Intra- and Intergeneric Similarities of *Chromobacterium* and *Janthinobacterium* Ribosomal Ribonucleic Acid Cistrons

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We prepared hybrids between ^{14}C -labeled ribosomal ribonucleic acid (rRNA) from either *Chromobacterium violaceum* NCTC 9757 or *Chromobacterium lividum* NCTC 9796 and deoxyribonucleic acid (DNA) from a great variety of named gram-negative bacteria, including many type and reference strains. Each hybrid was described by two parameters: (i) $T_{m(e)}$, the temperature at which 50% of the hybrid was denatured; (ii) the percent rRNA binding, the amount (micrograms) of [^{14}C]rRNA duplexed in stringent conditions per 100 μg of filter-fixed homologous or heterologous DNA. Each taxon occupied a definite area on the rRNA similarity maps. All *C. lividum* and *C. violaceum* strains formed tight separate clusters around their neotype strains. The rRNA parameters of both taxa were about as different from each other as was the case for many genera. Both taxa are phenotypically and genotypically (by DNA-DNA hybridization) likewise very different. We proposed to elevate each cluster to genus rank as *C. violaceum* and *Janthinobacterium lividum*. The following strains were misnamed and belonged in neither genus: "*C. lividum*" = "*C. folium*" NCTC 10590 and 10591; "*C. lividum*" GA; "*C. marismortui*" ATCC 17056; "*Pseudomonas*" ("*Chromobacterium*") *iodinum* ATCC 9897, 15728, and 15729; "*C. indicum-rubrum*" (now *Serratia marcescens*) NCTC 2847; and "*C. viscosum*" (now *Corynebacterium* sp.) NCTC 2416. The rRNA cistrons of *Chromobacterium* and *Janthinobacterium* resembled most closely those of *Pseudomonas* section II and III, the authentic *Alcaligenes*, *Bordetella bronchiseptica*, the H_2 -oxidizing *Alcaligenes eutrophus* and *Alcaligenes paradoxus*, and *Comamonas percolans* NCIB 8193. These taxa displayed a number of phenotypical similarities. We suggested that all these taxa are the closest taxonomic relatives of *Chromobacterium* and *Janthinobacterium*.

In a previous paper (16) one of us reported that the quantitative comparison of ribosomal ribonucleic acid (rRNA) cistron similarities is a powerful and quick tool to detect the degree of intergeneric relationships between remotely related taxa, when other methods frequently fail (e.g., deoxyribonucleic acid [DNA]-DNA hybridization) or are very time consuming (e.g., numerical analysis of phenotypic features). There seemed to be a distinct correlation between the thermal stability of heterologous DNA-rRNA hybrids and the overall phenotypic similarities of the organisms concerned. These findings were discovered and explored with the *Rhizobiaceae* (16).

In the present paper we shall describe the rRNA similarities of *Chromobacterium*, both within the genus and with other genera. *Chromobacterium* is a particularly interesting genus for several reasons. (i) Its internal classification is very well known from the numerical analysis of its phenotypical features (29-32). Two taxa were proposed, *C. violaceum* and *C. lividum*. (ii) Sneath (32) pointed out that this genus is not a very natural taxon, since both species are almost

as different from each other as are many genera. (iii) The exact taxonomic position and affiliation of *Chromobacterium* is unknown. In *Bergey's Manual*, 7th ed. (4), it was grouped together with *Agrobacterium* and *Rhizobium* in the *Rhizobiaceae*. This was not justified (20), and *Chromobacterium* is now temporarily listed in *Bergey's Manual*, 8th ed. (32), as a genus of uncertain affiliation in an appendix to the *Enterobacteriaceae* and *Vibrionaceae*, a position that is likewise totally unjustified because of considerable differences in phenotypical features and DNA base composition (guanine-plus-cytosine [G+C] content). As a conclusion of part of our data we propose in the Discussion a new genus name *Janthinobacterium* for the strains of the *C. lividum* taxon. For the sake of clarity we shall use this genus name from now on instead of *C. lividum*. The nomenclature of *C. violaceum* will be maintained.

MATERIALS AND METHODS

Bacterial strains and growth media. The strains used are listed in Table 1. The bacteriological purity was checked by plating and by examination of living

TABLE 1. *List of organisms used*^a

Se- quence no. in Fig. 3 or 4	Organism used for DNA prep	Origin and strain no.	Mol% G+C	Strain status	Hybridization with [¹⁴ C]rRNA from:			
					<i>J.</i> <i>lividum</i> NCTC 9796		<i>C.</i> <i>violaceum</i> NCTC 9757	
					<i>T_m(e)</i> (°C)	rRNA bind- ing (%)	<i>T_m(e)</i> (°C)	rRNA bind- ing (%)
Proposed genus <i>Chromobac-</i> <i>terium</i> emend.								
1	<i>C. violaceum</i>	NCTC 9757	67.2	Neotype (5)	67.5	0.17	80.0	0.27
2	<i>C. violaceum</i>	NCTC 8683	65.2		69.5	0.18	76.5	0.28
3	<i>C. violaceum</i>	NCTC 8684	66.7		70.5	0.18	78.0	0.27
4	<i>C. violaceum</i>	NCTC 8685	66.3		69.0	0.14	77.5	0.26
5	<i>C. violaceum</i>	NCTC 9370	66.4		71.0	0.20	76.5	0.25
6	<i>C. violaceum</i>	NCTC 9371	66.1		70.0	0.16	76.5	0.29
7	<i>C. violaceum</i>	NCTC 9372	66.2		70.0	0.18	78.0	0.26
8	<i>C. violaceum</i>	NCTC 9373	65.8		68.5	0.15	78.0	0.25
9	<i>C. violaceum</i>	NCTC 9374	65.9		70.0	0.17	80.0	0.26
10	<i>C. violaceum</i>	NCTC 9377	66.9		70.0	0.17	78.5	0.27
11	<i>C. violaceum</i>	NCTC 9695	65.2		68.0	0.17	76.5	0.29
12	<i>C. violaceum</i>	NCTC 9696	66.5		67.0	0.18	80.0	0.29
Proposed genus <i>Janthinobac-</i> <i>terium</i>								
13	<i>J. lividum</i>	NCTC 9796	65.5	Neotype (this pa- per)	78.5	0.21	66.5	0.19
14	<i>J. lividum</i>	DA (Sneath)	66.1		79.0	0.21	67.5	0.18
15	<i>J. lividum</i>	MB (Sneath)	66.1		79.0	0.18	68.5	0.19
16	<i>J. lividum</i>	NCTC 8661	65.4		79.0	0.22	69.0	0.17
17	<i>J. lividum</i>	HA (Sneath)	64.5		77.5	0.23	68.0	0.175
18	<i>J. lividum</i>	P1			77.0	0.225	67.5	0.16
19	<i>J. lividum</i>	ATCC 14487	61.2		78.0	0.23	69.0	0.16
20	<i>J. lividum</i>	RU (Sneath)	65.5		77.5	0.19	68.5	0.15
21	<i>J. lividum</i>	NCTC 7150	65.5		77.5	0.18	68.0	0.17
22	<i>J. lividum</i>	HD (Sneath)	66.0		78.5	0.21	68.0	0.16
23	<i>J. amethystinum</i>	ATCC 6915	65.1		78.0	0.23	68.0	0.16
Misnamed chromobacteria								
24	" <i>C. lividum</i> " (= " <i>C. folium</i> ")	NCTC 10590	61.1	Holotype (33)	59.0	0.02	57.5	0.05
25	" <i>C. lividum</i> " (= " <i>C. folium</i> ")	NCTC 10591	63.0		61.0	0.03	58.0	0.04
26	" <i>C. lividum</i> "	GA (Sneath)	71.4		69.5	0.03	66.5	0.045
27	" <i>C.</i> " <i>marismortui</i>	ATCC 17056	61.8		61.5	0.12	61.5	0.15
Other genera								
28	<i>Pseudomonas acidovorans</i>	ATCC 15668	66.5	Type (5)	71.0	0.09	66.5	0.08
29	<i>P. acidovorans</i>	ATCC 17476	65.0		70.5	0.09		
30	<i>P. delafieldii</i>	ATCC 17506 t ₂	64.3		74.0	0.11	70.0	0.12
31	<i>P. desmolytica</i>	ATCC 15005	66.6		71.0	0.08		
32	<i>P. testosteroni</i>	NCTC 10698	62.5	Type (5)	70.5	0.19	67.0	0.18
33	<i>P. testosteroni</i>	ATCC 17510 t ₂	63.0		70.5	0.16	67.5	0.15
34	<i>P. testosteroni</i>	ATCC 17407	64.5		70.5	0.20	66.5	0.17
35	<i>P. testosteroni</i>	ATCC 17409	63.0		70.5	0.16	66.5	0.15
36	<i>P. testosteroni</i>	ATCC 11996	62.5		71.5	0.18		
37	<i>P. palleronii</i>	ATCC 17724 t ₁	65.9	Type (5)	72.0	0.07	67.5	0.05
38	<i>P. facilis</i>	ATCC 11228	64.7	Type (5)	73.0	0.09		
39	<i>P. facilis</i>	ATCC 15376	63.7		70.0	0.09	66.0	0.09
40	<i>P. facilis</i>	ATCC 17695 t ₁	65.2		72.5	0.10	68.0	0.10
41	<i>P. facilis</i>	ATCC 17695 t ₂	65.7		71.5	0.09		
42	<i>P. solanacearum</i>	NCPPB 325	66.1	Proposed neotype (5)	71.0	0.07	71.0	0.08
43	<i>P. solanacearum</i>	NCPPB 282	67.4		70.0	0.06	71.0	0.07
44	<i>P. solanacearum</i>	NCPPB 1019	68.0				70.0	0.09
45	<i>P. solanacearum</i>	NCPPB 613	68.1		68.5	0.07	70.5	0.07
46	<i>P. solanacearum</i>	NCPPB 253					70.0	0.09
47	<i>P. cepacia</i>	NCTC 10661	67.4	Proposed holotype (34) for <i>P. mul-</i> <i>tivorans</i>	72.0	0.09	70.5	0.10
48	<i>P. cepacia</i>	ATCC 25416	67.5		Type (5)	71.5	0.10	70.5
49	<i>P. marginata</i>	ATCC 10248	68.6	Type (5)			70.5	0.07
50	<i>P. ruhlandii</i>	ATCC 15749	64.9	Type (5)	70.0	0.06	70.0	0.06
51	<i>P. fluorescens</i>	ATCC 13525	60.2	Neotype (5)	63.5	0.11	61	0.13

