Journal of General Microbiology (1983), **129**, 3057–3074. *Printed in Great Britain*

Intra- and Intergeneric Similarities of the rRNA Cistrons of *Alteromonas*, *Marinomonas* (gen. nov.) and Some Other Gram-negative Bacteria

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(*Received 6 December 1982; revised 28 March 1983*)

^{14}C-labelled rRNA was prepared from *Alteromonas macleodii* ATCC 27126 and from *Alteromonas haloplanktis* ATCC 14393. ^{3}H-labelled rRNA was isolated from *Alteromonas vaga* ATCC 27119 and *Alteromonas putrefaciens* ATCC 8071 colony type t1. These rRNAs were hybridized under stringent conditions with filter-fixed DNA from various *Alteromonas* strains and from organisms of marine origin and/or with mol % G + C values in the range 40 to 50. Each hybrid was described by its $T_{m(e)}$ and percentage of rRNA binding. From rRNA similarity maps and $T_{m(e)}$ dendrograms the following conclusions were drawn. The genus *Alteromonas* is very heterogeneous and consists of four rRNA branches: (1) *Alt. macleodii* alone; (2) the *Alt. haloplanktis* cluster, containing most of the named *Alteromonas* species and a number of organisms which should be renamed *Alteromonas* (*'Pseudomonas marinoglutinosa'*, *'Pseudomonas nigrifaciens'*, *'Pseudomonas atlantica'* ATCC 19262, *'Pseudomonas carrageenovora'*, *'Pseudomonas piscicida'*, and several unnamed alginolytic bacteria); we propose to limit the genus *Alteromonas* to the former two clusters; (3) *Alt. putrefaciens*, the rRNA cistrons of which resemble those of the *Vibrionaceae* and are as different from the above two *Alteromonas* rRNA branches as are those of the *Vibrionaceae*, the *Enterobacteriaceae* and *Aeromonas*; *'Pseudomonas rubescens'* belongs to this branch and *Alteromonas hanedai* seems to be a remote relative; (4) *Alteromonas communis* and *Alt. vaga* constitute another, separate rRNA branch; in conjunction with their special phenotypic features, we propose to create a new genus, *Marinomonas*, for them. The exact taxonomic position of *'Alteromonas thalassomethanolica'* could not be established.

INTRODUCTION

Baumann *et al.* (1972) created the genus *Alteromonas* for a group of Gram-negative, aerobic, polarly flagellated, heterotrophic marine bacteria. The main difference from *Pseudomonas* was the range of the mean molar percentage of G + C (mol % G + C) of the DNA, extending from 40 to 50 for *Alteromonas*. They described four species: *Alteromonas vaga, Alteromonas communis, Alteromonas macleodii* (type species of the genus), and *'Alteromonas marinopraesens'* [subsequently changed by Reichelt & Baumann (1973) to *Alteromonas haloplanktis*]. Several additional species have since been proposed: *Alteromonas luteoviolacea* (Gauthier, 1976*a*, 1982), *Alteromonas rubra* (Gauthier, 1976*b*) and *Alteromonas citrea* (Gauthier, 1977), *Alteromonas espejiana* and *Alteromonas undina* (Chan *et al.*, 1978), *Alteromonas aurantia* (Gauthier & Breittmayer, 1979), *Alteromonas hanedai* (Jensen *et al.*, 1980), and *'Alteromonas thalassomethanolica'* (Yamamoto *et al.*, 1980). Taxon names in quotation marks are not in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) nor on the validation lists, and have not been published since 1 January 1980 in the *International Journal of Systematic Bacteriology*.

'Pseudomonas' putrefaciens has been regarded as a very important organism in the spoilage of chilled protein. It has been isolated from fish, butter, oil brine and human clinical specimens. It has been associated with infections (Debois *et al.*, 1975). *'Pseudomonas rubescens'* has been isolated from cutting oil (Pivnick, 1955). Lee *et al.* (1977) proposed to rename both species as

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Alteromonas putrefaciens (Anonymous, 1981). The mol % G + C values of the latter organisms fall within the range of *Alteromonas*. The genetic structure of *Alt. putrefaciens* was studied by Owen *et al.* (1978) who found some correlation between four DNA homology groups and their sources of isolation.

DNA/rRNA hybridizations are a powerful tool to reveal genetic homogeneity or heterogeneity in a named genus or family, and the establishment of relationships between genera (De Ley *et al.*, 1978; De Smedt *et al.*, 1980; De Smedt & De Ley, 1977; Gillis & De Ley, 1980; De Vos & De Ley, 1983). We have examined the degree of heterogeneity within *Alteromonas*, the eventual validity of this genus, and its taxonomic neighbours by this technique. We also examined a number of other organisms of marine origin and/or with low mol % G + C values, which might eventually be related to the present alteromonads. These included some *Pseudomonas* organisms which are not on the Approved Lists and whose taxonomic status is still uncertain, and some unnamed alginolytic bacteria isolated by Mrs S. Meland from northern Norway. The purified DNA from all these organisms was hybridized with labelled rRNA from selected *Alteromonas* strains. A number of taxonomically well-located strains were included as controls.

METHODS

Bacterial strains and growth media. Table 1 lists the bacterial strains used and their corresponding growth media. The purity of the strains was checked by plating and by microscopic examination of living and Gram-stained cells. Four *Alteromonas* strains and three *Pseudomonas* strains showed two or three colony types on plates. The different types are indicated in the text by t1, t2 and t3. The compositions of the growth media are given in De Smedt & De Ley (1977), Gillis & De Ley (1980) and De Vos & De Ley (1983). The compositions of additional growth media are summarized in Table 2.

