Intra- and Intergeneric Similarities of the Ribosomal Ribonucleic Acid Cistrons of *Acetobacter* and *Gluconobacter*

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Hybrids between ^{14}C -labeled ribosomal ribonucleic acid (rRNA) from either *Gluconobacter oxydans* subsp. *oxydans* NCIB 9013, *Acetobacter aceti* subsp. *aceti* NCIB 8621t₁, or *Zymomonas mobilis* subsp. *mobilis* ATCC 29191 and deoxyribonucleic acid (DNA) from acetic acid bacteria and representative strains of possibly related and other gram-negative bacteria were prepared. Each hybrid was described by two parameters: $T_{m(e)}$, the temperature at which 50% of the hybrid was denatured, and the percentage of rRNA binding, the amount of ^{14}C -labeled rRNA (in micrograms) duplexed under stringent conditions per 100 μg of filter-fixed homologous or heterologous DNA. Each taxon occupied a definite area on the rRNA similarity maps. Parameters of hybrids formed with rRNA from *G. oxydans* subsp. *oxydans* NCIB 9013 showed that the acetic acid bacteria consist of two separate but closely related groups corresponding to the genera *Acetobacter* and *Gluconobacter*. When compared with rRNA from *A. aceti* subsp. *aceti* NCIB 8621t₁, both genera were indistinguishable, showing that there were many strains of *Acetobacter* whose rRNA cistrons are as different from the reference *Acetobacter* rRNA as from *Gluconobacter* rRNA. The rRNA cistrons of *Acetobacter* were more heterogeneous than these of *Gluconobacter*. The great similarities among the $T_{m(e)}$'s of the heterologous hybrids and among the numerous phenotypic features stress that both genera are more closely related to each other than to any other genus. The parameters of the DNA:rRNA hybrids located the acetic acid bacteria as a separate branch in an rRNA superfamily consisting of *Rhodopseudomonas*, *Beijerinckia*, *Agrobacterium*, *Rhizobium*, some *Spirillum* species, and *Paracoccus denitrificans*. Parameters of hybrids formed with rRNA from *Z. mobilis* subsp. *mobilis* ATCC 29191 showed that the genus *Zymomonas* forms a separate branch in the same rRNA superfamily. We detected a number of misnamed organisms. "*Acetobacter*" *aceti* subsp. *xylinum* NCIB 4112, "*Acetobacter*" *aceti* subsp. *orleanensis* NCIB 6426, and "*Acetobacter*" *lermae* NRRL B-1810 belong in the genus *Gluconobacter*. "*Gluconobacter*" *industrius* IFO 3261, "*Gluconobacter*" *dioxyaceticus* IAM 1814, "*Gluconobacter*" sp. strains A4.1 and M28, "*Pseudomonas*" *melophthora* NCPPB 461 and 462, and "*Pseudomonas*" *pomi* NCPPB 463 are all regular members of *Acetobacter*. Our evidence is against the maintenance of "intermediate" strains of acetic acid bacteria. "*Gluconobacter*" *liquefaciens* NCIB 9505 and IAM 1834 and "*G.*" *melanogenus* IAM 1835 and IAM 1836 are genetically regular members of the genus *Acetobacter*. "*Acetobacter*" *aurantius* IFO 3246 is a *Gluconobacter*. "*A.*" *aurantius* IFO 3249, 3247, 13330, and 13333 are not acetic acid bacteria at all. We propose to unite *Acetobacter* and *Gluconobacter* in the family *Acetobacteraceae*. The ranges of the moles percent guanine plus cytosine of the DNAs have been determined for the different taxa in both genera.

Bacterial ribosomal ribonucleic acid (rRNA) cistron similarities (expressed as the midpoint of the thermal denaturation curve [$T_{m(e)}$] and the percentage of rRNA binding of the deoxyribonucleic acid [DNA]:rRNA hybrids) are valuable parameters for revealing taxonomic relatedness at the generic and suprageneric levels, for detecting misnamed taxa, and for identifying unknown isolates (16, 19). There seemed to be a

distinct correlation between the thermal stability of heterologous DNA:rRNA hybrids, expressed as $T_{m(e)}$, and the overall phenotypic similarities of the organisms concerned. These findings were discovered and explored in the *Rhizobiaceae* (19) and in *Chromobacterium* (16).

It is the purpose of the present paper to report the results of the application of this new method to an exploration of the internal taxonomic

structure of the acetic acid bacteria *Acetobacter* and *Gluconobacter* and their relationship to each other and to other genera. This is part of an extensive genotypic and phenotypic study of these organisms in our laboratory. We also examined the similarity between the rRNA cistrons of the acetic acid bacteria and *Zymomonas* in view of the considerable phenotypic similarities between these genera (39).

MATERIALS AND METHODS

Bacterial strains and growth media. The strains used in this study are listed in Tables 1 and 2 under the names with which they were received. The acetic acid bacteria recently received from the National Collection of Industrial Bacteria (NCIB) were named according to the nomenclature used in *Bergey's Manual*, eighth edition (8). The bacteriological purity of all strains was checked by plating and by examining living and gram-stained cells. All petri dish cultures were incubated aerobically, except for the *zymomonads*, which were incubated anaerobically by the use of GasPak (BBL Microbiology Systems). Some strains were differently displayed two different stable colony types (t_1 , t_2) upon plating; both were examined by polyacrylamide gel electrophoresis of their soluble proteins to differentiate between colony variants and contaminants (28). Either one or both types were used depending on the similarity of their electrophoregrams. For mass cultures, cells were grown as described before (19) except when stated otherwise. *Zymomonads* were grown without aeration at 28°C in 600 ml of liquid medium Y (19) in 1-liter Erlenmeyer flasks. The compositions of some of the growth media used are summarized in Table 3. After growth, the cells were harvested, washed, and stored at -12°C except for those of *Acetobacter peroxidans* and *A. pasteurianus* subsp. *paradoxus*, which were lysed immediately after harvesting.

Preparation of DNA. High-molecular-weight native DNA was prepared by the method described by Marmur (32). The final purification step was carried out through a CsCl gradient (16).

Fixation of single-stranded, high-molecular-weight DNA on membrane filters. The procedure used for fixing single-stranded, high-molecular-weight DNA on membrane filters was that described by De Ley and Tytgat (17). Sartorius membrane filters SM 11309 were used. The DNA-containing filters were preserved at 4°C in vacuo (13).

Preparation of [14 C]rRNA. Because the protein gel electrophoretograms of both colony types t_1 (dull) and t_2 (glistening) of NCIB 8621, the suggested neotype strain of *Acetobacter aceti* subsp. *aceti*, were nearly identical, we used t_1 for the preparation of [14 C]-labeled rRNA. From this strain and from NCIB 9013, the suggested neotype strain of *Gluconobacter oxydans* subsp. *oxydans*, [14 C]rRNA was prepared as previously described (19). We used medium N (19) with a block of marble instead of powdery CaCO_3 . [14 C]rRNA from the phenotypic centrostrain *Zymomonas mobilis* subsp. *mobilis* ATCC 29191 was prepared from 60 ml of cell suspension from a culture grown without aeration in medium Y (19) containing

30 μCi of filter-sterilized L-[14 C]aspartate (The Radiochemical Center, Amersham, Buckinghamshire, England). CO_2 liberated during growth was trapped in 40% KOH. The specific activities (expressed as counts per minute per microgram of rRNA) of [14 C]rRNA of *G. oxydans* subsp. *oxydans* NCIB 9013, *A. aceti* subsp. *aceti* NCIB 8621 t_1 , and *Z. mobilis* subsp. *mobilis* ATCC 29191 were 4,250, 6,500, and 345, respectively.

Saturation hybridization between [14 C]rRNA and filter-fixed DNA. Thermal stability of the DNA:rRNA hybrids. For hybridizations with [14 C]-rRNA from *Gluconobacter* and *Acetobacter*, we followed the methods as described in previous papers from this department (13, 16, 17, 19). In view of the very low specific activity of *Zymomonas* [14 C]rRNA, some modifications were required in the hybridization technique: two filters, each with approximately 80 μg of DNA, were hybridized separately with 20 μg of [14 C]-rRNA. The thermal stability of the hybrids was determined by counting for 100 min the amount of labeled material released from both filters together, in 2 ml of 1.5 $\times$ SSC in 20% formamide, in 10° steps from 50 to 90°C. SSC buffer contains 0.15 M NaCl and 0.015 M sodium citrate, pH 7. All labeled material was counted in a Beckman liquid scintillation spectrometer LS 100°C at room temperature. $T_{m(e)}$ is the temperature at which 50% of the hybrid was eluted. The total amount of [14 C]rRNA bound, after RNase treatment, expressed in micrograms of rRNA per 100 μg of DNA retained on the filter, was called "the percentage of rRNA binding."

Chemical determination of DNA on the filter. For determining the amount of DNA on the filter, we used a previously described method (19).

Determination of the G+C content of the DNA. Several of the moles percent guanine plus cytosine (G+C) in Tables 1 and 2 were previously published (16, 19, 38); the other G+C values were determined by the thermal denaturation method (18).