TABLE 1—Continued

Se- quence no. in Fig. 3 or 4	Organism used for DNA prepn	Origin and strain no.	Mol% G+C	Strain status	Hybridization with [¹⁴ C]rRNA from:			
					<i>J.</i> <i>lividum</i> NCTC 9796		<i>C.</i> <i>violaceum</i> NCTC 9757	
					<i>T_m(e)</i> (°C)	rRNA bind- ing (%)	<i>T_m(e)</i> (°C)	rRNA bind- ing (%)
52	<i>P. aeruginosa</i>	ATCC 7700	66.8		62.5	0.08		
53	<i>P. aeruginosa</i>	CCEB 481	66.8	Neotype (5)	62.5	0.07		
54	<i>P. putida</i>	ATCC 23467	63.4	Representative (5)	62.0	0.09		
55	<i>P. cichorii</i>	NCPPB 1512	60.3		62.5	0.10		
56	<i>P. stutzeri</i>	ATCC 17588	64.7	Suggested neotype (5)	64.5	0.10		
57	<i>P. oleovorans</i>	NCTC 10692		Cotype (33)	63.0	0.09		
58	<i>P. maltophilia</i>	ATCC 13637	65.0	Type (5)	62.5	0.09		
59	<i>P. mucidolens</i>	NCTC 8068	61.0	Cotype (33)	62.5	0.09		
60	<i>P. rubescens</i>	NCTC 10695	45.9	Cotype (33)	61.0	0.10		
61	" <i>P.</i> " <i>atlantica</i>	CCEB 506	66.5	Holotype (33)	60.0	0.05		
62	" <i>P.</i> " <i>diminuta</i>	CCEB 513	67.3	Type (5)	60.0	0.04		
63	" <i>P.</i> " <i>iodinum</i>	ATCC 9897	63.2	Holotype (33)	55.5	0.05	53.0	0.06
64	" <i>P.</i> " <i>iodinum</i>	ATCC 15728	62.0		57.0	0.06	53.5	0.06
65	" <i>P.</i> " <i>iodinum</i>	ATCC 15729	61.8				51.5	0.04
66	<i>Comamonas percolans</i>	NCIB 8193	64.3	Proposed paratype (33) for <i>Vibrio</i> <i>percolans</i>	66.0	0.18	68.5	0.18
67	<i>Bordetella bronchiseptica</i>	ATCC 10580	68.2		69.0	0.08	69.5	0.09
68	<i>B. bronchiseptica</i>	NCTC 8761	69.5		69.0	0.07	69.5	0.08
69	<i>B. bronchiseptica</i>	NCTC 452	68.9	Type (5)	68.5	0.07	67.0	0.08
70	<i>Achromobacter denitrifi- cans</i>	M250	67.7		71.0	0.06	68.0	0.07
71	<i>Ach. xylosoxidans</i>	KM 583	69.5		70.0	0.08	67.0	0.06
72	<i>Alcaligenes faecalis</i>	AB 1286	57.2		70.0	0.09		
73	<i>Alc. faecalis</i>	ATCC 8750	57.3	Reference (5)	69.5	0.09	66.5	0.10
74	<i>Alc. odorans</i>	GIL 117	57.9		70.0	0.10	67.5	0.08
75	<i>Alc. eutrophus</i>	ATCC 17697	67.2	Type (5)	71.5	0.09	67.5	0.08
76	<i>Alc. eutrophus</i>	ATCC 17698	66.9		72.0	0.09		
77	<i>Alc. paradoxus</i>	ATCC 17713 t ₁	67.7	Type (5)	68.5	0.04		
78	<i>Alc. paradoxus</i>	ATCC 17712	67.9		69.0	0.05		
79	<i>Alc. paradoxus</i>	ATCC 17549 t ₁	67.1		70.5	0.04	67.0	0.03
80	<i>Alc. paradoxus</i>	ATCC 17549 t ₂	67.9		68.0	0.04		
81	<i>Alc. paradoxus</i>	ATCC 17719 t ₂	66.9		69.5	0.04	67.0	0.04
82	<i>Alc. pacificus</i>	ATCC 27122	66.7	Type ATCC (1974 Catalog)	60.5	0.10		
83	<i>Alc. venustus</i>	ATCC 27125	53.2	Type ATCC (1974 Catalog)	62.0	0.09		
84	<i>Alc. aestus</i>	ATCC 27128	57.4	Type ATCC (1974 Catalog)	62.5	0.14		
85	<i>Alc. aquamarinus</i>	NCMB 557	57.9	Reference (5)	62.5	0.125		
86	<i>Azotobacter vinelandii</i>	NCIB 8660	65.0		62.0	0.12		
87	<i>Azo. paspali</i>	22B	63.7		61.0	0.10		
88	<i>Azomonas agilis</i>	SS4	52.8		62.0	0.06		
89	<i>Derxia gummosa</i>	D					68.0	0.13
90	<i>Derxia</i> sp.	D12	72.6		71.0	0.09		
91	<i>Xanthomonas campestris</i>	ICPB T11	64.3	Cotype for <i>X. tar- axaci</i> (33)	64.0	0.04	60.0	0.07
92	<i>X. campestris</i>	ICPB V136	66.4		62.5	0.04		
93	<i>X. campestris</i>	ICPB P121	66.5		62.5	0.05	61.5	0.06
94	<i>X. fragariae</i>	NCPPB 1822	63.3		65.0	0.07	61.5	0.06
95	<i>X. albilineans</i>	NCPPB 2503	64.5		63.0	0.07	59.5	0.04
96	<i>X. axonopodis</i>	NCPPB 457	65.0	Cotype (33)	64.5	0.04		
97	<i>Aplanobacter populi</i>	Bt3	65.0		64.0	0.05	61.0	0.06
98	<i>Apl. populi</i>	Spm 11	65.2		63.0	0.05	60.0	0.06
99	<i>Spirillum sinuosum</i>	NCIB 9010	59.0	Type (5)			68.0	0.14
100	<i>S. polymorphum</i>	NCIB 9072	63.7	Type (5)	60.0	0.07		
101	<i>Rhodopseudomonas gelati- nosa</i>	NCIB 8290	70.1	Neotype (5)			67.5	0.06
102	<i>R. palustris</i>	NCIB 8252	67.2	Neotype (5)	59.5	0.03		
103	<i>R. sphaeroides</i>	NCIB 8253	68.4	Type (5)	58.5	0.05		