Mass cultures for the extraction of DNA were grown as described by De Smedt & De Ley (1977). Cells were harvested in 0·01 M-phosphate buffer pH 7, containing the same percentage of NaCl as in the medium used for growth. In some cases, to prevent lysis of the cells, it was necessary to harvest and wash the cells using a solution with the same ionic composition as the growth medium.

Extraction of DNA and fixation of the single-stranded DNA on membrane filters. The procedures of Gillis & De Ley (1980) were used.

Chemical determination of DNA fixed on filters. Early in the work the method of Burton (1956) was used, as described by De Smedt & De Ley (1977); later, a modified method was used (Richards, 1974) involving a more stable solution of 2·25% (w/v) diphenylamine and 0·005% (w/v) paraldehyde instead of 1·5% (w/v) diphenylamine and 0·005% (w/v) acetaldehyde.

Preparation of labelled rRNA. [^{14}C]rRNA was prepared from the type strains of *Alt. macleodii* ATCC 27126 and *Alt. haloplanktis* ATCC 14393. [^{3}H]rRNA was prepared from the type strain of *Alt. vaga* ATCC 27119 and *Alt. putrefaciens* ATCC 8071. The latter strain gave two colony types: t1 gave larger, more shiny and yellow colonies than t2. Microscopically the cells were the same and their DNAs had an identical mol % G + C of 45·5. The electrophoretic protein patterns of both types were very similar and DNA/DNA reassociation revealed that t1 was 100% related to t2. Colony type t1 was used for the preparation of [^{3}H]rRNA.

The growth medium for the preparation of rRNA from *Alt. putrefaciens* ATCC 8071 t1 was Z25 whereas Z24 (Table 2) was used for the preparation of labelled rRNA from all other type strains. The preparation of labelled rRNAs was as described by De Ley & De Smedt (1975), except that the rRNA solutions contained 0·2% (w/v) bentonite during the whole procedure (Midgley, 1965).

Saturation hybridization between labelled rRNA and filter-fixed DNA: thermal stability of the DNA/rRNA hybrids. The method was described by De Smedt & De Ley (1977). Two parameters were measured: (1), $T_{m(e)}$, which is the temperature at which 50% of the hybrid is denatured; (2) the percentage of rRNA binding, which represents the amount of labelled rRNA duplexed, in μg per 100 μg DNA fixed on the filter, after ribonuclease treatment. Both parameters are derived from the melting curves of the hybrids.

RESULTS

16S and 23S fractions

In Fig. 1 the distribution of the labelled rRNA in sucrose gradients is shown. The 23S [^{14}C]rRNA fraction of *Alt. macleodii* ATCC 27126 was used for DNA/rRNA hybridizations. There is no evidence that the 23S fraction was contaminated by the 16S fraction. The specific activity [c.p.m. (μg rRNA)$^{-1}$] of this rRNA was 5800. The 16S fraction of [^{14}C]rRNA from *Alt.*

Table 1. *Organisms used, their strain number, growth media, DNA base composition and properties of the DNA/rRNA hybrids with labelled rRNA from four Alteromonas type strains*

The composition of the growth media are given either in Table 2, in De Smedt & De Ley (1977), Gillis & De Ley (1980) or De Vos & De Ley (1983). Unmarked DNA base composition values have been determined in our laboratory by thermal denaturation. Values from other authors have a reference letter: *a*, Gauthier (1976*a*); *b*, Chan *et al.* (1978); *c*, Gauthier & Breittmayer (1979); *d*, Yamamoto *et al.* (1980); *e*, Owen *et al.* (1978); *f*, Mandel (1966); *g*, Weeks (1974). The letter *h* denotes our mol % G + C values determined from absorbance ratios (De Ley, 1967); *i* denotes mol % values from Dr L. Lizarraga-Partida (personal communication). Asterisks denote type strains; square brackets indicate that the strain has been misnamed, and quotation marks that the taxon name is not contained in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980). Culture collection abbreviation definitions are given in Skerman *et al*, (1980) except for the following: MMCA, Medical Microbiology Culture Collection, Institute of Medical Microbiology, Aarhus University, Denmark; ICPB, International Collection of Phytopathogenic Bacteria, Department of Bacteriology, University of California, Davis, Calif., U.S.A.