RESULTS

16S and 23S fractions. The distribution of the rRNA fractions in sucrose gradients is shown in Fig. 1. No 23S rRNA peak was detectable from *G. oxydans* subsp. *oxydans* NCIB 9013, and there was but a small peak in the rRNA preparation from *A. aceti* subsp. *aceti* NCIB 8621 t_1 . The 16S rRNA peaks are quite distinct in both cases. This distribution is quite different from that obtained with *Escherichia coli* (19), where approximately equal 16S and 23S peaks indicate approximately equal yields of each fraction. The low ratio of 23S to 16S with NCIB 8621 t_1 is approximately the same as in *Agrobacterium* (19). In the latter genus, nicking of the 23S rRNA (24, 35) explains its low yield; we assume that nicking explains the absence of 23S in *Gluconobacter* and its low yield in *Acetobacter* reference strains. Although the 16S peaks are probably heavily contaminated with fragmented 23S fractions as in *Agrobacterium* (19, 35), we shall continue to use both symbols for the sake

TABLE 1. List of strains studied, the growth media for bacterial growth, the DNA base compositions, and the properties of the DNA:rRNA hybrids with reference [¹⁴C]rRNA from the type strains of the type species of *Acetobacter* and *Gluconobacter*

Sequence no. in Fig. 3 and 4	Organisms used for DNA preparation"	Origin and strain no.	Growth medium (Table 2 and [19])	Mol % G + C	Hybridization with rRNA from			
					<i>Gluconobacter</i> <i>oxydans</i> subsp. NCIB 9013		<i>Acetobacter</i> <i>aceti</i> subsp. NCIB 8621 t ₁	
					<i>T_m</i> ^(a) (°C)	% Binding	<i>T_m</i> ^(a) (°C)	% Binding
1	<i>Gluconobacter oxydans</i> subsp. <i>oxydans</i>	NCIB 9013	N	57.9	81.5	0.27	77.5	0.22
2	<i>G. oxydans</i> subsp. <i>oxydans</i>	NCIB 9014	N	60.0	81.3	0.28	78.8	0.19
3	<i>G. oxydans</i> subsp. <i>suboxydans</i>	NCIB 621	N	61.2	80.8	0.26	77.0	0.21
4	<i>G. oxydans</i> subsp. <i>suboxydans</i>	NCIB 7069	N	62.9	81.0	0.30	77.5	0.21
5	<i>G. oxydans</i> subsp. <i>suboxydans</i>	NCIB 8035	N	63.6	81.0	0.25	77.5	0.20
6	<i>G. oxydans</i> subsp. <i>suboxydans</i>	NCIB 8036	N	62.0	80.3	0.22	75.8	0.18
7	<i>G. oxydans</i> subsp. <i>suboxydans</i>	NCIB 9119	N	60.6	80.5	0.23	76.3	0.18
8	<i>G. oxydans</i> subsp. <i>suboxydans</i>	NCIB 3734	N	61.7	81.8	0.27	77.3	0.20
9	<i>G. oxydans</i> subsp. <i>suboxydans</i>	NCIB 6723	N	63.3	81.8	0.28	77.5	0.10
10	<i>G. oxydans</i> subsp. <i>suboxydans</i>	NCIB 4739	N	61.4	80.3	0.19	75.5	0.17
11	<i>G. oxydans</i> subsp. <i>suboxydans</i>	NCIB 9137	N	59.4	81.0	0.23	77.0	0.19
12	<i>G. oxydans</i> subsp. <i>suboxydans</i>	NCIB 9498	N	62.8	81.8	0.26	77.5	0.22
13	<i>G. oxydans</i> subsp. <i>suboxydans</i>	NCIB 9108	N	56.0	80.3	0.22	76.0	0.19
14	<i>G. oxydans</i> subsp. <i>suboxydans</i>	NCIB 8131	N	63.4	80.3	0.24	75.3	0.18
15	<i>G. oxydans</i> subsp. <i>industrius</i>	NCIB 9099	N	60.7	81.8	0.19	78.8	0.13
16	<i>G. oxydans</i> subsp. <i>industrius</i>	NCIB 8624	N	56.1	80.8	0.25	77.0	0.22
17	<i>G. oxydans</i> subsp. <i>industrius</i>	NCIB 5595	N	64.0	80.8	0.25	77.0	0.20
18	<i>G. oxydans</i> subsp. <i>industrius</i>	NCIB 4943	N	61.9	80.8	0.27	77.3	0.19
19	<i>G. oxydans</i> subsp. <i>industrius</i>	IFO 3261	N	61.1	77.8	0.18	77.3	0.18
20	<i>G. oxydans</i> subsp. <i>melanogenes</i>	NCIB 8086	N	60.6	81.0	0.21	77.0	0.16
21	<i>G. oxydans</i> subsp. <i>melanogenes</i>	NCIB 8085	N	59.4	81.5	0.23	77.3	0.17
22	<i>G. oxydans</i> subsp. <i>melanogenes</i>	NCIB 8084	N	60.3	80.8	0.22	76.3	0.20
23	<i>G. oxydans</i> subsp. <i>melanogenes</i>	49	N	61.1	81.3	0.24	76.8	0.18
24	<i>G. melanogenus</i>	116	N	60.6	81.0	0.21	77.8	0.18
25	" <i>G. dioxycetonicus</i> "	IAM 1814	N	56.8	76.5	0.16	81.0	0.14
26	<i>G. nonoxygluconicus</i>	IFO 3276	N	56.5	80.8	0.28	76.5	0.21
27	<i>G. oxydans</i> subsp. <i>sphaericus</i>	IFO 12467	N	56.3	80.8	0.21	77.3	0.17
28	<i>G. rubiginosus</i>	IFO 3244	N	61.2	81.5	0.24		
29	<i>G. oxydans</i> subsp. <i>hoyerianum</i>	LMD 29.7 t ₁	Z2	60.5	80.5	0.27		
30	" <i>Gluconobacter</i> " sp.	A 4.1	N	57.7	75.5	0.21	75.8	0.23
31	" <i>Gluconobacter</i> " sp.	A 13.1	N	55.6	59.5	0.10	61.0	0.13
32	" <i>Gluconobacter</i> " sp.	M 28	N	57.2	76.5	0.25	76.5	0.24
33	<i>Acetobacter aceti</i> subsp. <i>aceti</i>	NCIB 8621 t ₁	N	58.7	76.0	0.13	81.0	0.17

TABLE 1. —continued

Sequence no. in Fig. 3 and 4	Organisms used for DNA preparation ^a	Origin and strain no.	Growth medium (Table 2 and [19])	Mol % G + C	Hybridization with rRNA from			
					Gluconobacter oxydans subsp. NCIB 9013		Acetobacter aceti subsp. NCIB 8621 t ₁	
					T _m (^c) (°C)	% Binding	T _m (^c) (°C)	% Binding
34	<i>A. aceti</i> subsp. <i>aceti</i>	NCIB 8621 t ₂	N	57.4	76.0	0.12	81.0	0.17
35	<i>A. aceti</i> subsp. <i>aceti</i>	NCIB 8940	N	55.4	75.0	0.18	80.8	0.16
36	<i>A. aceti</i> subsp. <i>aceti</i>	NCIB 8941	N	57.9	77.0	0.10	80.3	0.10
37	<i>A. aceti</i> subsp. <i>aceti</i>	NCIB 8554	N	55.9	75.5	0.18	81.5	0.15
38	<i>A. ketogenum</i>	24 wt	N	56.1	74.5	0.12	79.0	0.16
39	<i>A. aceti</i>	A2	N	59.4	77.0	0.10	81.5	0.13
40	<i>A. aceti</i>	CH 31	N	59.5	75.8	0.12	80.8	0.13
41	<i>A. aceti</i> subsp. <i>muciparum</i>	5	N	59.5	75.8	0.10	80.3	0.10
42	<i>A. aceti</i> subsp. <i>orleanensis</i>	NCIB 8622	N	56.8	77.0	0.22	78.3	0.22
43	<i>A. aceti</i> subsp. <i>orleanensis</i>	NCIB 8746	N	59.7	76.3	0.24	75.8	0.19
44	" <i>A. aceti</i> subsp. <i>orleanensis</i> "	NCIB 6426	N	59.6	81.3	0.30	76.5	0.24
45	<i>A. aceti</i> subsp. <i>orleanensis</i>	NCIB 8747	N	60.7	76.5	0.18	77.3	0.21
46	<i>A. mesoxydans</i>	P20	N	56.2	78.3	0.12	80.8	0.13
47	<i>A. aceti</i> subsp. <i>xylinum</i>	NCIB 613	N	54.9	76.5	0.16	76.5	0.14
48	<i>A. aceti</i> subsp. <i>xylinum</i>	NCIB 1375	N	58.1	77.3	0.24	77.0	0.17
49	" <i>A. aceti</i> subsp. <i>xylinum</i> "	NCIB 4112	N	62.4	81.8	0.26	77.3	0.19
50	<i>A. aceti</i> subsp. <i>xylinum</i>	NCIB 4940	N	62.4	76.0	0.22	76.0	0.18
51	<i>A. aceti</i> subsp. <i>xylinum</i>	NCIB 5346	N	58.4	76.8	0.25	77.0	0.21
52	<i>A. aceti</i> subsp. <i>xylinum</i>	NCIB 6656	N	59.1	77.8	0.22	77.8	0.18
53	<i>A. aceti</i> subsp. <i>xylinum</i>	NCIB 7029	N	58.9	77.8	0.20	76.5	0.19
54	<i>A. aceti</i> subsp. <i>xylinum</i>	NCIB 8034	N	55.1	77.8	0.22	77.8	0.23
55	<i>A. aceti</i> subsp. <i>xylinum</i>	NCIB 8132	N	60.4	77.8	0.25	76.5	0.16
56	<i>A. aceti</i> subsp. <i>xylinum</i>	NCIB 8246	N	55.2	76.3	0.20	78.0	0.20
57	<i>A. aceti</i> subsp. <i>xylinum</i>	NCIB 8623	N	55.1	76.5	0.14	76.3	0.15
58	<i>A. aceti</i> subsp. <i>xylinum</i>	NCIB 8752	N	62.6	77.8	0.20	77.8	0.18
59	<i>A. xylinum</i>	12	N	57.8	75.8	0.20	75.8	0.17
60	<i>A. xylinum</i>	25	N	62.8	77.3	0.24	77.8	0.20
61	<i>A. aceti</i> subsp. <i>liquefaciens</i>	NCIB 9505	N	64.0	76.2	0.13	77.3	0.10
62	<i>A. aceti</i> subsp. <i>liquefaciens</i>	NCIB 9417	N	63.7	75.8	0.17	76.0	0.20
63	<i>A. aceti</i> subsp. <i>liquefaciens</i>	NCIB 9418	N	64.6	76.0	0.18	77.8	0.16
64	<i>A. aceti</i> subsp. <i>liquefaciens</i>	NCIB 9136	N	62.3	77.3	0.17	78.0	0.16
65	<i>A. pasteurianus</i>	NCIB 8757	N	55.4	75.5	0.21	77.8	0.21
66	<i>A. pasteurianus</i>	NCIB 8856	Z1	53.8	77.5	0.28	79.8	0.19
67	<i>A. pasteurianus</i>	NCIB 2224	N	57.5	76.5	0.22	76.8	0.14