TABLE 1—Continued

Se- quence no. in Fig. 3 or 4	Organism used for DNA prep	Origin and strain no.	Mol% G+C	Strain status	Hybridization with [¹⁴ C]rRNA from:			
					<i>J.</i> <i>lividum</i> NCTC 9796		<i>C.</i> <i>violaceum</i> NCTC 9757	
					<i>T</i> _{m(e)} (°C)	rRNA bind- ing (%)	<i>T</i> _{m(e)} (°C)	rRNA bind- ing (%)
104	<i>Escherichia coli</i>	B	52.2		62.0	0.10	60.0	0.14
105	<i>Salmonella typhimurium</i>	1	55.2		61.5	0.13	63.0	0.14
106	<i>Enterobacter aerogenes</i>	NCTC 10006	53.8	Type (5)			60.0	0.15
107	<i>Ent. agglomerans</i>	IPO 445	60.6		62.0	0.13	61.5	0.18
108	<i>Edwardsiella tarda</i>	NCTC 10396	56.7	Type (5)	61.5	0.18		
109	<i>Klebsiella aerogenes</i>	NCTC 8172	55.9	Proposed neotype ATCC (1974 Catalog)	63.0	0.14		
110	<i>K. rubiacearum</i>		59.6		64.0	0.15	60.5	0.14
111	<i>Hafnia protea</i>	540	49.3		63.0	0.11		
112	<i>Proteus morganii</i>	NCTC 2815	52.3		63.5	0.14	63.0	0.16
113	<i>Serratia marcescens</i>	ATCC 10759	59.2	Suggested working strain (33)	64.0	0.12		
114	<i>S. marcescens</i>	CCEB 293	59.9		62.5	0.09		
115	<i>S. marcescens</i> (<i>Chromobac- terium indicum-rubrum</i>)	NCTC 2847	55.3		63.5	0.10	62.5	0.13
116	<i>Yersinia rodentium</i>	NCTC 1102	48.9		64.0	0.13	61.5	0.14
117	<i>Vibrio albensis</i>	NCMB 41	47.8	Proposed reference (5)	60.5	0.14		
118	<i>V. fischeri</i>	NCMB 1281	40.9	Neotype (5)	61.0	0.14		
119	<i>V. marinus</i>	NCMB 1143	42.3		61.0	0.18		
120	<i>V. marinus</i>	ATCC 15382	43		61.5	0.14		
121	<i>V. parahaemolyticus</i>	Sak 3	47.6		61.0	0.17		
122	<i>V. parahaemolyticus</i>	FC 1011	47.9		62.5	0.16		
123	<i>V. alginolyticus</i>	ATCC 17749	45.5	Suggested working strain for <i>Vibrio parahaemolyti- cus</i> (5)	64.0	0.18		
124	<i>V. anguillarum</i>	ATCC 19264	44.9	Suggested working strain (5)	62.0	0.13		
125	<i>V. non-cholera</i>	E 509	49.0		62.5	0.17	63.0	0.23
126	<i>Beneckeia natriegens</i>	ATCC 14048	44.9	Type ATCC (1974 Catalog)	62.0	0.12		
127	<i>B. nigrapulchrituda</i>	ATCC 27043	46.1	Type ATCC (1974 Catalog)	62.5	0.09		
128	<i>Lucibacterium harveyi</i>	NCMB 1280	45.5	Type (5)	61.5	0.15	61.5	0.17
129	<i>L. harveyi</i>	NCMB 1	45.0		62.0	0.15		
130	<i>L. harveyi</i>	NCMB 24	46.5		62.0	0.14		
131	<i>Photobacterium phospho- reum</i>	NCMB 844	40.5	Suggested working type strain (5)	62.0	0.22	61.5	0.26
132	<i>Ph. mandapamensis</i>	NCMB 1198	41.8	Suggested working type strain (5)	63.0	0.22	62.0	0.27
133	<i>Ph. mandapamensis</i>	NCMB 391	40.7	Suggsted working type strain (5)	63.0	0.21	62.0	0.24
134	<i>Plesiomonas shigelloides</i>	NCTC 10360	52.0	Holotype (33)	63.0	0.20	61.0	0.28
135	<i>Aeromonas punctata</i> subsp. <i>caviae</i>	NCIB 9232	59.1		61.5	0.16	62.0	0.20
136	<i>Aer. hydrophila</i>	AB 833	59.5		60.5	0.16	63.0	0.22
137	<i>Aer. hydrophila</i> subsp. <i>hy- drophila</i>	NCIB 9233	58.9		63.0	0.19		
138	<i>Aer. salmonicida</i>	NCMB 833	57.0	Suggested neotype (33)	62.0	0.18		
139	<i>Agarbacterium alginicum</i>	NCMB 886	53.2		63.0	0.13	63.5	0.12
140	<i>Cellvibrio vulgaris</i>	NCIB 8633	51.5	Cotype (33)	62.0	0.06		
141	<i>C. vulgaris</i>	NCIB 8975	48.1				61.5	0.07
142	<i>Acetobacter aceti</i> subsp. <i>aceti</i>	NCIB 8621 t ₁	57.9	Suggested neotype (5)			60.0	0.06
143	<i>Ace. aceti</i> subsp. <i>aceti</i>	NCIB 8554	56.9		60.5	0.07		
144	<i>Ace. aceti</i> subsp. <i>xylinum</i>	NCIB 8246	60.7		59.5	0.08		
145	<i>Ace. pasteurianus</i> subsp. <i>pasteurianus</i>	23 kl+	55.5		58.5	0.11		

TABLE 1—Continued

Se- quence no. in Fig. 3 or 4	Organism used for DNA prep	Origin and strain no.	Mol% G+C	Strain status	Hybridization with [¹⁴ C]rRNA from:			
					<i>J.</i> <i>lividum</i> NCTC 9796		<i>C.</i> <i>violaceum</i> NCTC 9757	
					<i>T</i> _{m(e)} (°C)	rRNA bind- ing (%)	<i>T</i> _{m(e)} (°C)	rRNA bind- ing (%)
146	<i>Ace. pasteurianus</i> subsp. <i>pasteurianus</i>	NCIB 8945	58.3				61.0	0.06
147	<i>Gluconobacter oxydans</i> subsp. <i>suboxydans</i>	NCIB 621	61.7		59.5	0.10	59.5	0.12
148	<i>G. oxydans</i> subsp. <i>melano-</i> <i>genus</i>	NCIB 8086	60.6		57.5	0.08		
149	<i>G. oxydans</i> subsp. <i>melano-</i> <i>genus</i>	NCIB 8085	59.5				59.5	0.11
150	<i>Rhizobium leguminosarum</i>	USDA 316c10a	61.4		61.5	0.05		
151	<i>Rhi. leguminosarum</i>	5.0	61.1		59.5	0.06		
152	<i>Rhi. meliloti</i>	USDA 3DOa30	62.3		58.5	0.06		
153	<i>Rhi. japonicum</i>	USDA 316n10	63.3		57.5	0.02		
154	<i>Rhi. japonicum</i>	3.1	63.9		58.5	0.02		
155	<i>Phyllobacterium</i> <i>rubiacearum</i>	LMG1 t ₁	61.3		59.5	0.04		
156	<i>Phy. myrsinacearum</i>	LMG2 t ₁	60.3		60.5	0.05		
157	<i>Phy. myrsinacearum</i>	LMG3 t ₂	59.6				57.5	0.05
158	<i>Agrobacterium tumefaciens</i>	ICPB TT111	60.4	Reference (J. De Ley, unpub- lished)	59.5	0.07		
159	<i>Agr. tumefaciens</i>	B _{3a}	61.1		59.5	0.07		
160	<i>Agr. rhizogenes</i>	ICPB TR7	61.4	Reference (J. De Ley, unpub- lished)	57.5	0.06		
161	<i>Agr. rhizogenes</i>	ICPB TR101	61.3				59.5	0.04
162	<i>Agrobacterium</i> sp.	NA	58.9		58.0	0.05		
163	<i>Zymomonas mobilis</i> subsp. <i>mobilis</i>	Z1	48.9		57.5	0.10		
164	<i>Paracoccus denitrificans</i>	ATCC 17741	66.4	Holotype (5)	60.5	0.05	58.0	0.05
165	<i>Par. denitrificans</i>	ATCC 19367	67.4	Suggested cotype for <i>Micrococcus</i> <i>denitrificans</i> (33)	60.5	0.03		
166	<i>Mycoplasma dimorpha</i>	NCIB 9439	63.8		58.5	0.06	57.5	0.05
167	<i>Flavobacterium meningosepticum</i>	NCTC 10588	37.3		56.5	0.05		
168	<i>F. arborescens</i>	NCIB 8185	70.0	Reference (5)	58.5	0.04	55.0	0.04
169	<i>F. esteraromaticum</i>	NCIB 8186	65.1	Reference (5)	58.0	0.08		
170	<i>F. flavescens</i>	NCIB 8187	67.2	Reference (5)	59.5	0.04		
171	<i>F. suaveolans</i>	NCIB 8188	67.1	Reference (5)	62.0	0.03		
172	<i>Arthrobacter oxydans</i>	CBRI 21010	62.4		58.0	0.07		
173	<i>Corynebacterium michiganense</i>	P 1468	70.5		57.0	0.06		
174	<i>Cor. sp. (Chromobacterium viscosum)</i>	NCTC 2416	58.0	Holotype for <i>C. viscosum</i> (33)	59.0	0.06	57.5	0.08
175	<i>Microcycylus aquaticus</i>	NCIB 9271		Type (5)			56.5	0.02
176	<i>Campylobacter fetus</i>	B5			55.0	0.12		
177	<i>Cam. jejuni</i>	M2	34.0		57.0	0.10		
178	<i>Cytophaga marinoflava</i>	ATCC 19326	38.4	Type ATCC (1974 Catalog)	56.0	0.04	54.0	0.04
179	<i>Bacillus megaterium</i>	899 thy ⁻	39.9		56.5	0.17		
180	<i>Bac. subtilis</i>	BQ2	47.7		56.0	0.20		
181	HeLa					0.01		

