* (data not shown). *Alcaligenes* and *Flavobacterium* are genotypically and phenotypically extremely heterogeneous and will be reported on separately (Kerstens et al., manuscript in preparation; Bauwens and De Ley, unpublished data). The *Pseudomonas fluorescens* rRNA branch belongs in the second rRNA superfamily and is neatly separated (Fig. 8); its closest relatives are *Azotobacter* and *Azomonas*. These organisms are linked at a $T_{m(e)}$ of 76°C. In view of their disparate morphologies and physiologies, the rRNA closeness of these organisms seems unexpected. However, this cannot be a coincidence. Indeed, Ambler (1) showed that other gene products (cytochrome c_{551} molecules) from several members of *Pseudomonas* section I and *Azotobacter vinelandii* have considerable sequence homology. This point has been discussed by De Smedt et al. (20). The similarities between the rRNA and cytochrome c_{551} cistrons indicate an ancestral, close phylogenetic relationship between these taxa.

The *Pseudomonas acidovorans* and *Pseudomonas solanacearum* rRNA branches (Fig. 7) remain separate branches in the third rRNA superfamily (Fig. 8); these branches are removed from each other and from *Derxia*, *Janthinobacterium*, and authentic *Alcaligenes* by a $T_{m(e)}$ of about 10°C, but are separated by a $T_{m(e)}$ gap of 19°C from the *Pseudomonas fluorescens* rRNA branch. The closest relative of the *Pseudomonas acidovorans* rRNA branch is *Alcaligenes paradoxus* at a $T_{m(e)}$ of about 76.5°C, and the closest relative of the *Pseudomonas solanacearum* rRNA branch is *Alcaligenes eutrophus*

at a $T_{m(e)}$ of about 75.5°C. DNA-rRNA hybridizations, extensive phenotypic analyses, and comparisons of electrophoretic protein profiles have confirmed that *Alcaligenes paradoxus* and *Alcaligenes eutrophus* are quite different from the real members of the genus *Alcaligenes*, such as *Alcaligenes faecalis* and *Alcaligenes denitrificans* (Kerstens et al., unpublished data). *Alcaligenes paradoxus* includes rod-shaped, H₂-oxidizing, and nonautotrophic strains with degenerate peritrichous flagella and a typical carotenoid pigment (10). *Alcaligenes eutrophus* consists of rod-shaped, H₂-oxidizing, denitrifying bacteria which do not have carotenoids but do have peritrichous flagella (10). It is surprising that a peritrichously flagellated species with different phenotypic characteristics is closely related to a group of polarly flagellated species. rRNA similarity maps based on reverse hybridizations with labeled rRNAs from both *Alcaligenes eutrophus* and *Alcaligenes paradoxus* (J. De Ley and P. Segers, unpublished data) and DNAs from members of the *Pseudomonas acidovorans* and *Pseudomonas solanacearum* rRNA branches show that each *Alcaligenes* species is closely related to, but separate from, the corresponding *Pseudomonas* complex. This situation is quite similar to that observed in the acetic acid bacteria. *Acetobacter* is peritrichous, and *Gluconobacter* is polarly flagellated; when they are hybridized with labeled *Acetobacter* rRNA, these two genera overlap, but when they are hybridized with labeled *Gluconobacter* rRNA, they are closely related but clearly separate (26). No DNA homology was observed between *Alcaligenes eutrophus* and other facultatively autotrophic hydrogen-oxidizing bacteria, such as *Pseudomonas saccharophila*, *Pseudomonas palleronii*, and *Pseudomonas facilis*, or the heterotrophs *Pseudomonas acidovorans*, *Pseudomonas testosteroni*, *Pseudomonas delatfieldii*, *Pseudomonas stutzeri*, *Pseudomonas mendocina*, and *Pseudomonas aeruginosa* (50). We performed a limited number of DNA-DNA hybridizations with two strains of *Pseudomonas cepacia* and two *Alcaligenes eutrophus* strains (Table 3). The level of DNA relatedness between the type strain of *Alcaligenes eutrophus* and both *Pseudomonas cepacia* strains was 15 to 18%, indicating that these strains have at most a small amount of genomic DNA in common (14).

It is not surprising that *Pseudomonas* is very heterogeneous. Indeed, for decades this genus has been a dumping ground for a variety of strains as long as they were gram-negative, aerobic, nonsporeforming rod-shaped organisms with polar flagella; new organisms have often been put in the genus *Pseudomonas* without a thorough examination. A considerable improve-

TABLE 3. DNA-DNA hybridizations between two strains of *Alcaligenes eutrophus* and two strains of *Pseudomonas cepacia*^a

Strain	DNA similarity (%) with strain:			
	ATCC 17698	ATCC 17697 ^T	ATCC 17759	ATCC 25416
<i>Alcaligenes eutrophus</i> ATCC 17698	100			
<i>Alcaligenes eutrophus</i> ATCC 17697 ^T	93	100		
<i>Pseudomonas cepacia</i> ATCC 17759	18		100	
<i>Pseudomonas cepacia</i> ATCC 25416	15		63	100

^a We used the method of De Ley et al. (14). The results are expressed as the degree of DNA similarity and are shown in a half matrix.

ment and simplification of *Pseudomonas* is given in the 8th edition of *Bergey's Manual* (22), and a number of doubtful species have been temporarily moved to the addenda. In this paper we simplify the classification of *Pseudomonas*, more by proving that *Pseudomonas maltophilia*, *Pseudomonas diminuta*, and *Pseudomonas vesicularis* are misnamed species, thus eliminating completely section IV of *Pseudomonas* as defined in *Bergey's Manual*, 8th ed. (22). Only three rRNA branches remain, the *Pseudomonas fluorescens* branch, the *Pseudomonas solanacearum* branch, and the *Pseudomonas acidovorans* branch, which correspond reasonably well, but not completely, to sections I, II, and III, respectively. From our results it follows quite clearly that these three rRNA branches do not correspond to differences at the species or sub-genus level, that the differences are at least at the genus level, and that the present genus *Pseudomonas* Migula 1894, 237 should be split to form at least two, and perhaps three, genera. However, with the present information it is not yet possible to make a nomenclatural proposal. One fact is certain: an emended genus *Pseudomonas* with *Pseudomonas aeruginosa* as type species must be retained. This taxon corresponds to our *Pseudomonas fluorescens* rRNA branch and to group I of Palleroni (45). We are working on an extensive treatment of the phenotypic properties of the three rRNA branches, and descriptions of these taxa and their differentiating characteristics will be presented elsewhere.

When *Xanthomonas* rRNAs are compared with the DNAs of many gram-negative taxa (or the reverse), this genus remains quite separate (Fig. 8).

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