					Hybridization with:							
					[^{14}C]rRNA from:				[^{3}H]rRNA from:			
					Alt. macleodii ATCC 27126		*Alt. haloplanktis* ATCC 14393		*Alt. putrefaciens* ATCC 8071 t1		*Alt. vaga* ATCC 27119	
No. in Figs. 2,3,4, and 5	Organism used for DNA isolation	Origin and strain no.	Growth medium	Mean mol % G + C	$T_{m(e)}$ (°C)	Percentage binding	$T_{m(e)}$ (°C)	Percentage binding	$T_{m(e)}$ (°C)	Percentage binding	$T_{m(e)}$ (°C)	Percentage binding
1	*Alteromonas macleodii*	ATCC 27126*	Z11	46·4	79·5	0·14	70·0	0·12	69·0	0·12	67·0	0·12
2	*Alt. haloplanktis*	ATCC 14393*	Z11	42·8	71·0	0·16	79·0	0·28	70·5	0·20	67·0	0·20
3	*Alt. haloplanktis*	ATCC 19648	Z11	41·7	70·0	0·15	78·0	0·28	70·0	0·20	68·0	0·19
4	*Alt. haloplanktis*	ATCC 19855	Z11	43·3	71·0	0·12	77·0	0·25	69·0	0·18	68·0	0·20
5	*Alt. haloplanktis*	ATCC 29127	Z11	42·7	71·0	0·12	76·5	0·18			66·0	0·19
6	*Alt. haloplanktis*	ATCC 23821 t1	Z11				79·0	0·28				
7	*Alt. haloplanktis*	ATCC 23821 t2	Z11				79·0	0·27				
8	*Alt. haloplanktis*	ATCC 23821 t3	Z11				79·0	0·27				
9	*Alt. luteoviolacea*	NCMB 1893*	Z11	42·1[a]	70·5	0·15	76·0	0·20				
10	*Alt. luteoviolacea*	NCMB 1942	Z10	41·5[a]			73·5	0·19				
11	*Alt. luteoviolacea*	NCMB 2035	Z10				75·0	0·31				
12	*Alt. luteoviolacea*	NCMB 2036 t1	Z10				76·0	0·24				
13	*Alt. luteoviolacea*	NCMB 2036 t2	Z10				77·0	0·24				
14	*Alt. rubra*	ATCC 29570*	Z11	48·6	71·0	0·15	76·0	0·24				
15	*Alt. citrea*	ATCC 29719*	Z11	42·8	71·5	0·18	75·5	0·27				
16	*Alt. espejiana*	ATCC 29659*	T	43·1[b]	70·0	0·14	77·5	0·24				
17	*Alt. espejiana*	NCMB 1879	Z11	43·0[b]			73·5	0·17				
18	*Alt. undina*	ATCC 29660*	T	43·1[b]	70·0	0·17	77·0	0·28			66·0	0·19
19	*Alt. aurantia*	ATCC 33044	Z20	42·5[c]			76·0	0·25				
20	*Alt. aurantia*	ATCC 33045	Z20	40·8[c]			76·5	0·25				
21	*Alt. aurantia*	ATCC 33046*	Z20	38·8[c]			76·0	0·21				
22	*Alt. vaga*	ATCC 27119*	T	47·9	67·5	0·10	65·5	0·12	69·0	0·17	79·5	0·23
23	*Alt. communis*	ATCC 27118*	T	46·7	67·5	0·11	67·0	0·14	68·0	0·19	78·0	0·24
24	*Alt. hanedai*	ATCC 33224*	Z22	43·7	68·5	0·18	68·5	0·21	72·0	0·21	67·5	0·20

Table 1 (*continued*)

No. in Figs. 2,3,4, and 5	Organism used for DNA isolation	Origin and strain no.	Growth medium	Mean mol % G + C	Hybridization with: [^{14}C]rRNA from: *Alt. macleodii* ATCC 27126 $T_{m(e)}$ (°C)	Percentage binding	*Alt. haloplanktis* ATCC 14393 $T_{m(e)}$ (°C)	Percentage binding	[^{3}H]rRNA from: *Alt. putrefaciens* ATCC 8071 t1 $T_{m(e)}$ (°C)	Percentage binding	*Alt. vaga* ATCC 27119 $T_{m(e)}$ (°C)	Percentage binding
25	*'Alteromonas* sp.'	YK 2031	Z23	45·3[d]	71·0	0·08	70·0	0·10	67·5	0·08	69·0	0·07
26	*'Alt. thalassomethanolica'*	YK 4007	Z23	45·5[d]	70·0	0·08	64·0	0·10	66·0	0·07	68·0	0·14
27	*'Alt. thalassomethanolica'*	YK 2021	Z23	46·9[d]	68·0	0·07	63·0	0·08	65·5	0·07	68·0	0·06
28	*Alt. putrefaciens*	ATCC 8071 t1*	Z5	45·5	70·0	0·15	69·5	0·21	79·5	0·23	68·5	0·23
29	*Alt. putrefaciens*	ATCC 8071 t2*	Z5	45·5	69·5	0·16			79·5	0·26	68·5	0·21
30	*Alt. putrefaciens*	ATCC 8073	Z5	46·1[e]			68·5	0·20	77·5	0·25	67·0	0·19
31	*Alt. putrefaciens*	NCTC 10735	Z5	46·3[e]	69·5	0·16	68·0	0·18	77·5	0·28		
32	*Alt. putrefaciens*	NCTC 10737	Z5	47·9	69·0	0·16	68·0	0·17	77·0	0·26		
33	*Alt. putrefaciens*	CL 256/73 t1	Z5	48·4[e]	69·0	0·17			77·0	0·24		
34	*Alt. putrefaciens*	CL 256/73 t2	Z5	48·4[e]					76·5	0·22		
35	*Alt. putrefaciens*	NCTC 10763	Z5	52·8[e]	69·5	0·15			75·5	0·22		
36	*Alt. putrefaciens*	NCTC 10738	Z5	53·3	70·0	0·15	69·0	0·17	75·0	0·22		
37	*Alt. putrefaciens*	NCTC 10736	Z5	44·4[e]					74·5	0·28		
38	Alginolytic bacterium	SM 132	Z11	40·7			76·5	0·26				
39	Alginolytic bacterium	SM 75	T	40·9			77·5	0·29				
40	Alginolytic bacterium	SM 95	T	40·7			77·0	0·29				
41	Alginolytic bacterium	SM 77	T	40·7			77·0	0·29				
42	*'Pseudomonas rubescens'*	NCTC 10695	B	46·1	69·0	0·14	69·0	0·18	79·0	0·28		
43	*'Ps. nigrifaciens'*	NCTC 10691	Z11	41·0	70·0	0·15	74·0	0·17				
44	*'Ps. nigrifaciens'*	ATCC 25013 t1	Z20	40·4			76·5	0·25				
45	*'Ps. nigrifaciens'*	ATCC 25013 t2	Z20				76·0	0·25				
46	*'Ps. piscicida'*	ATCC 15251	Z11	44·4			75·5	0·19				
47	*'Ps. piscicida'*	NCMB 848	Z11	44·5	66·5	0·11	71·0	0·14	66·5	0·11	63·0	0·10
48	*'Ps. piscicida'*	NCMB 2037 t1	Z11	42·4	66·0	0·12	71·5	0·17	65·5	0·11	65·0	0·11
49	*'Ps. piscicida'*	NCMB 2037 t2	Z11	41·9			69·0	0·11	65·0	0·10	63·0	0·11
50	*'Ps. carrageenovora'*	NCMB 302	Z21	40·0			76·0	0·28				
51	*'Ps. marinoglutinosa'*	NCMB 1770 t1	Z11	42·2			76·0	0·25				
52	*'Ps. marinoglutinosa'*	NCMB 1770 t2	Z11	42·1			76·5	0·28				
53	*'Ps. atlantica'*	ATCC 19262	T	42·3	70·0	0·14	77·5	0·21				
54	*'Ps. atlantica'*	CIP 59.31	Z19	45·4	67·0	0·21	69·5	0·25	71·0	0·27	65·0	0·22
55	*'Methylomonas thalassica'*	YK 2004	Z23	43·9[d]	70·0	0·08	65·0	0·10	66·5	0·08	67·0	0·08
56	*'Methylomonas thalassica'*	YK 4015	Z23	43·8[d]	68·0	0·08	63·5	0·09	65·0	0·07	66·0	0·07
57	*Ps. putida*	Biotype B no. 53	B	60·2[f]	65·5	0·09	66·0	0·09	67·0	0·11	70·0	0·13
58	*Ps. aeruginosa*	CCEB 481*	Z5	66·8	65·5	0·13					72·0	0·13
59	*Ps. fluorescens*	MMCA 40*	B	60·2	65·0	0·10	65·0	0·09	67·0	0·11	70·5	0·15