^a Included in the table are strain number, DNA base composition (G+C content), eventual type strain status, and properties of the DNA-rRNA hybrids with reference ¹⁴C-labeled rRNA from *Chromobacterium violaceum* NCTC 9757 and the renamed *Janthinobacterium lividum* NCTC 9796. The sequence numbers are not strain numbers; their purpose is only to locate the strains more easily on the similarity maps in Fig. 3 and 4. The symbols t₁ or t₂ in some strain numbers indicate the colony type used, when more than one was observed in the original culture. Organisms with taxon names in quotation marks do not belong in that taxon. DNA base composition (mol% G+C) was determined by thermal denaturation. The nature of the different type and reference strains was checked in *Bergey's Manual*, 8th ed. (5), Sneath and Skerman (33), and the various collection catalogs.

and Gram-stained cells. Cells were grown in mass cultures on the media as described before (16). We were unable to test *C. marinum* ATCC 19699, as it died both in our collection and in the ATCC before use. An authentic strain of *C. chocolateum* was not available for inclusion in the present study.

Preparation of [14 C]rRNA. Reference [14 C]rRNA was prepared from the neotype strains *J. lividum* NCTC 9796 and *C. violaceum* NCTC 9757; it was purified and separated in 23S and 16S fractions as described before (12, 16, 27). The organisms were grown under aeration at 28°C in 100 ml of a liquid medium containing (weight per volume): 0.52% K_2HPO_4 , 0.27% KH_2PO_4 , 0.2% NaCl, 0.012% $MgSO_4 \cdot 7H_2O$, 0.5% yeast extract (Difco), 0.6% glucose, and 100 μ Ci of [14 C]uracil. The specific activity of *J. lividum* 23S and 16S [14 C]rRNA was close to 4,000 cpm/ μ g; for *C. violaceum* 23S and 16S [14 C]rRNA it was close to 3,000 cpm/ μ g.

Preparation of high-molecular-weight DNA. DNA was prepared by a combination of the methods of Marmur (25) and Kirby et al. (21, 22) as described by De Ley et al. (11). The final purification was carried out through a CsCl gradient in a Ti rotor for 20 h at 40,000 rpm and at 20°C in a Beckman L2-65B preparative ultracentrifuge. The gradient was separated in a Isco fractionator, model 640. The DNA fractions were pooled and dialyzed against 0.1 concentration of standard saline citrate (SSC) buffer at 4°C. SSC buffer contains 0.15 M NaCl and 0.015 M trisodium citrate (pH 7.0).

Fixation of single-stranded, high-molecular-weight DNA on membrane filters. For the fixation of DNA on membrane filters, we followed the procedure as described by De Ley and Tytgat (14) by using Sartorius membrane filters (SM 11309). The DNA-containing filters were preserved at 4°C in vacuo (12).

Saturation hybridization between [14 C]rRNA and filter-fixed DNA; thermal stability of the DNA-rRNA hybrids. For saturation hybridization, we followed the methods of De Ley et al. (12, 16). We determined two parameters: (i) $T_{m(e)}$, the temperature at which half of the DNA-rRNA hybrid was denatured, as a measure of its thermal stability, and (ii) the percent rRNA binding, i.e., the amount of [14 C]rRNA (in micrograms) hybridized in stringent conditions to 100 μ g of homologous or heterologous single-stranded, high-molecular-weight DNA.

Chemical determination of DNA on the filter. In each series of hybridizations a vial was included with buffer and a DNA filter, but without [14 C]rRNA. After the simulated hybridization step, the remaining fixed DNA was released from the filter by the method of Meys and Schilperoort (26) and determined by the method of Burton (6).

Determination of DNA base composition (G+C content). Several of the G+C content data in Table 1 have been published on previous occasions by this laboratory (7-10, 13, 15). Other data were determined for the present paper by the thermal denaturation method (15).

Genome DNA similarities; genome DNA molecular weight. The degree of genome DNA similarities (expressed as % D, degree of duplexing) was determined from the initial renaturation rate method

(11). The molecular weight of the genome DNA was determined by the method of Gillis et al. (19; equation 15 in reference 18).

RESULTS

The 16S and 23S fractions. *C. violaceum* and *J. lividum* [14 C]rRNA were readily separated in a 23S and a 16S fraction (Fig. 1). Most of our experiments were carried out with the 23S fraction. Both fractions probably have identical or very similar base sequences as 16S fractions can be used without any noticeable differences in the results.

Comparison of DNA-rRNA cistrons. All the data are compiled in Table 1. They will be evaluated extensively in Discussion. The data on $T_{m(e)}$ and percent rRNA binding were all obtained from the melting curves of the hybrids. These curves displayed a variety of shapes, varying from high to low $T_{m(e)}$, from high to low percent rRNA binding, and many values in between. Some examples are given in Fig. 2. One of us pointed out previously (16) that the percent rRNA binding, as defined above, is not a measure of the "rRNA cistron homology." The percent rRNA binding depends not only on the actual rRNA homology, but also on the size of the genome, its state of replication, and the number of rRNA cistrons per genome; the percent rRNA binding is a very useful parameter in separating taxa. The correlations between the data were better revealed by plotting $T_{m(e)}$ against percent RNA binding for each DNA-rRNA hybrid (Fig. 3 and 4). Table 2 summarizes data on reciprocal hybridizations between DNA from various *Chromobacterium* and *Janthino-*

FIG. 1. Distribution of [14 C]rRNA in a linear 15 to 30% sucrose gradient, in 0.1 M acetate buffer (pH 5.1), plus 0.05 M NaCl; all solutions were treated with bentonite before use.

FIG. 2. Examples of melting curves of hybrids between ^{14}C -labeled 23S rRNA from *Janthinobacterium lividum* NCTC 9796 and filter-fixed DNA from various bacteria. The total amount of counts/50 min per 100 μg of DNA is a measure of the amount of hybrid denatured. The melting point ($T_{m(e)}$) is the temperature at which half of the hybrid has been denatured and is represented by the central dot on each curve.

bacterium strains and ^{14}C -rRNA from a variety of other genera.

Degree of genome DNA similarity. Six strains of the *J. lividum* taxon and four strains of the *J. violaceum* taxon were selected at random for DNA-DNA hybridizations. The main purpose was to determine the DNA similarity between both taxa: it is $(24 \pm 4)\%$ D in stringent conditions (Table 3). The *J. violaceum* taxon seems to be more homogeneous (83 to 100% D) than the *J. lividum* taxon (48 to 95% D).