TABLE 1. —continued

Sequence no. in Fig. 3 and 4	Organisms used for DNA preparation ^a	Origin and strain no.	Growth medium (Table 2 and [19])	Mol % G + C	Hybridization with rRNA from			
					Gluconobacter oxydans subsp. NCIB 9013		Acetobacter aceti subsp. NCIB 8621 t ₁	
					T _m (^a) (°C)	% Binding	T _m (^a) (°C)	% Binding
68	<i>A. pasteurianus</i>	NCIB 8090	N	57.9	77.0	0.18	78.5	0.17
69	<i>A. pasteurianus</i>	NCIB 6249	Z1	58.5	76.5	0.20	77.3	0.19
70	<i>A. pasteurianus</i>	NCIB 6423	Z1	60.0	77.3	0.19	77.0	0.16
71	<i>A. pasteurianus</i>	NCIB 6424	Z1	59.7	76.0	0.19	78.5	0.20
72	<i>A. pasteurianus</i>	NCIB 6425	N	55.0	76.5	0.17	78.8	0.18
73	<i>A. pasteurianus</i>	NCIB 6427	N	60.7	75.8	0.16	77.5	0.19
74	<i>A. pasteurianus</i>	NCIB 6429	Z1	58.0	75.3	0.19	77.3	0.18
75	<i>A. pasteurianus</i>	NCIB 6430	Z1	60.3	77.0	0.18	78.5	0.17
76	<i>A. pasteurianus</i>	NCIB 7030	Z1	52.6	75.5	0.23	78.3	0.22
77	<i>A. pasteurianus</i>	NCIB 7113	N	58.2	77.3	0.17	78.8	0.17
78	<i>A. pasteurianus</i>	NCIB 7212	Z1	52.8	77.3	0.21	77.8	0.21
79	<i>A. pasteurianus</i>	NCIB 7214	Z1	56.2	77.5	0.19		
80	<i>A. pasteurianus</i>	NCIB 7215	N	54.8			79.5	0.18
81	<i>A. pasteurianus</i>	NCIB 8088	Z1	56.4	77.0	0.21	78.0	0.21
82	<i>A. pasteurianus</i>	NCIB 8089	N	56.6	75.2	0.19	78.8	0.18
83	<i>A. rancens</i>	11	Z8	51.7			79.0	0.16
84	<i>A. rancens</i>	NCIB 4939	N	57.4	77.0	0.25		
85	<i>A. rancens</i>	NCIB 6428	N	58.9	76.8	0.16	77.0	0.17
86	<i>A. rancens</i>	23 kl ⁺	N	55.4	77.0	0.21	78.8	0.19
87	<i>A. pasteurianus</i> subsp. <i>pasteurianus</i>	NCIB 8133	Z1	52.9	78.0	0.18	79.5	0.16
88	<i>A. pasteurianus</i> subsp. <i>pasteurianus</i>	NCIB 8148	Z1	56.2	78.0	0.17	78.8	0.14
89	<i>A. pasteurianus</i> subsp. <i>pasteurianus</i>	NCIB 8555	N	56.2	77.3	0.18		
90	<i>A. pasteurianus</i> subsp. <i>pasteurianus</i>	NCIB 8619	Z1	55.2	76.8	0.24	78.8	0.20
91	<i>A. pasteurianus</i> subsp. <i>pasteurianus</i>	NCIB 8758	Z1	53.2	76.8	0.20	77.3	0.22
92	<i>A. pasteurianus</i> subsp. <i>pasteurianus</i>	NCIB 8894	Z1	56.8	77.3	0.15	79.5	0.16
93	<i>A. pasteurianus</i> subsp. <i>pasteurianus</i>	NCIB 8945 t ₁	N	57.9	76.0	0.11	78.5	0.11
94	<i>A. pasteurianus</i> subsp. <i>pasteurianus</i>	NCIB 8945 t ₂	N	59.3	76.8	0.13	78.3	0.13
95	<i>A. pasteurianus</i> subsp. <i>pasteurianus</i>	NCIB 8163	N	55.0	75.5	0.25	77.8	0.23
96	<i>A. pasteurianus</i> subsp. <i>pasteurianus</i>	NCIB 8956	N	57.6	75.8	0.17	78.5	0.11
97	<i>A. pasteurianus</i> subsp. <i>paradoxus</i>	NCIB 9015	Z8	52.8	77.0	0.21	78.5	0.22
98	<i>A. pasteurianus</i> subsp. <i>estunensis</i>	NCIB 8935	N	60.4	78.0	0.14	80.5	0.18
99	<i>A. estunensis</i>	E	N	62.2	77.8	0.13	78.8	0.14
100	<i>A. pasteurianus</i> subsp. <i>ascendens</i>	NCIB 8138	Z8	54.1	78.0	0.20	79.5	0.17
101	<i>A. ascendens</i>	asc. kl.	Z8	54.3			78.5	0.18

TABLE 1. —continued

Sequence no. in Fig. 3 and 4	Organisms used for DNA preparation ^a	Origin and strain no.	Growth medium (Table 2 and [19])	Hybridization with rRNA from			
				Mol % G + C		Gluconobacter oxydans subsp. oxydans NCIB 9013	
				<i>T_m</i> (^o C)	% Binding	<i>T_m</i> (^o C)	% Binding
102	<i>A. pasteurianus</i> subsp. <i>lovaniensis</i>	NCIB 8620	N	58.0	0.20	76.3	0.19
103	<i>Acetobacter</i> sp.	NCIB 1345	N	58.4	0.20	75.3	0.20
104	<i>Acetobacter</i> sp.	ATCC 15487	N	53.8	0.24	76.5	0.20
105	<i>A. schuezenbachii</i>	A1	N	57.3	0.14	76.0	0.11
106	"A." <i>lermae</i>	NRRL B-1810	N	59.6	0.29	81.3	0.20
107	<i>A. vini</i>	LMD 31.4	N	53.3	0.17	77.3	0.12
108	"A." <i>aurantium</i>	IFO 3249	N	63.4	0.12	61.8	0.10
109	"A." <i>aurantium</i>	IFO 3247	N	63.6	0.15	60.5	0.09
110	"A." <i>aurantium</i>	IFO 3248	N	58.4	0.19	77.3	0.18
111	"A." <i>aurantium</i>	IFO 13333	N	62.2	0.10	60.0	0.08
112	"A." <i>aurantium</i>	IFO 13330	N	63.1	0.10	57.8	0.11
113	"A." <i>aurantium</i>	IFO 3246 t ₁	N	56.3	0.14	80.3	0.13
114	<i>A. peroxydans</i>	NCIB 8087	Z8	60.6	0.20	76.3	0.15
115	<i>A. peroxydans</i>	NCIB 8618	Z8	62.5	0.23	77.5	0.18
116	<i>A. peroxydans</i>	LMD 53.9	Z8	61.0		79.5	0.18
117	" <i>Pseudomonas melophthora</i> "	NCPPB 461	N	57.2	0.17	77.0	0.17
118	" <i>P. melophthora</i> "	NCPPB 462	N	57.5	0.14	76.8	0.15
119	" <i>P. pomi</i> "	NCPPB 463	N	58.5	0.14	79.3	0.14
120	<i>Spirillum itersonii</i> subsp. <i>vulgatum</i>	NCIB 9071	Z7	61.8	0.15	67.5	0.13
121	<i>S. polymorphum</i>	NCIB 9072	Z7	63.7	0.10	69.5	0.07
122	<i>S. sinuosum</i>	NCIB 9010	Z7	59.3	0.11	57.5	
123	<i>Zymomonas mobilis</i> subsp. <i>mobilis</i>	ATCC 29191	Y	48.8		66.0	0.15
124	<i>Z. mobilis</i> subsp. <i>mobilis</i>	Z8	Y	49.0	0.12	65.5	
126	<i>Z. mobilis</i> subsp. <i>mobilis</i>	5.5	Y	48.0	0.11	66.0	
128	<i>Z. mobilis</i> subsp. <i>mobilis</i>	AG 11	Y	46.5			
130	<i>Z. mobilis</i> subsp. <i>pomaceae</i>	ATCC 29192	Y	48.2	0.12	67.5	0.13
131	<i>Z. mobilis</i> subsp. <i>pomaceae</i>	NCIB 10565	Y	46.4		65.3	0.12
132	<i>Rhodopseudomonas capsulata</i>	NCIB 8254	Z4	65.2	0.08	65.8	0.12
133	<i>R. palustris</i>	NCIB 8252	Z4	67.2	0.06	64.0	0.07
134	<i>R. sphaeroides</i>	NCIB 8253	Z4	68.4	0.06	68.0	0.04
135	" <i>Agrobacterium</i> " <i>aggregatum</i>	Ahrens B1	T	58.7	0.06	67.5	0.05
136	"A." <i>ferrugineum</i>	Ahrens A43	T	64.8	0.06	66.5	0.05
137	"A." <i>luteum</i>	Ahrens B14	T	52.5		66.0	0.03
139	<i>Rhizobium japonicum</i>	3.1	I	64.0	0.04	69.8	0.02