60	*Ps. solanacearum*	NCPPB 952	B	67·1[h]						64·0	0·10
61	*Ps. solanacearum*	No. 3 (Kelman)	Z5	67·7	62·0	0·08	61·0	0·06			
62	*Ps. solanacearum*	NCPPB 1029	Z5	66·4[h]	63·0	0·09					
63	*Ps. acidovorans*	ATCC 15668*	B	66·6					61·5	0·08	
64	*Ps. acidovorans*	ATCC 17406	Z5	68·4			60·5	0·09			
65	*Ps. acidovorans*	ATCC 17476	Z5	68·4	62·0	0·09					
66	*Escherichia coli*	K12 C 600	B	51·1	70·5	0·14	67·5	0·15	70·0	0·17	67·0	0·17
67	*E. coli*	B	R	52·2	68·0	0·14	69·5	0·16	70·0	0·15	67·5	0·16
68	*E. coli*	NCTC 9001*	R	50·8	68·0	0·16	69·0	0·19	70·0	0·13		
69	*Alcaligenes aquamarinus*	ATCC 14400*	B	57·9	65·5	0·11	65·0	0·15	68·0	0·16	70·0	0·22
70	*Vibrio parahaemolyticus*	SAK 3	Z5	47·6	69·5	0·19	69·0	0·29	71·5	0·27	68·0	0·28
71	*V. parahaemolyticus*	ATCC 17802*	Z5	46·4	69·0	0·22	68·5	0·24	71·5	0·26	67·5	0·23
72	*V. albensis*	NCMB 41*	Z10	47·8					71·0	0·19		
73	*V. fischeri*	NCMB 1143	Z11	42·3					71·0	0·28		
74	*Photobacterium phosphoreum*	NCMB 1275	T	42·0					70·5	0·45		
75	*'Cellvibrio fulvus'*	NCIB 8634	Z17		65·5	0·08					69·5	0·10
76	*'Cell. vulgaris'*	NCIB 8633	Z17	51·5	64·0	0·06						
77	*'Cell. vulgaris'*	NCIB 8975	Z18	48·2	65·0	0·07					68·5	0·12
78	*Xanthomonas fragariae*	NCPPB 1822	X	63·3	65·5	0·05	65·5	0·05	64·5	0·06	65·5	0·05
79	*Xanth. axonopodis*	NCPPB 457*	B	65·0	65·0	0·04	66·0	0·04	63·0	0·04	65·5	0·05
80	*Xanth. campestris*	NCPPB 528*	T	65·2[h]	65·0	0·06					65·5	0·07
81	*Xanth. campestris*	ICPB H 110	X	68·5			65·0	0·06	62·5	0·05		
82	*Azomonas insignis*	WR 59	E	57·8	68·0	0·12						
83	*Azom. insignis*	WR 31	E	57·1	67·5	0·13			67·0	0·13	72·5	0·17
84	[*Azom. insignis*]	ATCC 12523	E	43·4	64·0	0·19					71·5	0·12
85	*Azotobacter chroococcum*	DSM 281_{II}	E	66·3							70·5	0·19
86	*Azotobacter chroococcum*	NCIB 8515	E	67·5							72·5	0·20
87	*Chromobacterium violaceum*	NCTC 9757*	X	67·2			60·5	0·15	62·5	0·12	63·0	0·20
88	*Janthinobacterium lividum*	NCTC 9796*	X	65·5	62·5	0·11					63·0	0·15
89	*Flavobacterium ferrugineum*	DSM 30193*	Z5	42·6[g]			62·5	0·02				
90	*Fl. okeanokoïtes*	CCM 320*	Z5	45·3			56·5	0·17				
91	*'Flavobacterium* sp.'	CCM 1048	Z5	39·7			54·5	0·07				
92	*'Flavobacterium* sp.'	ATCC 9491 t1	Z5	50·6			56·5	0·11			59·5	0·13
93	*Agrobacterium tumefaciens*	ICPB TT111	A	60·6	57·5	0·08			59·0	0·10		
94	*Agr. tumefaciens*	B6	A	60·2			57·0	0·05			60·0	0·07
95	*Acetobacter aceti* subsp. *aceti*	NCIB 8621 t1*	N	57·4			57·0	0·05	58·5	0·05		
96	*Gluconobacter oxydans* subsp. *oxydans*	NCIB 4739	N	61·4	57·0	0·10						
97	*Gluc. oxydans* subsp. *suboxydans*	NCIB 9137	N	59·4							60·0	0·16
98	Marine organism	Liz. 349		64·0[i]			67·0	0·14			70·5	0·17
99	Marine organism	Liz. 2128			65·0	0·05	68·5	0·07			69·0	0·08
100	Marine organism	Liz. 2145		56·8[i]			67·0	0·06				
101	Marine organism	Liz. 2026					70·0	0·07				
102	Marine organism	Liz. 1613		57·8[i]			68·5	0·07				
103	Marine organism	Liz. 1371					68·0	0·08				
104	Alginolytic bacterium	SM 64	T	55·1			64·5	0·16	67·0	0·18		