DNA genome size. The molecular complexity of the *C. lividum* genome DNA, with a molecular weight of 3.2×10^9 , is some 15% larger than that of the *C. violaceum* genome, which has a molecular weight of 2.7×10^9 .

DISCUSSION

rRNA cistron similarities. To make our results taxonomically more meaningful, we included as many type and reference strains as possible (Table 1).

For basic aspects of the discussion on the rRNA similarity maps (Fig. 3 and 4), we refer to a previous contribution from this laboratory (16). Each strain had a fixed position on each map within the limits of reproducibility of $T_{m(e)} \pm 0.5^\circ\text{C}$ and percent rRNA binding $\pm 0.015\%$. Strains of the same taxon, which are phenotypically and genotypically very similar, were close together on the maps. There are many examples in Fig. 3 and 4, such as the strains of *C. violaceum*, *J. lividum*, *Pseudomonas testosteroni*, *Alcaligenes paradoxus*, etc. When many strains were included, the size and shape of the rRNA similarity area was a rough measure of the in-

ternal heterogeneity of the taxon; for example, the *Vibrionaceae* appeared to be more heterogeneous than the *Enterobacteriaceae*. In several cases only one or a few strains of a taxon were included merely to locate the position of the genus on the map, not its area shape. A few genera were dispersed over several clearly separated rRNA areas. *Pseudomonas* was one example, it being divided over at least three areas: (i) the *P. acidovorans-solanacearum*- H_2 oxidizers group; (ii) *P. testosteroni*; (iii) the *P. fluorescens* group; and (iv) some misnamed organisms such as "*P.*" *atlantica*, "*P.*" *diminuta*, and "*P.*" *iodinum*, which do not belong in *Pseudomonas*. The explanation for the division in three main groups is that the present genus *Pseudomonas* is genetically and phenotypically very heterogeneous, and might in the future have to be split into at least two genera (De Ley and De Vos, manuscript in preparation). Another example was the *Alcaligenes-Achromobacter* group which was divided over the following rRNA areas: (i) the *Alcaligenes-Achromobacter* group sensu stricto (authentic *Alcaligenes faecalis*, *Alc. odorans*, *Alc. denitrificans*, *Ach. xylosoxidans*, and a few more); (ii) the H_2 -oxidizing *Alcaligenes paradoxus*; and (iii) the marine organisms "*Alc.*" *pacificus*, "*Alc.*" *venustus*, "*Alc.*" *aestus*, and "*Alc.*" *aquamarinus*. The explanation for the apparent heterogeneity of *Alcaligenes* is that this genus has always been, and still is, a taxonomic wastebasket full of misnamed organisms. Extensive phenotypical data (Kerstens and De Ley, manuscript in preparation) allowed us to predict that group (iii) does not belong in *Alcaligenes*. It is interesting

FIG. 3. Similarity map of hybrids between the ^{14}C -labeled 23S rRNA fraction of *Janthinobacterium* (*Chromobacterium*) *lividum* NCTC 9796 and DNA from a variety of bacteria. $T_m(e)$ and percent rRNA binding are as defined in the text. Strains are represented by their sequence number (Table 1), which is not the strain number. To simplify the drawing below 65°C , the sequence numbers of strains were not shown in areas where several taxa overlapped. The positions of all strains belonging phenotypically to the same taxon (usually a genus) are surrounded by a closed line; this line is not the ultimate border. Abbreviations: Acet: *Acetobacter*; Agr: *Agarobacterium*; Agr: *Agrobacterium*; Alc: *Alcaligenes*; Alc. par: *Alcaligenes paradoxus*; Am: *Azomonas*; Apl: *Aplanobacter*; Ar: *Arthrobacter*; Azot: *Azotobacter*; Bac: *Bacillus*; Bord: *Bordetella*; Camp: *Campylobacter*; Chr. f: "*Chromobacterium folium*," Chr. m: "*Chromobacterium marismortui*," Chr. viol: *Chromobacterium violaceum*; Com: *Comamonas*; Cor: *Corynebacterium*; Cv: *Cellvibrio*; Cyt: *Cytophaga*; Der: *Derxia*; Enterob: *Enterobacteriaceae*; Fl: *Flavobacterium*; GA: "*Chromobacterium lividum*" GA; Glu: *Gluconobacter*; Jan. liv: *Janthinobacterium lividum*; mar. Alc: marine "*Alcaligenes*," Micr: *Microcycus*; Myc: *Mycoplana*; Par: *Paracoccus*; Phyl: *Phyllobacterium*; Ps. acid., solan., H₂-oxid: *Pseudomonas acidovorans*, *Ps. solanacearum*, H₂-oxidizing *pseudomonads*; Ps. I: *Pseudomonas* section I; Ps. iod: "*Pseudomonas iodinum*," Ps. test: *Pseudomonas testosteroni*; Rh: *Rhizobium*; Rh. jap: *Rhizobium japonicum*; Rhps: *Rhodopseudomonas*; Sp. sin: *Spirillum sinuosum*; Vibrion: *Vibrionaceae*; Xan: *Xanthomonas*; Zym: *Zymomonas*.

FIG. 4. Similarity map of hybrids between the ^{14}C -labeled 23S rRNA fraction of *Chromobacterium violaceum* NCTC 9757 and DNA from a variety of bacteria. See legend to Fig. 3 for additional information.

to note that *Bordetella bronchiseptica*, which is phenotypically so similar to authentic *Alcaligenes*, is in the same rRNA area, and that the H_2 -oxidizing *Pseudomonas* and *Alcaligenes* strains are both in the same rRNA region. *Xanthomonas* and *Aplanobacter populi* were located in the same rRNA area, in excellent agreement with conclusions from other experiments (De Ley and De Vos, manuscript in preparation) that both genera are either very closely related or perhaps identical. *Spirillum* and *Rhodopseudomonas* rRNA areas were heterogeneous, in agreement with phenotypic heterogeneity (5).

The position of other genera will be commented on below.

Proposal for an improved nomenclature of the chromobacteria. The present taxonomy of *Chromobacterium* is almost exclusively the result of the numerical analysis of phenotypic features of these organisms and has been described extensively and thoroughly by Sneath (29-32). The chromobacteria contain two taxa which were presented up to now as two species, *C. violaceum* and *C. lividum*, of one genus. Sneath repeatedly stressed that the phenotypic data did not unequivocally allow a decision to

TABLE 2. Hybridizations between DNA from various *C. violaceum* and *J. lividum* strains and from a few misnamed chromobacteria, with [¹⁴C]rRNA from various reference strains^a

Source of [¹⁴ C]rRNA	<i>Pseudomonas solanacearum</i> NCPPB 325		<i>Pseudomonas acidovorans</i> ATCC 15668		<i>Alcaligenes faecalis</i> NCIB 8156		<i>Pseudomonas fluorescens</i> ATCC 13525		<i>Xanthomonas campestris</i> NCPPB 528		<i>Azotobacter chroococcum</i> NCIB 8002		<i>Escherichia coli</i> B		<i>Agrobacterium rhizogenes</i> ICPB TR7		<i>Agrobacterium tumefaciens</i> ICPB TT111		<i>Gluconobacter oxydans</i> NCIB 9013		<i>Acetobacter aceti</i> NCIB 8621	
DNA from:	<i>T_m(e)</i> ^b	% ^c	<i>T_m(e)</i>	%	<i>T_m(e)</i>	%	<i>T_m(e)</i>	%	<i>T_m(e)</i>	%	<i>T_m(e)</i>	%	<i>T_m(e)</i>	%	<i>T_m(e)</i>	%	<i>T_m(e)</i>	%	<i>T_m(e)</i>	%	<i>T_m(e)</i>	%
<i>Chromobacterium violaceum</i>																						
NCTC 8683			67	0.14									63	0.14								
NCTC 9370			70	0.14									64.5	0.16								
NCTC 9371			70	0.18							65.5	0.16										
NCTC 9373																			59	0.12		
NCTC 9374	70.5	0.15			68	0.19																
NCTC 9695			67	0.13																		
NCTC 9696	70.5	0.15																				
NCTC 9757	72	0.20			67	0.21	64.5	0.11	64	0.14	64.5	0.17	61.5	0.11		60	0.15					
<i>Janthinobacterium lividum</i>																						
NCTC 8661	73	0.13	70.5	0.12									64.5	0.14								
NCTC 9796	72.5	0.16	71	0.12	70.5	0.16	61.5	0.07	62.5	0.11	64	0.14	65	0.15		59	0.11	58	0.12			
ATCC 14487											63	0.16										
DA (Sneath)							62.5	0.10					66	0.16	57.5	0.17	58	0.10				
HD (Sneath)					71	0.18	64.5	0.11														
MB (Sneath)					70.5	0.16							64	0.11								
RU (Sneath)	72	0.13	71	0.08							63.5	0.12										
" <i>C. lividum</i> " NCTC 10590													62.5	0.08		66.5	0.06	66	0.08	65	0.09	
" <i>C. lividum</i> " NCTC 10591													59	0.09		67.5	0.08	66	0.18	66	0.12	
" <i>C. marismortui</i> " ATCC 17056			62.5	0.12			70.5	0.17					65	0.15				58	0.13			
" <i>C. lividum</i> " GA (Sneath)	66.5	0.01	75.5	0.04	66.0	0.05																

^a From unpublished data of J. De Ley, J. De Smedt, P. De Vos, M. Gillis, P. Segers, and R. Tytgat.^b *T_m(e)* is expressed in °C.^c %, Percent rRNA binding.