TABLE 1. —continued

Sequence no. in Fig. 3 and 4	Organisms used for DNA preparation ^a	Origin and strain no.	Growth medium (Table 2 and [19])	Mol % G + C	Hybridization with rRNA from			
					Gluconobacter oxydans subsp. NCIB 9013		Acetobacter aceti subsp. aceti NCIB 8621 t ₁	
					T _m (^a) (°C)	% Binding	T _m (^a) (°C)	% Binding
140	<i>R. japonicum</i>	USDA 311b59	I	64.0	67.0	0.03		
141	<i>Agrobacterium tumefaciens</i>	ICPB TT111	A	60.6			67.0	0.07
142	<i>A. tumefaciens</i>	CIP B6	A	60.2	67.5	0.08	67.3	0.06
143	<i>A. rhizogenes</i>	ICPB TR101	A	60.7	66.5	0.07		
144	" <i>Agrobacterium</i> " sp.	NA	B	58.9	66.3	0.09		
145	<i>Agrobacterium</i> sp.	Pérombélou 175/1	A	57.4	67.0	0.10		0.10
146	<i>Agrobacterium</i> sp.	Pérombélou 177/4	A	57.6	66.5	0.10	67.5	0.11
147	" <i>Azotomonas</i> " <i>fluorescens</i>	NCIB 9884	B	64.8	66.5	0.06	68.3	0.08
148	<i>Mycoplana dimorpha</i>	NCIB 9439	Z5	63.8	68.5	0.13	65.0	0.11
149	<i>Phyllobacterium rubiacearum</i>	LMG 1t1	B	61.3	66.8	0.07	65.8	0.06
150	<i>P. rubiacearum</i>	LMG 1t2	B	60.3	65.5	0.09		
151	<i>P. myrsinacearum</i>	LMG 2t2	B	59.8	66.8	0.07	66.8	0.06
152	<i>P. myrsinacearum</i>	LMG 3t2	B	59.6	65.8	0.08	66.0	0.06
153	<i>P. myrsinacearum</i>	LMG 3t1	B	60.4	65.8	0.06		
154	" <i>Pseudomonas</i> " <i>diminuta</i>	CCEB 513	Z3	67.3	65.0	0.08	67.8	0.07
155	<i>Paracoccus denitrificans</i>	ATCC 17741	Z5	66.3	65.0	0.09	65.8	0.08
156	<i>P. denitrificans</i>	ATCC 19367	Z5	67.4	66.5	0.07	65.0	0.07
159	<i>Beijerinckia fluminensis</i>	Hilger	J	56.2	65.0	0.10		
160	" <i>Chromobacterium</i> " <i>folium</i>	NCTC 10590	X	61.0	66.0	0.08	65.0	0.09
161	" <i>C.</i> " <i>folium</i>	NCTC 10591	X	63.0	66.3	0.08	66.3	0.08
162	" <i>Alcaligenes</i> " <i>paradoxus</i>	ATCC 17549 t ₂	A	67.5	57.0	0.02		
163	" <i>A.</i> " <i>paradoxus</i>	ATCC 17712	A	67.9	57.3	0.03		
164	<i>Bordetella bronchiseptica</i>	NCTC 8761	B	69.5	59.0	0.06		
165	<i>Pseudomonas facilis</i>	ATCC 15376	Z5	63.7	58.0	0.09		
166	<i>P. fluorescens</i>	ATCC 13525	Z5	60.2	58.0	0.15		
167	<i>Chromobacterium violaceum</i>	NCTC 9373	X	65.8	59.0	0.12		
168	<i>Janthinobacterium lividum</i>	NCTC 9796	X	65.5	58.0	0.12		

^a Name of organisms as received; quotation marks are used to indicate that the organism as received was misidentified.

TABLE 2. List of selected strains, the growth media used, the DNA base compositions, and the properties of the DNA:rRNA hybrids with reference [¹⁴C] rRNA from the type strain of the type species of *Zymomonas*

Sequence no. in Fig. 3, 4, and 5	Organisms used for DNA preparation	Origin and strain no.	Growth medium (Table 3 and [19])	Mol % G + C	Hybridization with rRNA from <i>Z. mobilis</i> subsp. <i>mobilis</i> ATCC 29119	
					<i>T_m</i> (°C)	% Binding
27	<i>Gluconobacter oxydans</i> subsp. <i>sphaericus</i>	IFO 12467	N	56.3	63.0	0.13
28	<i>G. rubiginosus</i>	IFO 3244	N	61.2	63.5	0.17
71	<i>Acetobacter pasteurianus</i>	NCIB 6424	Z1	59.7	62.5	0.16
73	<i>Ace. pasteurianus</i>	NCIB 6427	N	60.7	63.0	0.14
80	<i>Ace. pasteurianus</i>	NCIB 7215	N	54.8	61.0	0.14
120	<i>Spirillum itersonii</i> subsp. <i>vulgatum</i>	NCIB 9071	Z7	61.8	65.0	0.15
123	<i>Zymomonas mobilis</i> subsp. <i>mobilis</i>	ATCC 29191	Y	48.8	78.0	0.24
125	<i>Z. mobilis</i> subsp. <i>mobilis</i>	B70	Y		77.0	0.22
126	<i>Z. mobilis</i> subsp. <i>mobilis</i>	5.5	Y	48.0	77.5	0.22
127	<i>Z. mobilis</i> subsp. <i>mobilis</i>	5.3	Y	49.3	76.0	0.21
128	<i>Z. mobilis</i> subsp. <i>mobilis</i>	AG 11	Y	46.5	78.0	0.25
129	<i>Z. mobilis</i> subsp. <i>mobilis</i>	CP 3	Y	47.0	78.0	0.24
130	<i>Z. mobilis</i> subsp. <i>pomaceae</i>	ATCC 29192	Y	48.2	78.0	0.21
131	<i>Z. mobilis</i> subsp. <i>pomaceae</i>	NCIB 10565	Y	46.4	76.5	0.20
132	<i>Rhodopseudomonas capsulata</i>	NCIB 8254	Z4	65.2	63.5	0.12
133	<i>R. palustris</i>	NCIB 8252	Z4	67.2	63.5	0.05
138	" <i>Agrobacterium</i> " <i>gelatinovorum</i>	Ahrens B6	T	57.6	64.5	0.04
139	<i>Rhizobium japonicum</i>	3.1	I	64.0	63.5	0.02
140	<i>Rhi. japonicum</i>	USDA 311b59	I	64.0	63.5	0.03
141	<i>Agrobacterium tumefaciens</i>	ICPB TT111	A	60.6	66.0	0.10
142	<i>Agr. tumefaciens</i>	CIP B6	A	60.2	65.0	0.09
154	" <i>Pseudomonas</i> " <i>diminuta</i>	CCEB 513	Z3	67.3	64.5	0.09
155	<i>Paracoccus denitrificans</i>	ATCC 17741	Z5	66.3	63.0	0.08
156	<i>Par. denitrificans</i>	ATCC 19367	Z5	67.4	63.5	0.06
157	<i>Beijerinckia indica</i>	NCIB 8005	J	58.2	65.0	0.07
158	<i>Beij. indica</i>	LMD 38.7	J	57.6	65.0	0.09
161	" <i>Chromobacterium</i> " <i>folium</i>	NCTC 19591	X	63.0	71.5	0.10

TABLE 3. Compositions of growth media^a

Ingredient	Medium							
	Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8
Glucose			1 ^b					
Mannitol		2.5						
Yeast extract (Oxoid)	2	0.5	0.5	1	0.2	0.2		2
Meat extract (Oxoid)					0.1	0.1		
Peptone (Oxoid)		0.3			0.5	0.5	1	
Proteose peptone (Oxoid)			0.5					
(NH ₄) ₂ SO ₄							0.1	
K ₂ HPO ₄				0.1				
NaCl					0.5	3.5		
FeCl ₃ ·6H ₂ O							0.0002	
MnSO ₄ ·H ₂ O							0.0002	
MgSO ₄ ·7H ₂ O				0.05			0.1	
CaCO ₃								2
Ca-lactate	2							1
Succinic acid							0.1	
Red wine (in % vol/vol)								50
Tap water	+			+				
Distilled water		+	+		+	+	+	
pH adjusted to			6.5-7	7-7.2	7.4		6.8	

^a The compositions of media A, B, N, T, V, X, and Y have been given previously (16, 19).

^b Figures are percentages (wt/vol) unless stated otherwise. Symbol: +, Indicates the kind of water used.