Table 2. *Composition (%, w/v) of growth media*

Component Medium ...	Z17	Z18	Z19	Z20	Z21	Z22	Z23	Z24	Z25
Glucose						0·2		0·6	0·6
Lactose		1							
Methanol							1		
Starch	1								
Yeast extract				0·1	0·3			0·5	0·5
Meat extract	1	1							
Peptone	1	1		0·5	0·5				
Proteose peptone						1			
Calf brain infusion						1·25			
Beef heart infusion						0·5			
Casamino acids			0·1						
Vitamin B_{12}							10^{-7}		
$(NH_4)_2SO_4$							0·2		
KH_2PO_4							0·1		0·27
K_2HPO_4			0·01				0·2		0·52
Na_2HPO_4						0·25			
NaCl	0·5	0·5	2·5			0·5	2	3	0·5
KCl			0·1						
$FeSO_4.7H_2O$			0·002				$2·8 \times 10^{-5}$		
$Na_2MoO_4.2H_2O$							$2·4 \times 10^{-5}$		
$MnSO_4.H_2O$							$8·4 \times 10^{-5}$		
$MgSO_4.7H_2O$			0·5				0·03		0·012
$CaCl_2.2H_2O$			0·027				$1·5 \times 10^{-5}$		
$ZnSO_4$							$7·8 \times 10^{-5}$		
$CuSO_4.5H_2O$							$2·5 \times 10^{-5}$		
$CoCl_2.6H_2O$							$2·4 \times 10^{-5}$		
Na_2EDTA			0·05						
Tris			0·4						
Tap water*	+	+						+	+
Distilled water*			+		25% (v/v)	+	+		
Artificial seawater*				+	75% (v/v)				
pH	~7	~7	7·25	7·3	7·3	7·4	7	~7	7

* The plus sign indicates presence.

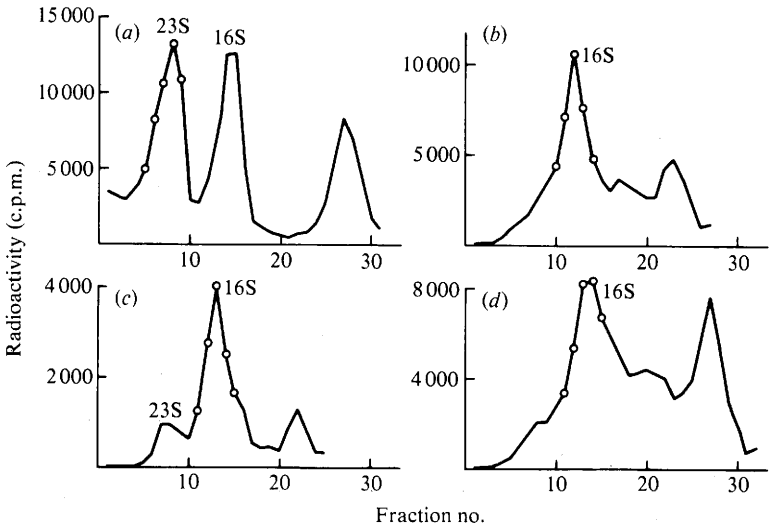

Fig. 1. Distribution of about 1 mg ^{14}C-labelled or ^{3}H-labelled rRNA in a linear 15 to 30% sucrose gradient. The procedure was as described in De Ley & De Smedt (1975). The radioactivity of the fractions was measured after addition of 5 ml scintillation liquid to 10 µl of each fraction. The 23S or 16S fractions, collected for use in DNA/rRNA hybridizations, are marked with open circles. (*a*) *Alt. macleodii* ATCC 27126, (*b*) *Alt. vaga* ATCC 27119, (*c*) *Alt. haloplanktis* ATCC 14393, (*d*) *Alt. putrefaciens* ATCC 8071 t1.