TABLE 3. Genome DNA similarities within and between the *J. lividum* and *C. violaceum* taxa^a

Strain no.										
NCTC 9796	100									
HA (Sneath)	95	100								
ATCC 14487	54	57	100							
MB (Sneath)	66	66	55	100						
RU (Sneath)	65	48		70	100					
NCTC 7150	58	74	51			100				
NCTC 9757	24	21	28				100			
NCTC 9372	32	23					87	100		
NCTC 9377	25	30					100		100	
NCTC 8684	18	20	26				83		100	100

^a Expressed in percent versus homologous degree of binding (100%). Initial renaturation rate method in stringent conditions (11).

be made whether both taxa are different at the species or at the genus level. We quote: "It is possible that the genus is not a very natural taxon . . ." (32); "The species are almost as different from one another as are many genera" (32); "One might therefore consider whether the two species of *Chromobacterium* should be raised to the rank of genera, ..." (31). Leifson (24) likewise pointed out that the species of *Chromobacterium* were very different and suggested that they might be raised to generic rank.

The rRNA similarity maps (Fig. 3 and 4) showed clearly that the *C. violaceum* and *C. lividum* clusters were likewise different, that they exchanged places depending on the reference [¹⁴C]rRNA used, and that all other taxa remained approximately in about the same position. All *C. lividum* strains and all *C. violaceum* strains formed tight separate clusters around their respective neotype strains. The rRNA cistrons of both taxa were quite different both in nucleotide sequence ($T_m(e)$) and in percent rRNA binding. In fact they were about as different from each other as from *Pseudomonas* sections II and III, or as *Agrobacterium*, *Acetobacter*, and *Zymomonas* are from each other (16), or as *Escherichia* is from *Vibrio* (De Ley, Tytgat, De Smedt, and De Vos, manuscript in preparation). The conclusion was therefore unavoidable that both taxa *C. violaceum* and *C. lividum* are not species of one genus, but are separate genera. The long list of phenotypic differences

(Table 4) and the low degree of DNA relatedness (about 24% D, Table 3) between both taxa strongly supported this suggestion.

We propose to retain and emend the name *Chromobacterium* Bergonzini 1881, 153 for the *C. violaceum* taxon only (Rule 39a and 35 of the *International Code of Nomenclature of Bacteria* [23]). Its diagnostic characters as given in *Bergey's Manual*, 8th ed. (32), have to be emended by the features from Table 4. The type and sole species is *C. violaceum* Bergonzini 1881, 153, emend. mut. char. Sneath 1956, 79, emend. mut. char. De Ley et al. (present paper), (Rule 39b) with the same description as the genus. The neotype strain remains MK = NCTC 9757 = NCIB 9131 = ATCC 12472 = Sneath D 252.

For the *C. lividum* taxon we propose a new genus name. Previously this taxon belonged in the genera *Bacillus*, *Bacterium*, and *Chromobacterium*. None of these is obviously suited as a new genus name. The most striking feature of these organisms is the violet color. Therefore we propose *Janthinobacterium*, genus novum, from *janthinus*, L. adj. violet-colored. The basic description is the same as for *Chromobacterium* in *Bergey's Manual*, 8th ed. (32), but has to be modified by the typical features in Table 4. The type and sole species is *J. lividum* comb. nov. (Rule 41a) with a description as for the genus. The neotype strain is NCTC 9796 = NCIB 9130 = ATCC 12473 = CCM 160 = Sneath HB and D303 = Hans H-24 (32). Some other members of this genus are listed in Table 1. DNA-rRNA hybridizations with strain ATCC 14487 showed it to be a regular member of *J. lividum*. Nevertheless it is an unusual organism, as it grows preferentially at 4°C, not at room temperature, and has a low G+C content of 61.2 mol%; its DNA-DNA hybridizations are the lowest of the taxon. We assume all this to be due to considerable deletions in the genome, since the genome DNA molecular weight is 2.65×10^9 , 18% smaller than the average genome size of the *J. lividum* taxon. The facts that both genera *Chromobacterium* emend. and *Janthinobacterium* have many phenotypic features in common (violaecin biosynthesis is but one of them) and that their genome DNAs hybridize for about 24% are good indications that they descended from common ancestors.

Misnamed strains. *C. amethystinum* ATCC 6915 has to be renamed and belongs in *J. lividum* (Table 1) in agreement with phenotypic findings of Sneath (personal communication). The rRNA cistrons of the following organisms were quite different from authentic *Chromobacterium* and *Janthinobacterium* rRNA cistrons. These organisms have been misnamed and do not belong in either *Chromobacterium* or *Jan-*

TABLE 4. *Phenotypic features differentiating the genera Chromobacterium and Janthinobacterium*^a

Feature	<i>Chromobacterium</i>	<i>Janthinobacterium</i>
Occurrence	Predominates in the tropics	Predominates in temperate regions
Pathogenicity	Occasionally responsible for pyogenic or septicemic infections of man and animals	Probably not pathogenic
Grows best at:	30–35°C ("mesophilic")	25°C ("psychrophilic")
Growth at 37°C	+	—
Aerobiosis	Facultatively aerobic	Strictly aerobic
Size of rods (μm)	0.6–0.9 by 1.5–3	0.8 – 1.2 by 2.5 – 6
Violet ring in broth	+	Usually viscous and frondlike
Production of HCN	+	—
Turbidity from egg-yolk	+	—
Proteolysis, hemolysis, and chitinolysis	Moderate to strong	Poor
Acid from (in Hugh & Leifson medium):		
L-Arabinose	—	Almost always +
Galactose	—	Almost always +
m-Inositol	—	Almost always +
Xylose	—	Almost always +
Lactose	—	Often +
Mannitol	—	Often +
Cellobiose	—	Often +
Inulin	—	Often +
Sucrose	Rarely +	Often +
Glycerol	Rarely +	Often +
Trehalose	+	—
Hydrolysis of esculin	—	+
Arginine decarboxylase	+	Rarely +
Arginine dihydrolase	Variable	—
Melanine from phenylalanine	Often	Usually —
Visible gas from NO ₃	—	Sometimes +
Methylene blue reduction	+	Seldom +
Growth on citrate and NH ₃	+	Usually rapid
Growth on:		
L-Leucine	—	+
Ornithine	+	—
Phenylalanine	+	—
G+C range (mol %)	65.2 – 67.6	65.1 – 66.1
Average mol wt of genome DNA	2.7×10^9	3.2×10^9

^a The phenotypic data are from Sneath (29–32) the G+C content values are from De Ley et al. (7, 15; unpublished results), and the genome sizes are from the present paper.

thinobacterium. This conclusion is corroborated by other data.

"*C. lividum*" ("*C. folium*") NCTC 10590 and NCTC 10591 were isolated from germinated seeds, and are present in leaf nodules of *Psychotria nairobiensis* and *Ardisia crispa* (1). Their rRNA cistrons were more closely related to the agrobacterial and rhizobial rRNA cistrons (16; Tables 1 and 2). These and other features (16) suggested that both strains belong in the family of the *Rhizobiaceae*, together with *Agrobacterium*, *Rhizobium*, *Mycoplana*, *Phyllobacterium*, and a few other organisms.