FIG. 1. [¹⁴C]rRNA distributions in a linear 15 to 30% sucrose gradient.

of simplicity. There is no evidence that the 23S fraction would be contaminated by the 16S fraction. From *Z. mobilis* subsp. *mobilis* ATCC 29191, we isolated almost equal amounts of 23S and 16S [¹⁴C]rRNA. Although about 50% of the added L-[¹⁴C]aspartate had been consumed by the zymomonad, only 5% of this amount was assimilated in the bulk rRNA fraction. Other labeled rRNA precursors such as [2-¹⁴C]uracil and [6-¹⁴C]orotic acid were not taken up at all by zymomonads in medium Y (19). Hybridizations were carried out with 16S rRNA from *G. oxydans* subsp. *oxydans* NCIB 9013 and with either 23S or 16S from *A. aceti* subsp. *aceti* NCIB 8621t₁ and from *Z. mobilis* subsp. *mobilis* ATCC 29191. Homologous and heterologous hybridizations with 23S and 16S rRNA from *A. aceti* subsp. *aceti* (Table 4) and from *Zymomonas* gave similar results within the limits of reproducibility of $\pm 0.5^\circ\text{C}$ for $T_{m(e)}$ and $\pm 0.015\%$ for the percentage of rRNA binding (19).

Comparison of DNA:rRNA hybrids. The DNA:rRNA hybridization data are compiled in Tables 1 and 2. They will be evaluated extensively in the Discussion. $T_{m(e)}$ and percentage of rRNA binding were obtained from the melting curves of the hybrids. A few examples are given in Fig. 2. For each labeled reference rRNA, $T_{m(e)}$ was plotted versus the percentage of rRNA binding for each DNA:rRNA hybrid resulting in rRNA similarity maps (Fig. 3 through 5). $T_{m(e)}$ is the most useful of both parameters (16, 19). It

TABLE 4. $T_{m(e)}$ and the percent rRNA binding of hybrids formed between either 16S or 23S rRNA from *A. aceti* subsp. *aceti* NCIB 8621 t_1 and DNA from several other acetic acid bacteria

Organism used for DNA preparation	rRNA from <i>A. aceti</i> subsp. <i>aceti</i> NCIB 8621 t_1			
	16S		23S	
	$T_{m(e)}$ (°C)	% Binding	$T_{m(e)}$ (°C)	% Binding
<i>A. aceti</i> subsp. <i>aceti</i> NCIB 8621 t_1	80.8	0.16	81.0	0.19
<i>A. aceti</i> A ₂	81.8	0.11	81.5	0.14
<i>A. aceti</i> subsp. <i>liquefaciens</i> NCIB 9136	77.8	0.14	78.0	0.17
<i>A. aceti</i> subsp. <i>aceti</i> NCIB 8940	80.8	0.17	81.0	0.17
<i>G. oxydans</i> subsp. <i>oxydans</i> NCIB 9013	77.3	0.20	77.5	0.24

FIG. 2. Examples of melting curves of hybrids between 16S [^{14}C]rRNA from *G. oxydans* subsp. *oxydans* NCIB 9013 and filter-fixed DNA from various bacteria. The total amount of cp50m per 100 μg of DNA (counts per 50 min per 100 μg of filter-fixed DNA) is a measure of the amount of hybrid denatured. The melting point, $T_{m(e)}$, is identified by the dot on each curve.

has to be stressed that the percentage of rRNA binding is not a measure of rRNA cistron homology because it depends not only on the actual rRNA homology but also on the number of rRNA cistrons per genome, the size of the genome, and its state of replication; practice has shown that it is a useful parameter only in separating taxa (16, 19). Data on reciprocal hybridizations between DNA from *Acetobacter*, *Gluconobacter*, and *Zymomonas* and [¹⁴C]rRNA from other genera are summarized in Table 5; they are in excellent agreement with the hybridizations reported in Tables 1 and 2 and strengthen our discussion given below.

DISCUSSION

rRNA similarities (Fig. 3 to 5). The $T_{m(e)}$ similarity between rRNA cistrons from different bacteria is a measure of their relationship at the inter- and suprageneric level and can be used for taxonomic and identification purposes. The basic aspects of the discussion on the rRNA similarity maps were given earlier (16, 19). Each strain has a fixed position on each map within the limits of reproducibility. Each taxon occupies a definite area on a rRNA similarity map (13, 16, 19). When many strains are included, the size and shape of the rRNA similarity area provide a rough measure of the internal phenotypic and genetic heterogeneity of the taxon. A few strains suffice to localize a genus on the map.

The genus *Gluconobacter*. According to *Bergey's Manual*, eighth edition (8), the genus *Gluconobacter* consists of one species, *G. oxydans*, and four subspecies, *G. oxydans* subsp. *oxydans*, *G. oxydans* subsp. *industrius*, *G. oxydans* subsp. *suboxydans*, and *G. oxydans* subsp. *melanogenes*. Representative strains of each of these subspecies were included as well as variously named strains from several collections. We included a total of 27 authentic *Gluconobacter* strains (Fig. 3 and 4 and Table 1). When their DNAs were hybridized with [¹⁴C]rRNA of the type strain of *G. oxydans* subsp. *oxydans* NCIB 9013, they all fell into a well-defined area with $T_{m(e)}$ from 80.3 to 81.8°C and 0.18 to 0.30% rRNA binding. When their DNA was hybridized with [¹⁴C]rRNA from *A. aceti* subsp. *aceti* NCIB 8621t₁, they fell into an area between $T_{m(e)}$ 75.3 and 78.8°C and between 0.13 and 0.22% rRNA binding. The area delineated by hybridization with NCIB 9013 [¹⁴C]rRNA was clearly separated from all other genera. Into that area fell, in addition to the named *Gluconobacter* strains, a number of differently named strains (*A. aceti* subsp. *xylinum* NCIB 4112, *A. aceti* subsp. *orleanensis* NCIB 6426, *A. lermæ* NRRL B-1810, and *A. aurantius* IFO 3246t₁). On the basis of

our previous experience (16, 19) that the position of a strain in the rRNA similarity maps and in particular the value of $T_{m(e)}$ are powerful aids for identification, we concluded that the latter four strains were misnamed and belong in *Gluconobacter*. There is additional evidence supporting this conclusion.

"A. lermæ NRRL B-1810, isolated from fermenting agave juice near lake Lerma in Mexico in 1950 (23), is phenotypically a typical *Gluconobacter* (J. De Ley, K. Kersters, and J. Swings, manuscript in preparation). Its exact taxonomic position in this genus is being worked out.

A. aceti subsp. *orleanensis* NCIB 6426, isolated from a ropy sample of top-fermented beer in the United Kingdom, was originally named *A. viscosum* (42). It was found in our department to be phenotypically a *gluconobacter*; its protein electrophoretogram located it in *G. oxydans* subsp. *suboxydans* (K. Kersters and J. De Ley, manuscript in preparation).

The third strain, NCIB 4112, is an interesting taxonomic case. We received it from the NCIB as *Acetobacter aceti* subsp. *xylinum*. According to the NCIB catalogue, this strain is the original "la bactérie du sorbose" of Bertrand (6, 7, 21). However, this strain is located in the *Gluconobacter* area of the rRNA similarity map (Fig. 3). Phenotypically, it does not overoxidize acetic acid; it does not form a thick leathery pellicle; it effects no or very weak ketogenesis from mannitol, sorbitol, and glycerol (the very active ketogenesis of the authentic "bactérie du sorbose" was the origin of the discovery of this phenomenon); and in colony morphology and other cultural aspects, this strain is quite different from well-known *A. aceti* subsp. *xylinum* strains. Its protein electrophoretogram is very close to those of authentic *G. oxydans* subsp. *suboxydans* strains (J. De Ley, K. Kersters, and J. Swings, manuscript in preparation). *Acetobacter xylinum* CIP 57.14, from the Collection de l'Institut Pasteur, has exactly the same protein electrophoretogram as NCIB 4112. We concluded that NCIB 4112 and CIP 57.14 are members of *G. oxydans* subsp. *suboxydans* and not Bertrand's sorbose bacterium. The results of an attempt to identify Bertrand's sorbose bacterium will be described elsewhere.

The following strains fall outside the *Gluconobacter* area in the rRNA similarity map: "*Gluconobacter*" *industrius* IFO 3261, "*Gluconobacter*" *dioxyacetonicus* IAM 1814, and "*Gluconobacter*" strains A4.1 and M28. We conclude that these strains are misnamed and do not belong in *Gluconobacter*. They will be discussed below with *Acetobacter*. "*Gluconobacter*" strain 13.1 was presumably isolated from ripening honey

TABLE 5. Hybridizations between DNA from a number of acetic acid bacteria or *Zymomonas* with [¹⁴C]-RNA from various reference strains^a

Source of [¹⁴ C]RNA	Agrobacterium tumefaciens ICPB TT111			Beijerinckia indica NCIB 8172			Pseudomonas fluorescens ATCC 13525			Pseudomonas acidovorans ATCC 15668			Xanthomonas campestris NCPPB 528			Escherichia coli B			Janthinobacterium lividum NCTC 9796			Chromobacterium NCTC 9757		
	T _m (°C)	RNA bind-ing	%	T _m (°C)	RNA bind-ing	%	T _m (°C)	RNA bind-ing	%	T _m (°C)	RNA bind-ing	%	T _m (°C)	RNA bind-ing	%	T _m (°C)	RNA bind-ing	%	T _m (°C)	RNA bind-ing	%	T _m (°C)	RNA bind-ing	%
<i>Acetobacter aceti</i> Ch 31	66.5	0.09					58.5	0.06		58.5	0.04					60.5	0.07		58.0	0.05				
<i>A. rancens</i> 23 kl [*]				69.0	0.10		58.5	0.11											58.5	0.11				
<i>A. aceti</i> subsp. <i>estunensis</i> E							59.0	0.05		58.5	0.04					58.5	0.05							
<i>A. sp.</i> P20																								
<i>A. aceti</i> subsp. <i>aceti</i> NCIB 8621 t ₁																						60.0	0.06	
<i>A. aceti</i> subsp. <i>xylinum</i> NCIB 8246																								
<i>A. pasteurianus</i> subsp. <i>pasteurianus</i> NCIB 8945 t ₁																						61.0	0.06	
<i>Gluconobacter oxydans</i> subsp. <i>oxydans</i> NCIB 9013										58.5	0.07					59.0	0.10		59.2	0.10				
<i>G. oxydans</i> subsp. <i>suboxydans</i> NCIB 621				66.0	0.12														59.5	0.10		59.5	0.12	
<i>G. oxydans</i> subsp. <i>industrius</i> NCIB 8036							58.0	0.07								61.0	0.07		57.5	0.08				
<i>G. oxydans</i> subsp. <i>melanogenes</i> NCIB 8086	67.0	0.09																						
<i>G. melanogenus</i> 116	66.0	0.09																						
<i>Zymomonas mobilis</i> subsp. <i>mobilis</i> Z1	68.0	0.16					59.0	0.07								62.0	0.12		57.5	0.10				
<i>Z. mobilis</i> subsp. <i>mobilis</i> ATCC 29191				66.0	0.08																			
<i>Z. mobilis</i> subsp. <i>mobilis</i> AG 11				67.0	0.09																			
<i>Z. mobilis</i> subsp. <i>mobilis</i> CP 3				67.0	0.11																			
<i>Z. mobilis</i> subsp. <i>pomaceae</i> ATCC 29192				65.5	0.08																			
"A." <i>aurantius</i> IFO 13330													72.5	0.14										
"A." <i>aurantius</i> IFO 3249													72.0	0.11										