haloplanktis ATCC 14393, [^{3}H]rRNA from *Alt. vaga* ATCC 27119 and [^{3}H]rRNA of *Alt. putrefaciens* ATCC 8071 type t1 were used. The specific activities of these three rRNAs were 2400, 9800 and 10 900 c.p.m. (μg rRNA)$^{-1}$, respectively. The 23S fraction was either very small or totally lacking from three strains, presumably due to total or partial nicking. The 16S peaks may have been contaminated with fragmented 23S fractions, as in *Agrobacterium* (De Smedt & De Ley, 1977), but their sedimentation coefficients remained unchanged. It is our experience (Gillis & De Ley, 1980) that hybridizations with the 23S and the 16S fractions from the same organism give similar results, within the limits of reproducibility.

DNA/rRNA hybridizations

The results of the hybridizations ($T_{m(e)}$ and percentage of rRNA binding) are compiled in Table 1. For each labelled reference rRNA, the $T_{m(e)}$ values are plotted against the percentage of rRNA binding; the position of each DNA/rRNA hybrid is indicated. The resulting rRNA similarity maps are shown in Figs 2, 3, 4 and 5. It is our experience that taxa outside the rRNA superfamily examined always have about the same location on the similarity maps irrespective of the labelled reference rRNA from the rRNA superfamily used. Therefore we used only a limited number of strains from these 'foreign' taxa (*Pseudomonas, Escherichia, Chromobacterium,* etc.) (De Ley *et al.*, 1978; De Smedt *et al.*, 1980; De Smedt & De Ley, 1977; Gillis & De Ley, 1980; and this paper).

The parameters of additional and reciprocal DNA/rRNA hybridizations are given in Table 3. The results of the reciprocal experiments are in good agreement with the data of the hybridizations in Table 1. The dendrograms Figs 6 and 7 are constructed from the $T_{m(e)}$ values in Table 1 and from many other data in our laboratory. These data were clustered by the unweighted pair group method (Sokal & Sneath, 1963). Each vertical branch is from a labelled reference rRNA.

DISCUSSION

The most valid and useful parameter of a DNA/rRNA hybrid is $T_{m(e)}$ (De Smedt & De Ley, 1977). Its magnitude is a measure of the thermal stability of the hybrids and, as such, a measure of the base sequence similarities between the rRNA cistrons. An additional parameter is the percentage of RNA binding. This parameter is used to differentiate between taxa with the same $T_{m(e)}$. The $T_{m(e)}$ of the homologous duplex of the four reference strains in this paper is 79 °C or 79·5 °C. This is very close to the values for *Janthinobacterium* (78·5 °C; De Ley *et al.*, 1978), *Beijerinckia* (78 °C; De Smedt *et al.*, 1980) and *Zymomonas* (78 °C; Gillis & De Ley, 1980) but is lower than the $T_{m(e)}$ of the duplex of most other reference strains (De Ley *et al.*, 1978; De Smedt *et al.*, 1980; De Smedt & De Ley, 1977; Gillis & De Ley, 1980). The variation from 78 °C to 82·5 °C in the $T_{m(e)}$ of homologous duplexes may be attributed to mismatching in the duplex due to methylation of some bases and/or to the variation of the mol % G + C in the rRNA cistrons.

The four rRNA similarity maps (Figs 2, 3, 4 and 5) and the dendrograms (Figs 6 and 7) show that the genus *Alteromonas* is extremely heterogeneous. It divides into four groups and some species of uncertain taxonomic position. These four groups are (1) *Alt. macleodii*, (2) a large *Alt. haloplanktis* group, (3) the *Alt. putrefaciens* group and (4) the *Alt. vaga–Alt. communis* group. We shall first discuss briefly the content and the position of each group.

(*1*) *Alteromonas macleodii* (*Fig. 2*)

The type strain ATCC 27126 is on a branch separate from all other *Alteromonas* species examined here. Unfortunately no further strains of this species were available to us. *Alt. macleodii* has a 70·5 °C $T_{m(e)}$ against the *Alt. haloplanktis* group, a 69·1 °C $T_{m(e)}$ against the *Alt. putrefaciens* group, and a 66·5 °C $T_{m(e)}$ against the *Alt. vaga–Alt. communis* group.

(*2*) *The Alt. haloplanktis group* (*Fig. 3*)

This group is quite heterogeneous: it extends from 73·5 °C to 79·0 °C $T_{m(e)}$ and from 0·17 to 0·31 % rRNA binding against labelled rRNA of the type strain of *Alt. haloplanktis*. It is the