The exact taxonomic position of "*C. lividum*" GA has been a problem for several years, for its G+C content was noticeably different from the base composition of authentic *J. lividum* strains

(15) and it is yellow. The rRNA parameters of strain GA were outside the *Chromobacterium* and *Janthinobacterium* areas in the similarity maps. Strain GA belongs to neither of these genera. Since the parameters were more similar to *P. acidovorans* reference rRNA (Table 2), strain GA may be a member of *Pseudomonas* section II or III (17).

Several phenotypical features (halophily, flagellar arrangement, colorless to pale beige colonies, etc.) set *C. marismortui* ATCC 17056 apart from *Chromobacterium* and *Janthinobacterium*. In *Bergey's Manual*, 7th ed. (2), *C. marismortui* was still a regular species of *Chromobacterium*. In *Bergey's Manual*, 8th ed., *C. marismortui* was temporarily maintained as a species *incertae sedis* in the same genus (32). The rRNA

parameters (Tables 1 and 2) definitely move the holotype of this taxon outside *Chromobacterium* and *Janthinobacterium*. *C. marismortui* ATCC 17056 is either a border case of *Pseudomonas* section I or a member of a genus closely related to the latter taxon (Table 2).

"*Pseudomonas*" (*Chromobacterium*) *iodinum* continues to resist taxonomic identification. It is temporarily a species incertae sedis of *Chromobacterium* (32). Sneath (31) concluded that it was a gram-positive diphtheroid organism. Our rRNA data remove it definitely from *Chromobacterium*, *Janthinobacterium*, *Pseudomonas*, and nearly all of the other genera listed in Table 1. Its eventual relationship with the coryneforms remains to be explored.

"*C. indicum-rubrum*" NCTC 2847 has been renamed *Serratia marcescens*. The absorption peak of the extracted pigment was the same as that of prodigiosin from authentic *Serratia* (M. Gillis and De Ley, unpublished data). The properties of the rRNA hybrids of this strain fell correctly within the area of the *Enterobacteriaceae* (Table 1).

Sneath (31) found that "*C.*" *viscosum* NCTC 2416 does not belong to the chromobacteria. We found that the properties of its rRNA hybrids are quite different from *Chromobacterium* and *Janthinobacterium*. The exact taxonomic position of this strain is unknown. We received it as *Corynebacterium* sp.

Relationship of *Chromobacterium* and *Janthinobacterium* with other genera. The relationship between the former genus *Chromobacterium* Bergonzini and other genera has always been obscure. The early taxonomic wanderings of these bacteria are of historical interest only and will not be discussed here. In *Bergey's Manual*, 6th ed. (3) and 7th ed. (4), this genus turned up quite unjustifiably in the family of the *Rhizobiaceae* together with *Rhizobium* and *Agrobacterium*. Heberlein et al. (20) showed by DNA-DNA hybridization that both *J. lividum* and *C. violaceum* taxa were far removed from the latter genera and proposed to remove the chromobacteria from the family *Rhizobiaceae*. Our rRNA data confirmed this conclusion. In *Bergey's Manual*, 8th ed. (32), *Chromobacterium* Bergonzini was located in Part 8 (gram-negative, facultatively anaerobic rods) as a genus of uncertain affiliation in the vicinity of the *Enterobacteriaceae* and *Vibrionaceae*.

According to Fig. 3 and 4 the percent rRNA binding of both *Chromobacterium* and *Janthinobacterium* are most similar to the *Enterobacteriaceae*, the *Vibrionaceae*, *P. testosteroni*, *Comamonas percolans* NCIB 8193, and *Bacillus*. However, the percent rRNA binding cannot be a measure of overall taxon relatedness, be-

cause it is not a measure of ribosomal DNA homology (see above) and because of the enormous differences in phenotypic features and in G+C content between most of these taxa. On the contrary, a previous study with rRNA cistrons of *Agrobacterium* (16) demonstrated that $T_{m(e)}$ is a more valuable parameter as it displayed a clear-cut correlation with the overall phenotypic similarity. The rRNA cistron areas of both *Chromobacterium* and *Janthinobacterium* overlap (Fig. 3 and 4) with those of *P. testosteroni*. This taxon is a member of *Pseudomonas* section III (representative strains are *P. acidovorans*, *P. delafieldii*, *P. facilis*, and *P. solanacearum*) (17), both on phenotypic grounds (34) and by rRNA homology (competitive method) (28). At the same $T_{m(e)}$ level (Tables 1 and 2), we noticed representative strains of *Pseudomonas* section II (*P. cepacia* and *P. marginata*), of the authentic *Alcaligenes-Achromobacter* group (*Alc. faecalis*, *Alc. odorans*, *Alc. denitrificans*, *Ach. xylosoxidans*) (Kerstens and De Ley, manuscript in preparation), *Bordetella bronchiseptica*, and the H_2 -oxidizing *Alcaligenes eutrophus* and *Alcaligenes paradoxus*. All of these strains are at about the same $T_{m(e)}$ level, i.e., an average of 71°C versus *J. lividum* [^{14}C]rRNA and 68.5°C versus *C. violaceum* [^{14}C]rRNA. On the above grounds we concluded that the rRNA cistrons of *Chromobacterium*, *Janthinobacterium*, most or all of *Pseudomonas* sections II and III, the authentic *Alcaligenes-Achromobacter* group, *Bordetella bronchiseptica*, the H_2 -oxidizing polarly flagellated *Pseudomonas*, and peritrichously flagellated *Alcaligenes* are phylogenetically more similar to each other than to other genera; these taxa constitute one large rRNA family. Figure 5 summarizes the relationships between the $T_{m(e)}$ values. This similarity extends to the genotypic level, as most organisms have a high G+C content in the range 62 to 70 mol% (Table 5); the *Alc. faecalis* group and *P. lemoignei* are somewhat lower with 56.5 to 59.4 mol% G+C. Many of these genera share some common phenotypic features: they are gram-negative, rigidly bound rods, usually 0.5 to 1 by 1 to 4 μm , without resting stages, motile (by polar, peritrichous, or both types of flagella); they are chemoorganotrophic (some are facultatively chemolithotrophic for the oxidation of H_2); the metabolism is usually respiratory with O_2 as the universal electron acceptor; they frequently use organic acids, or glucose, and some other carbohydrates; growth factors are usually not required; they are soil and water bacteria, occasionally causing infections in mammals, in plants, or food spoilage. The phenotypic descriptions of some of these taxa are incompletely known. Our conclusions suggest that the phenotypic features of the taxa

FIG. 5. Levels of $T_{m(e)}$ similarities within the rRNA family of *Chromobacterium*, *Jantthinobacterium*, *Pseudomonas* (sections II and III), the authentic *Alcaligenes*, and *Bordetella bronchiseptica*. The next set of rRNA families joins at an average $T_{m(e)}$ of 62.1°C, and consists of the *Enterobacteriaceae*, the *Vibrionaceae*, *Pseudomonas* (section I), and a few other taxa. The next lower set of rRNA families is at an average $T_{m(e)}$ of 59.6°C and consists of the *Rhizobiaceae* (15), the acetic acid bacteria, and a few other taxa. All remaining taxa, both gram negative and gram positive, which we investigated are farther removed, with a $T_{m(e)}$ below 59°C. The data for the graph are from the present paper and from unpublished experiments from this laboratory (De Ley, De Vos, Segers). They were clustered by the unweighted average linkage method. The hatched areas on top of most branches represent the range of $T_{m(e)}$ values of the reference taxon.

in this rRNA family should be reexamined more comparatively.