^a Data from references 16 and 19 and from De Ley, De Vos, and Tytgat (unpublished).

FIG. 3. Similarity map of hybrids between the 16S [^{14}C]rRNA fraction of *G. oxydans* subsp. *oxydans* NCIB 9013 and DNA from a variety of bacteria. $T_{m(e)}$ and percent rRNA binding are as defined in the text. To simplify the drawing, each strain is represented by a sequence number (Table 1 and 2) which is not the strain number. The positions of all strains belonging phenotypically to the same taxon (usually a genus) are indicated by a closed line. These areas locate the taxa on the map. It is our experience that taxa which do not belong in an rRNA superfamily always have the same location on the similarity map no matter which ^{14}C reference rRNA from the rRNA superfamily is used. Therefore, we used but a limited number of strains from these taxa (*Pseudomonas*, *Chromobacterium*, *Escherichia*, etc.) (16, 19, the present paper, and many data to be published from this laboratory). Abbreviations: Ace, *Acetobacter*; "Ace" aur, "*Acetobacter*" aurantius; Agr, *Agrobacterium*; "Agr", "*Agrobacterium*"; "Al", par, "*Alcaligenes*" paradoxus; "Azot", "*Azotomonas*" fluorescens; Beij, *Beijerinckia*; Bord bronch, *Bordetella bronchiseptica*; Chr, *Chromobacterium violaceum*; "Chr" fol, "*Chromobacterium*" folium; Gluc, *Gluconobacter*; "Gluc", "*Gluconobacter*" sp.; Janth, *Janthinobacterium lividum*; Myc, *Mycoplana dimorpha*; P. fac, *Pseudomonas facilis*; P. fluor, *Pseudomonas fluorescens*; Par, *Paracoccus denitrificans*; Phyl, *Phyllobacterium*; Rhiz jap, *Rhizobium japonicum*; Rhps, *Rhodopseudomonas*; Spir, *Spirillum*; and Zym, *Zymomonas*.

and honey bees (32, 34). With a $T_{m(e)}$ of 61°C, this strain definitely does not belong with the acetic acid bacteria (Fig. 4). It is a fast-growing organism which produces copious amounts of polysaccharide and forms yellow, slimy colonies, characteristics which are in contradiction with the original description (33, 34) and with the characteristics (14) of acetic acid bacteria. The exact taxonomic position of this organism remains to be resolved.

The genus *Acetobacter*. According to *Bergey's Manual*, eighth edition, (8), *Acetobacter* consists of three species: *A. aceti*, *A. pasteurianus*, and *A. peroxydans*. The first of these was divided into four subspecies (*A. aceti* subsp. *aceti*, *A. aceti* subsp. *orleanensis*, *A. aceti* subsp.

xylinum, and *A. aceti* subsp. *liquefaciens*); and the second, into five subspecies (*A. pasteurianus* subsp. *pasteurianus*, *A. pasteurianus* subsp. *lovaniensis*, *A. pasteurianus* subsp. *estunensis*, *A. pasteurianus* subsp. *ascendens*, and *A. pasteurianus* subsp. *paradoxus*). Representative strains of all of these taxa as well as variously named strains from several collections, a total of 78 authentic *Acetobacter* strains (Table 1, Fig. 3 and 4), were included in this study. When their DNAs were hybridized with [^{14}C]rRNA of *Acetobacter aceti* subsp. *aceti* NCIB 8621t₁, they fell into a cluster with $T_{m(e)}$'s from 75.7 to 81.5°C and with 0.10 to 0.24% rRNA binding. The *Acetobacter* and *Gluconobacter* areas overlap (see below). When *Acetobacter* DNA was hybridized

FIG. 4. Similarity map of hybrids between the 16S [^{14}C]rRNA fraction of *A. aceti* subsp. *aceti* NCIB 8621 t_1 and DNA from a variety of bacteria. For further details, see legend to Fig. 3. x, *Acetobacter*; ●, *Gluconobacter*.

with [^{14}C]rRNA from *Gluconobacter* NCIB 9013, the *Acetobacter* area extended from $T_{m(e)}$ 74.5 to 78.3°C and from 0.10 to 0.28% rRNA binding.

A. peroxydans is an unusual acetic acid bacterium. It occurs in ditch water or household sewage as lightly tapered rods in pairs. By contrast to the other acetobacters, this species is unable to grow on and to produce acid from carbohydrates; it is not ketogenic and has no catalase. It appears to live mainly on ethanol or lactate or both (10, 22). Some strains have been reported to oxidize hydrogen (10, 41). Although this species overoxidizes ethanol and grows at a low pH of 4.5, it is quite different from authentic acetobacters, and there is no completely convincing proof that it really belongs in *Acetobacter*. The same is true of *A. paradoxus*, an organism that grows very poorly, has no catalase, lives mainly on ethanol or lactate, and displays

only a few of the features characteristic of acetic acid bacteria (22). The data presented here on the taxonomic position of these species are the first convincing arguments that both species really belong in *Acetobacter*.

Quasi-*Acetobacter* and quasi-*Acetomonas*. The former term has been used to denote acetic acid bacteria mutants which have lost the power of acetifying ethanol (37). The taxonomic position of these organisms remained vague, particularly since Shimwell (37) repeatedly stressed that the loss of this one feature (ability to oxidize ethanol to acetic acid) catapulted these organisms clear out of their original genus. However, a genus is a group of organisms characterized by a set of correlated phenotypic features and by distinct genetic similarity. Mutative loss in a strain of one or a few of the phenotypic features from this set does not change its genetic and taxonomic position. We

FIG. 5. Similarity map of hybrids between the 16S and 23S [^{14}C]rRNA fractions of *Zymomonas mobilis* subsp. *mobilis* ATCC 29191 and DNA from a variety of bacteria. For further details, see legend to Fig. 3.

examined one "quasi-acetobacter"—NCIB 8956 (received as *A. pasteurianus* subsp. *pasteurianus*). It fell within the *Acetobacter* area, and there is thus no reason to remove it from that genus. We also examined *G. oxydans* subsp. *suboxydans* NCIB 9498, which was originally received by the NCIB as a "quasi-acetomonas." It fell within the *Gluconobacter* area. The concept of "quasi-acetobacter" and "quasi-acetomonas" is taxonomically not justified and is redundant.

Into the *Acetobacter* area (Fig. 3) fell a few organisms which had been placed in genera other than *Acetobacter*. We submit that they have been misnamed, and we will present here phenotypic evidence to support this claim.

"*Gluconobacter industrius*" IFO 3261 (= Kondô 42). This organism falls into the *Acetobacter* cluster (Fig. 3). Phenotypic data which confirm our conclusion that this organism is an acetobacter and not a gluconobacter are (i) its oxidation of acetate (observed by Ameyama et al. [2] and in our laboratory) and (ii) its protein electrophoretogram differs from that of any known gluconobacter (K. Kersters and J. De Ley, manuscript in preparation).

"*Gluconobacter dioxyceticus*" IAM 1814 (= Asai A15). The following phenotypic data derived from this strain confirm our conclusion that it is an acetobacter: (i) the nature of the ubiquinone present (43), (ii) the presence of the Krebs cycle (27), (iii) acid formation from ethanol, overoxidation of ethanol, and growth

on lactate, and (iv) the protein electrophoretogram (J. De Ley, K. Kersters, and J. Swings, manuscript in preparation).

"*Gluconobacter*" sp. strains A4.1 and M28. A few years ago Ruiz-Argüeso and Rodríguez-Navarro (33, 34) isolated gluconic acid-producing bacteria from ripening honey and honey bees in the Madrid area. They seemed to fit best in *Gluconobacter*. Rodríguez-Navarro kindly sent us the strains labeled *Gluconobacter* A4.1 and M28. Their protein electrophoretograms do not correspond to those of any of numerous authentic *Gluconobacter* strains examined from all over the world. The phenotypic features of strain A4.1 indicate that it is an unusual acetic acid bacterium (K. Kersters, J. Swings, and J. De Ley, manuscript in preparation). On the basis of DNA:rRNA hybridization, both strains fall into the *Acetobacter*, not the *Gluconobacter*, area (Fig. 3). The taxonomic positions of these organisms are being investigated.