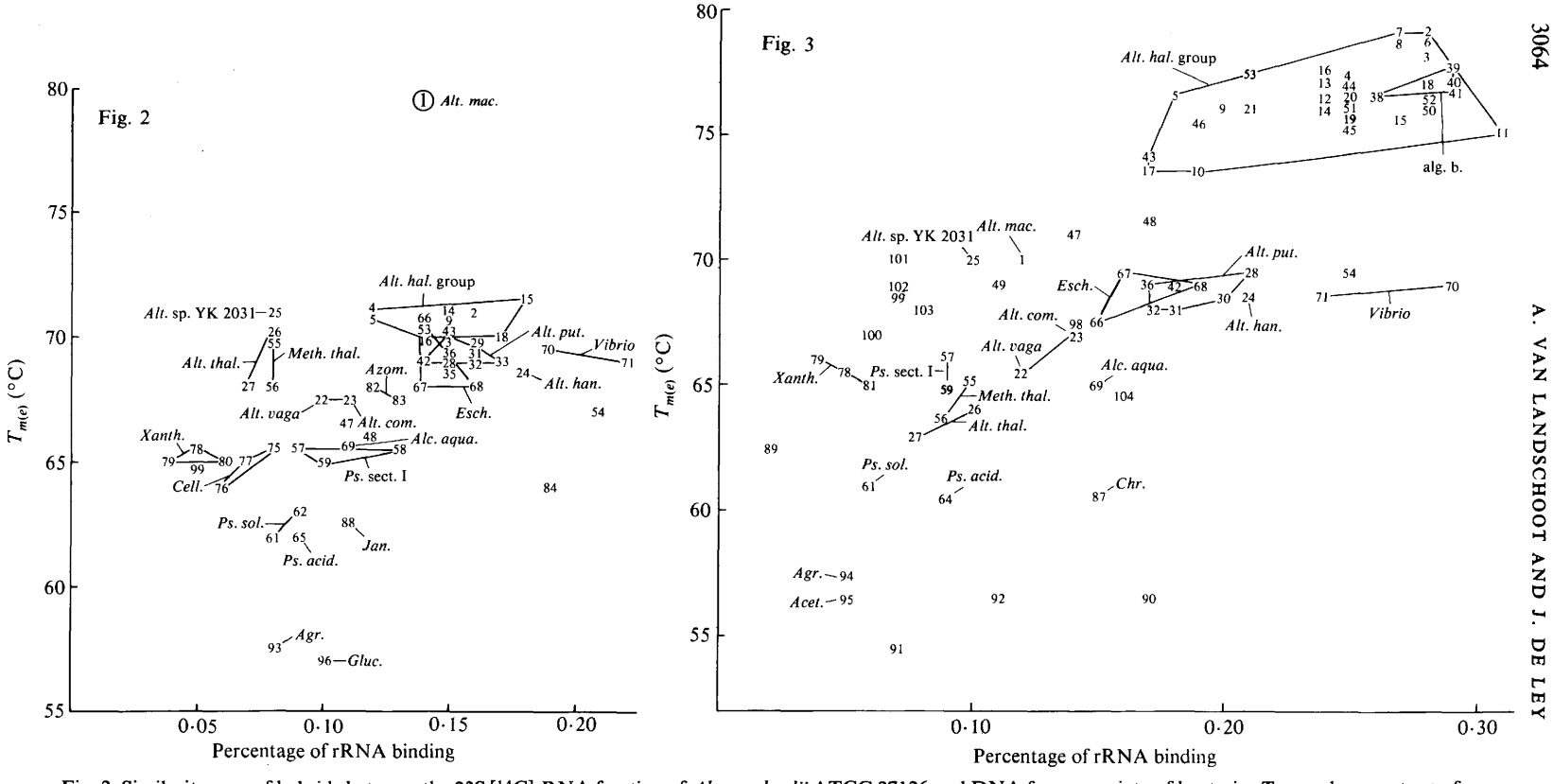

Fig. 2. Similarity map of hybrids between the 23S [^{14}C]rRNA fraction of *Alt. macleodii* ATCC 27126 and DNA from a variety of bacteria. $T_{m(e)}$ and percentage of rRNA binding are as defined in the text. To simplify the drawing each strain is represented by a sequence number (Table 1) which is not 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 taxon on the map.

Abbreviations: *Acet., Acetobacter; Agr., Agrobacterium; Alc. aqua., Alcaligenes aquamarinus;* alg. b., alginolytic bacteria; *Alt., Alteromonas; Alt. com., Alteromonas communis; Alt. hal., Alteromonas haloplanktis; Alt. han., Alteromonas hanedai; Alt. mac., Alteromonas macleodii; Alt. put., Alteromonas putrefaciens; Alt. thal., 'Alteromonas thalassomethanolica'; Azom., Azomonas; Azot., Azotobacter; Cell., Cellvibrio; Chr., Chromobacterium; Esch., Escherichia; Gluc., Gluconobacter; Jan., Janthinobacterium; Meth. thal., 'Methylomonas thalassica'; Ps., Pseudomonas; Ps. acid., Pseudomonas acidovorans* group; *Ps. sol., Pseudomonas solanacearum* group; *Ps.* sect. I, *Pseudomonas* section I; *Xanth., Xanthomomonas.*

Fig. 3. Similarity map of hybrids between ^{14}C-labelled 16S RNA of *Alt. haloplanktis* ATCC 14393 (number 2) and DNA from a variety of bacteria. See the legend to Fig. 2 for additional information.