The rRNA cistrons of all other taxa have a lower $T_{m(e)}$. They contain more mismatches and are thus less related to the *Chromobacterium* and *Jantthinobacterium* rRNA family. From the present paper and from unpublished experiments we can see three layers of $T_{m(e)}$ similarities in these remaining genera. (i) Be-

TABLE 5. DNA base composition range of taxa within the rRNA family of *Chromobacterium*, *Jantthinobacterium*, *Pseudomonas* sections II and III, *Alcaligenes* - *Achromobacter*, and *Bordetella bronchiseptica*^a

Taxon	Mol% G+C
<i>Chromobacterium</i>	65.2-67.6
<i>Jantthinobacterium</i>	65.1-66.1
<i>Pseudomonas</i>	
<i>acidovorans</i> group (<i>acidovorans</i> , <i>le-moinei</i> , <i>delafieldii</i>)	58-69
<i>testosteroni</i> group	62-64.5
H ₂ oxidizers (<i>facilis</i> , <i>flava</i> , <i>palleronii</i>)	61.7-67.3
<i>solanacearum</i> group	66-68
<i>cepacia</i> and <i>marginata</i>	66-68.6
<i>Alcaligenes</i> - <i>Achromobacter</i>	
Subgroup I (<i>Alc. faecalis</i>)	56.5-59.4
Subgroup II (<i>Alc. denitrificans</i>)	63.9-69.8
H ₂ -oxidizing <i>Alcaligenes</i> (<i>eutrophus</i> , <i>paradoxus</i>)	66.7-68.4
<i>Bordetella bronchiseptica</i>	68.2-69.5
<i>Comamonas percolans</i>	64.3

^a The data were determined in our laboratory by thermal denaturation. The subdivisions of *Pseudomonas* given are the combined results of the classification in *Bergey's Manual*, 8th ed. (17), and of unpublished hybridization data from this laboratory. The subdivisions in the *Alcaligenes* - *Achromobacter* group are due solely to data from this laboratory (13; unpublished data).

tween 60 and 65°C are the *Vibrionaceae*, the *Enterobacteriaceae*, the pseudomonads of section I (17), *Xanthomonas*, *Aplanobacter*, *Azotobacter*, and *Azomonas*, the marine "Alcaligenes" ("*Alc.*" *pacificus*, "*Alc.*" *venustus*, "*Alc.*" *aestus*, and "*Alc.*" *aquamarinus*), *Agarobacterium*, *Cellvibrio*, and a few other strains. (ii) Between 57 and 61.5°C are the *Rhizobiaceae* and their relatives (*Agrobacterium*, *Rhizobium*, *Mycoplana*, *Phyllobacterium*, "*Chromobacterium folium*"), *Gluconobacter*, *Acetobacter*, *Zymomonas*, *Paracoccus*, and some *Rhodopseudomonas*. (iii) All remaining taxa investigated are below $T_{m(e)}$ 62°C, in spite of the fact that some of them (e.g., *Bacillus*) have a high percent rRNA binding: *Flavobacterium*, *Arthrobacter*, *Corynebacterium*, *Microcycus*, *Campylobacter*, *Cytophaga*, and *HeLa*.

It is well known that there is excellent correlation between the dendrogram of amino acid sequences of cytochromes *c* from higher organisms and the phylogenetic relationship between these organisms, known from paleontological, comparative embryological, anatomical, and other data. We submit, as we did for the *Rhizobiaceae* (16), that a similar correlation may hold between the overall phenotypal and phylogenetic relationship of bacteria and the base sequences of their rRNA [here expressed as $T_{m(e)}$].

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LITERATURE CITED

- Bettelheim, K. A., J. F. Gordon, and J. Taylor. 1968. The detection of a strain of *Chromobacterium lividum* in the tissues of certain leaf nodulated plants by the immunofluorescence technique. *J. Gen. Microbiol.* 54:177-184.
- Breed, R. S. 1957. Genus III. *Chromobacterium* Bergonzini, 1881, p. 292-296. In R. S. Breed, E. G. D. Murray, and N. R. Smith (ed.), *Bergey's manual of determinative bacteriology*, 7th ed. The Williams & Wilkins Co., Baltimore, Md.
- Breed, R. S., E. G. D. Murray, and A. P. Hitchens (ed.). 1948. *Bergey's manual of determinative bacteriology*, 6th ed. The Williams & Wilkins Co., Baltimore, Md.
- Breed, R. S., E. G. D. Murray, and N. R. Smith (ed.). 1957. *Bergey's manual of determinative bacteriology*, 7th ed. The Williams & Wilkins Co., Baltimore, Md.
- Buchanan, R. E., and N. E. Gibbons (ed.). 1974. *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore, Md.
- Burton, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* 62:315-323.
- De Ley, J. 1964. Effect of mutation on DNA-composition of some bacteria. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 30:281-288.
- De Ley, J. 1968. DNA base composition of yellow *Erwinia* strains. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 34:257-262.
- De Ley, J. 1968. DNA base composition and hybridization in the taxonomy of phytopathogenic bacteria. *Annu. Rev. Phytopathol.* 6:63-90.
- De Ley, J. 1970. Reexamination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. *J. Bacteriol.* 101:738-754.
- De Ley, J., H. Cattoir, and A. Reynaerts. 1970. The quantitative measurement of DNA hybridization from renaturation rates. *Eur. J. Biochem.* 12:133-142.
- De Ley, J., and J. De Smedt. 1975. Improvements of the membrane filter method for DNA:rRNA hybridization. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 41:287-307.
- De Ley, J., K. Kersters, J. Khan-Matsubara, and J. M. Shewan. 1970. Comparative D-gluconate metabolism and DNA base composition in *Achromobacter* and *Alcaligenes*. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 36:193-207.
- De Ley, J., and R. Tytgat. 1970. Evaluation of membrane filter methods for DNA:DNA hybridization. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 36:461-474.
- De Ley, J., and J. Van Muylem. 1963. Some applications of deoxyribonucleic acid base composition in bacterial taxonomy. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 29:344-358.
- De Smedt, J., and J. De Ley. 1977. Intra- and intergeneric similarities of *Agrobacterium* ribosomal ribonucleic acid cistrons. *Int. J. Syst. Bacteriol.* 27:222-240.
- Doudoroff, M., and N. J. Palleroni. 1974. *Pseudomonas* Migula 1894, 237, p. 217-243. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore, Md.
- Gillis, M., and J. De Ley. 1975. Determination of the molecular complexity of double-stranded phage genome DNA from initial renaturation rates. The effect of DNA base composition. *J. Mol. Biol.* 98:447-464.
- Gillis, M., J. De Ley, and M. De Cleene. 1970. The determination of molecular weight of bacterial genome DNA from renaturation rates. *Eur. J. Biochem.* 12:143-153.
- Heberlein, G. T., J. De Ley, and R. Tytgat. 1967. Deoxyribonucleic acid homology and taxonomy of *Agrobacterium*, *Rhizobium*, and *Chromobacterium*. *J. Bacteriol.* 94:116-124.
- Kirby, K. S. 1957. A new method for the isolation of deoxyribonucleic acids; evidence on the nature of bonds between deoxyribonucleic acid and protein. *Biochem. J.* 66:495-504.
- Kirby, K. S., E. Fox-Carter, and M. Guest. 1967. Isolation of deoxyribonucleic acid and ribosomal ribonucleic acid from bacteria. *Biochem. J.* 104:258-262.
- Lapage, S. P., P. H. A. Sneath, E. F. Lessel, V. B. D. Skerman, H. P. R. Seeliger, and W. A. Clark (ed.). 1975. International code of nomenclature of bacteria. American Society for Microbiology, Washington, D.C.
- Leifson, E. 1956. Morphological and physiological characteristics of the genus *Chromobacterium*. *J. Bacteriol.* 71:393-400.
- Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. Mol. Biol.* 3:208-218.
- Meys, W. H., and R. A. Schilperoort. 1971. Determination of the amount of DNA on nitrocellulose membrane filters. *FEBS Lett.* 12:166-168.
- Moore, R. L., and B. J. McCarthy. 1967. Comparative study of ribosomal ribonucleic acid cistrons in enterobacteria and myxobacteria. *J. Bacteriol.* 94:1066-1074.
- Palleroni, N. J., R. Kunisawa, R. Contopoulou, and M. Doudoroff. 1973. Nucleic acid homologies in the genus *Pseudomonas*. *Int. J. Syst. Bacteriol.* 23:333-339.
- Sneath, P. H. A. 1956. Cultural and biochemical characteristics of the genus *Chromobacterium*. *J. Gen. Microbiol.* 15:70-98.
- Sneath, P. H. A. 1957. The application of computers to taxonomy. *J. Gen. Microbiol.* 17:201-226.
- Sneath, P. H. A. 1960. A study of the bacterial genus *Chromobacterium*. *Iowa State J. Sci.* 34:243-500.
- Sneath, P. H. A. 1974. *Chromobacterium* Bergonzini 1881, 153, p. 354-357. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore, Md.
- Sneath, P. H. A., and V. B. D. Skerman. 1966. A new list of type and reference strains of bacteria. *Int. J. Syst. Bacteriol.* 16:1-133.
- Stanier, R. Y., N. J. Palleroni, and M. Doudoroff. 1966. The aerobic pseudomonads: a taxonomic study. *J. Gen. Microbiol.* 43:159-271.