"*Pseudomonas*" *melophthora* NCPPB 461 (= Cole 2A) and 462 (= Cole 4A). In a separate project in our laboratory, the taxonomic positions of many of the pseudomonads cited in the addenda in *Bergey's Manual*, eighth edition were investigated by DNA:rRNA hybridization. "*P.*" *pomi* and "*P.*" *melophthora* did not belong in *Pseudomonas* (P. De Vos and J. De Ley, manuscript in preparation). The latter is listed in *Bergey's Manual*, eighth edition (8) as of uncertain position (*incertae sedis*) in Addendum I to *Pseudomonas*. Allen and Riker (1) described

a rot of apple fruit in the United States caused by *Phytophthora melophthora* sp. nov. after invasion by an apple maggot. Strains of this species are not known to exist. Cole (9) described a bacterial rotting of apple fruit in the United Kingdom. His two pathogenic isolates, 2A and 4A, were considered to be identical with *P. melophthora*. On the rRNA similarity map (Fig. 3), they fell in the *Acetobacter* area. Phenotypically, both strains belong in *Acetobacter* (acid from ethanol; overoxidation; growth on acetate, lactate, and glycerate; not ketogenic [J. Swings and J. De Ley, manuscript in preparation]). The protein electrophoretograms show that the two strains are identical and that they are not gluconobacters. We conclude that Cole's (9) strains are misnamed and belong in *Acetobacter*.

"*Pseudomonas*" *pomi* NCPPB 463 (= Cole A1). This strain is the type strain of *P. pomi* (9). It was isolated from and caused the rotting of apples in the United Kingdom. It was very similar to "*P.*" *melophthora*, except that it was nonmotile and capsulated. Its rRNA properties located it in the *Acetobacter* area (Fig. 3). Our conclusion that it is an acetobacter is strengthened by phenotypic characters: it produces acid from ethanol, grows on acetate, lactate, and glycerate, oxidizes acetate and lactate, grows at pH 3.55, and is not ketogenic (J. Swings and J. De Ley, manuscript in preparation). The same three strains were examined by Dhanvantari, et al. (20), who concluded from phenotypic features that they are members of *A. pasteurianus* (Hansen 1879) Beijerinck 1898.

A few strains received under the generic name *Acetobacter* were misnamed. We have already mentioned three of them (NRRL B-1810, NCIB 6426, and NCIB 4112) which belong in *Gluconobacter*. Several "*A.*" *aurantius* strains (IFO 3247, IFO 3249, IFO 13330, and IFO 13333) are not acetic acid bacteria at all and will be discussed below.

The "intermediate" strains. A number of strains of acetic acid bacteria display features of both *Acetobacter* and *Gluconobacter* and yet appear not to fit in with either genus. They have been kept as a separate group and have been referred to as "intermediate" strains (5). There are two groups of them, and they will be discussed separately.

The first group consists of four strains originally named *Gluconobacter liquefaciens* Asai G-1 (= NCIB 9136 = IAM 1834), *G. liquefaciens* NCIB 9505, *G. melanogenus* Asai A-8 (= NCIB 9418 = IAM 1835), and *G. melanogenus* Asai U-4 (= NCIB 9417 = IAM 1836), all isolated in Japan (sequence numbers 61 to 64, Table 1). These organisms resemble acetobacters in that

they are peritrichous, overoxidize ethanol, and grow at a low pH (3.5); they resemble gluconobacters because they are ketogenic, have a positive FeCl_3 -reaction, and produce a brown, water-soluble pigment, as do authentic strains of *G. melanogenus*. These organisms differ from many *Acetobacter* strains by the production of reducing substances from glucose, gluconate, glycerol, and mannitol and differ from authentic gluconobacters in their growth ratio on lactate and glucose, in the oxidation of lactate and acetate, in the deamination of amino acids, in their requirements for vitamins, and in the type of flagellation. One of us (14) considered that the small differences these strains had from the genus *Acetobacter* did not preclude these strains from membership in this genus as a separate subspecies—*A. aceti* subsp. *liquefaciens*. Our present results support this conclusion: the parameters of the rRNA hybrids of these four strains put these strains directly into the *Acetobacter* area (Fig. 3). Phenotypically, these strains are typical members of *Acetobacter*, and their protein electrophoretograms are almost identical (J. De Ley, K. Kersters, and J. Swings, manuscript in preparation). It is our conclusion that these four strains are authentic members of *Acetobacter*.

The second group consists of several brown pigment-producing strains named *A. aurantius* isolated from *Lilium auratum* Lindl. (3, 29) and from *Rubus parvifolius* (44). These organisms are similar to acetobacters in that they grow better on lactate than on glucose (with Casamino Acids), oxidize succinate, acetate, and amino acids, and do not require vitamins; on the other hand, they are polarly flagellated and are ketogenic with some sugars, just as are gluconobacters. Most or all of these strains grow at pH 3.5. On the basis of a number of phenotypic features, Ameyama and Kondô (3), Asai (4), and Yamada et al. (44) proposed that the *A. aurantius* strains are acetic acid bacteria which belong to neither *Acetobacter* nor *Gluconobacter* but which are intermediate between these genera. We find that the *A. aurantius* strains belong in three taxa: strain IFO 3248 is located in the *Acetobacter* area, strain IFO 3246 is located in the *Gluconobacter* area (Fig. 3), and all other "*A.*" *aurantius* strains that we examined (IFO 3249, 3247, 13330, and 13333) are very far removed from the genera *Acetobacter* and *Gluconobacter* because they have a $T_{m(e)}$ of about 60°C. The latter fall completely out of the acetic acid bacteria clusters, and they will be discussed extensively in a separate paper (J. De Ley, J. Swings, K. Kersters, M. Gillis, and P. De Vos, manuscript in preparation).

Relationships between *Acetobacter* and *Gluconobacter*. $T_{m(e)}$ and percentage of rRNA binding of the hybrids show that *Acetobacter* and *Gluconobacter* are closely related: when DNA of their strains is hybridized with rRNA from the type strain of the type species of *Gluconobacter*, *G. oxydans* NCIB 9013, both genera remain separated (Fig. 3), but they overlap when the same DNAs are hybridized with rRNA from the type strain of the type species of *Acetobacter*, *A. aceti* NCIB 8621t₁ (Fig. 4). Apparently there are many *Acetobacter* and *Gluconobacter* strains whose rRNA cistrons are equally different from the reference *Acetobacter* rRNA. The *Gluconobacter* area is much smaller than the *Acetobacter* area, indicating that the former genus is more homogeneous than the latter; this observation agrees very well with the known facts that *Acetobacter* has a much greater phenotypic variation and a wider range of moles percent G+C of the DNA (13).

When the DNA:rRNA hybridization method is to be used for identification purposes, hybridizations with [¹⁴C]rRNA from *Gluconobacter* strain NCIB 9013 in conjunction with Fig. 3 can decide whether an unknown strain belongs in *Gluconobacter* or in *Acetobacter*; hybridizations with [¹⁴C]rRNA from an *Acetobacter* reference strain cannot. One of us reported before (16, 19) that the DNA:rRNA hybridization method is of little use in distinguishing between species within a genus. This was confirmed in the present paper; strains belonging to different species and subspecies of *Acetobacter* and to different subspecies of *G. oxydans* are distributed throughout their respective genus areas.

Both genera link at an average $T_{m(e)}$ of $76.8 \pm 0.9^\circ\text{C}$. We examined many other organisms but found no other taxa which are so closely located to either genus. In previous contributions (16, 19) we brought evidence that $T_{m(e)}$ is a good measure of relatedness. This is confirmed here: phenotypically, *Acetobacter*, in particular *A. aceti*, and *Gluconobacter* are more similar to each other than to any other known genus. The main similarities are (we quote from [12] with the permission of the editor-in-chief, the publisher, and the author): "acid resistance; oxidation by both growing and resting cells of numerous hexoses and pentoses to the corresponding lactones and acids; oxidation of numerous polyols, i.e. glycerol, tetritols, pentitols, hexitols, heptitols, octitols, to ketocompounds according to the Rule of Bertrand-Hudson, unique for acetic acid bacteria, oxidation of primary and secondary alcohols and of aldehydes; the formation of 2- and 5-ketogluconate, the latter being formed in nature by *Gluconobacter* and *Acetobacter*

only; the oxidation of glycols; the numerous particulate enzymes; the dual enzyme systems, particulate and soluble, for the metabolism of many of the above compounds; the enzymic mechanism of lactate and pyruvate decomposition; the formation of pyrones; the inability to grow on proteinaceous material." On phenotypic grounds, one of us has previously deduced that *Acetobacter* and *Gluconobacter* are of a common phylogenetic origin (11). The presently reported similarity of rRNA cistrons in both genera confirms this conclusion. Unfortunately, in *Bergey's Manual*, eighth edition (8), more weight has been given to the difference in flagellation than to the numerous physiological, biochemical, and enzymic similarities taken together, and both genera were separated: *Gluconobacter* as Genus IV in the polarly flagellated family *Pseudomonadaceae* and *Acetobacter* as a genus of uncertain affiliation among the aerobic, gram-negative rods and cocci. Our evidence shows that the use of the type of flagellation as a dominant criterion to separate *Acetobacter* and *Gluconobacter* into different families is untenable. In fact, there is additional evidence from other taxa that the importance of the type of flagellation in establishing the higher ranks in bacterial taxonomy should be reevaluated and reduced to a lower level: thus, polarly flagellated and peritrichous species occur in *Chromobacterium*, *Janthinobacterium*, *Aeromonas* and *Vibrio*; there is considerable phenotypic and genetic similarity between the peritrichous *Enterobacteriaceae* and the polarly flagellated *Vibrionaceae*; and there is considerable phenotypic and genetic similarity between the peritrichous (*Alcaligenes eutrophus*, etc.) and the polarly (*P. palleronii*, etc.) flagellated hydrogen-oxidizing bacteria and between the peritrichous and the subpolarly flagellated rhizobia.

To do justice to the considerable similarities shared by *Acetobacter* and *Gluconobacter*, it seems unavoidable to unite them in the family *Acetobacteraceae* Henrici 1939, 99 (26) with *Acetobacter* Beijerinck 1898, 215 as the type genus. The description of this family is as follows. Cells ellipsoidal to rod-shaped, occurring singly or in pairs or in chains. Involution forms may occur and may be spherical, elongated, swollen, club shaped, curved, branched, or filamentous. Motile cells are peritrichous; cells with three to eight, rarely one, polar flagella and nonmotile cells also occur. Endospores are not produced. Gram negative or gram variable. A number of strains produce 2-, 5- or 2,5-diketogluconate, or both, water-soluble brown pigments, and γ -pyrones. Catalase is usually produced. Strictly aerobic. Temperature for optimum

growth: 25 to 30°C. Most strains do not grow at 37°C. The pH for optimum growth is 5.5 to 6.0. Growth and acetic acid production occur at pH 4 to 4.5. Gelatin is not liquefied (J. De Ley and J. Swings, manuscript in preparation). A number of the common biochemical and enzymatic characteristics of the organisms cited above have been quoted from reference 12.

Relationships of the acetic acid bacteria to other genera. Genera related to the acetic acid bacteria were detected by hybridizing rRNA from either *Gluconobacter* or *Acetobacter* with DNA from representative strains of a variety of gram-negative taxa. From previous papers (16, 19) and much unpublished data, we could deduce which taxa would constitute a rRNA superfamily with *Acetobacter* and *Gluconobacter*. Since reciprocal hybridizations always yield the same $T_{m(e)}$ values (within the limits of error), only a small number of strains per taxon had to be included, merely to locate the position of the genera on the map, not to determine their area shape. Table 1 shows that the strains fell into two groups: those with a $T_{m(e)}$ from 63 to 69.8°C and those from 57 to 61.8°C. None of the genera tested was found to map above 70°C, indicating that none of them

had a close relationship to the acetic acid bacteria. In the former group belong *Agrobacterium*, *Rhizobium*, *Phyllobacterium*, *Mycoplana*, some *Spirillum* species (this is a heterogeneous genus), *Zymomonas*, *Rhodopseudomonas*, *Beijerinckia*, *Paracoccus*, *Azotomonas fluorescens*, and the misnamed (16, 19) organisms "*Agrobacterium*" *aggregatum*, "*A.*" *ferrugineum*, "*A.*" *luteum*, "*Pseudomonas*" *diminuta*, and "*Chromobacterium*" *folium*. All these organisms, together with *Acetobacter* and *Gluconobacter*, constitute one large rRNA superfamily (Fig. 6) at a $T_{m(e)}$ of about 66.5°C. Many of these live in or on plants: the tumorigenic and rhizogenic *Agrobacterium*, the root nodule-forming *Rhizobium*, the leaf nodule-inhabiting *Phyllobacterium* and "*C.*" *folium*, the acetic acid bacteria, and *Zymomonas*, which prefers to grow in plant saps; others (*Mycoplana* and *Beijerinckia*) have been isolated from plant sources or live in the phytosphere. All of these organisms are gram negative, and the Entner-Doudoroff pathway has been detected in most of them. Extensive comparison of their phenotypic features is difficult for lack of data. The present results suggest that more comparative information at the generic level is required. It may seem unexpected

FIG. 6. Levels of $T_{m(e)}$ similarities within the rRNA superfamily consisting of the acetic acid bacteria, *Zymomonas*, *Agrobacterium*, *Rhizobium*, *Rhodopseudomonas*, *Paracoccus*, etc. The closest rRNA superfamilies are at an average $T_{m(e)}$ of 58°C and consist of *Chromobacterium*, *Pseudomonas*, *Bordetella bronchiseptica*, etc. (16). The data for the graph are from the present paper and from De Smedt and De Ley (19).

that representatives of the phototrophic *Rhodospseudomonas* and of the chemoorganotrophic, facultatively chemolithotrophic *Paracoccus denitrificans* figure in this rRNA superfamily. This seems to be contrary to a widely accepted but unproven assumption among biologists that the presently living phototrophic and chemolithotrophic bacteria are descendants of a primitive, separate, phylogenetic branch of microorganisms. Our data suggest that this is not the case for *Rhodospseudomonas* and *Paracoccus*. This is strengthened by an amino acid sequence comparison of cytochromes from both genera (36). N-terminal heme-bound cytochrome c_{550} of *Paracoccus denitrificans* is most similar to N-terminal heme-bound cytochrome c_2 of *Rhodomicrobium* and *Rhodospseudomonas*. The c-type cytochrome sequences place both genera in a portion of the evolutionary tree surrounded by strictly aerobic, obligately heterotrophic bacteria (36). There is a distinct similarity between the C-terminal heme-bound cytochrome c_{556} of *Agrobacterium* and the C-terminal heme-bound cytochrome c' of *Rhodospseudomonas* (J. Van Beeumen, P. Tempst, J. Van Damme, and J. De Ley, manuscript in preparation). When the *Rhodospirillaceae* grow chemoorganotrophically under aerobic conditions, they are very similar to nonphotosynthetic bacteria. For example, *Rhodospseudomonas sphaeroides* has the Entner-Doudoroff mechanism, an almost complete shunt mechanism, and several cytoplasmic membrane-linked, sugar-oxidizing enzymes (39) very much like the acetic acid bacteria. We conclude that the rRNA superfamily of taxa as shown in Fig. 6 represents a branch of the phylogeny of the aerobic, chemoorganotrophic organisms concerned as well as a measure of taxonomic relationships at the generic and suprageneric level. Taxa belonging to the *Chromobacterium*, *Janthinobacterium*, *P. acidovorans*, *P. solanacearum*, *Alcaligenes-Achromobacter*, *Bordetella bronchiseptica* rRNA superfamily (16) group at an average low $T_{m(e)}$ level of 58°C.

Zymomonas. Although some strains of *Zymomonas* can grow modestly aerobically, all strains prefer to grow anaerobically. This genus was recently extensively reviewed by Swings and De Ley (39). It is very homogeneous in spite of its wide geographic distribution. It contains but one species and two subspecies (39). Representative strains of each taxon were included. Figure 5 and Table 2 show variations on $T_{m(e)}$ and percentage of rRNA binding of $\pm 1^\circ\text{C}$ and $\pm 0.025\%$, respectively, which data confirm the compactness of the genus. Reverse hybridizations are reported in Table 5. *Zymomonas* is

about equidistant from *Gluconobacter*, *Acetobacter*, *Rhodospseudomonas*, *Rhizobium*, *Agrobacterium*, and *Paracoccus* [average $\Delta T_{m(e)}$ versus the homologous hybrid: -14.5°C]. It clearly belongs in the same rRNA superfamily as these taxa. At first glance it may seem surprising that a largely anaerobic genus belongs in a cluster together with many strictly aerobic genera. Nevertheless there are many phenotypic similarities, particularly between *Zymomonas* and the acetic acid bacteria (39): related ecologic niches in or on plants, joint occurrence in fermented tropical plant juices and beer, tolerance toward low pH, high ethanol or glucose concentration in the growth medium, aerobic oxidation of ethanol to acetic acid, and limited efficiency of C assimilation. *Zymomonas* is phenotypically more like *Gluconobacter* than *Acetobacter* (polar flagellation, incomplete Krebs cycle, occurrence of Entner-Doudoroff cycle, ready consumption of glucose). These considerable phenotypic and $T_{m(e)}$ similarities suggest that the ancestors of *Zymomonas* were aerobic organisms, closely related to the ancestors of the present acetic acid bacteria. We assume that these ancestors gave rise to the present zymomonads by loss of some Krebs cycle enzymes and perhaps also some electron transport enzymes (39).

DNA base composition of the acetic acid bacteria. The DNA base compositions of the acetic acid bacteria reported in *Bergey's Manual*, eighth edition (14), are almost solely derived from data obtained in this laboratory (15). In the present paper, we report a total of 116 G+C determinations, which we used to confirm and extend our previous conclusions. In *Bergey's Manual*, one of us (14) reported 55 to 64 mol% G+C for *Acetobacter* and 57 to 64 mol% G+C for *Gluconobacter*. Our present range is from 51 to 65 mol% G+C for *Acetobacter* and from 56 to 64 mol% G+C for *Gluconobacter*. The G+C ranges (in moles percent) for the individual species and subspecies are: *A. aceti* subsp. *aceti*, 55 to 60; *A. aceti* subsp. *orleanensis*, 56 to 60; *A. aceti* subsp. *xylinum*, 55 to 63; *A. aceti* subsp. *liquefaciens*, 61 to 65; *A. pasteurianus* subsp. *pasteurianus*, 51 to 61; *A. pasteurianus* subsp. *lovaniensis*, 58; *A. pasteurianus* subsp. *estunensis*, 60 to 62; *A. pasteurianus* subsp. *ascendens*, 54 to 56; *A. pasteurianus* subsp. *paradoxus*, 53; *A. peroxydans*, 50 to 53; *Gluconobacter oxydans* subsp. *oxydans*, 58 to 62; *G. oxydans* subsp. *industrius*, 61 to 64; *G. oxydans* subsp. *suboxydans*, 56 to 64; and *G. oxydans* subsp. *melanogenes*, 59 to 61.

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REPRINT REQUESTS

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