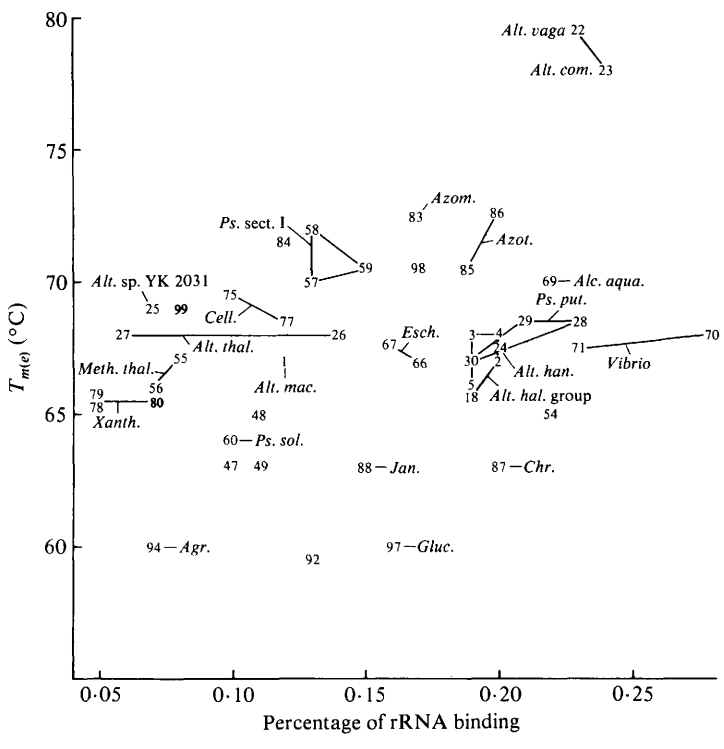

Fig. 4

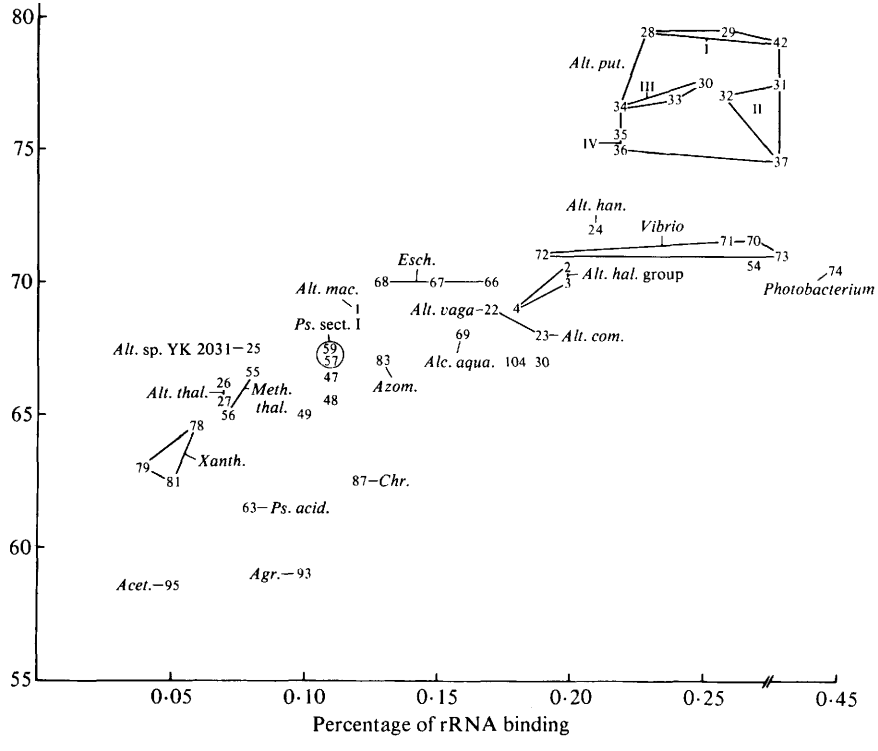

Fig. 5

Fig. 4. Similarity map of hybrids between ^{3}H-labelled 16S rRNA of *Alt. vaga* ATCC 27119 (number 22) and DNA from a variety of bacteria. See the legend to Fig. 2 for additional information.

Fig. 5. Similarity map of the hybrids between ^{3}H-labelled 16S rRNA of *Alt. putrefaciens* ATCC 8071 t1 (number 28) and DNA from a variety of bacteria. The four DNA/DNA homology groups I, II, III, IV of Owen *et al.* (1978) have been indicated. See the legend to Fig. 2 for additional information.

Table 3. *Results of hybridizations between DNA from several Alteromonas strains and ^{14}C-labelled 23S rRNA from various reference strains*

All results are from unpublished data of J. De Ley, P. De Vos, R. Tytgat & P. Segers, except for those obtained by using rRNA from *Azotobacter chroococcum* NCIB 8002, which are from De Smedt *et al.* (1980).

	Source of ^{14}C-labelled 23S rRNA:											
	Escherichia coli B		*Vibrio parahaemolyticus* ATCC 17802		*Pseudomonas fluorescens* ATCC 13525		*Azotobacter chroococcum* NCIB 8002		*Alcaligenes aquamarinus* NCMB 557 B		*Pseudomonas acidovorans* ATCC 15668	
Source of DNA on filter	$T_{m(e)}$ (°C)	Percentage binding	$T_{m(e)}$ (°C)	Percentage binding	$T_{m(e)}$ (°C)	Percentage binding	$T_{m(e)}$ (°C)	Percentage binding	$T_{m(e)}$ (°C)	Percentage binding	$T_{m(e)}$ (°C)	Percentage binding
Alteromonas macleodii ATCC 27126					67·0	0·09	68·0	0·12	68·5	0·06	62·0	0·10
Alt. haloplanktis ATCC 14393									67·5	0·14		
Alt. haloplanktis ATCC 19648					62·5	0·13	68·5	0·21	66·5	0·13		
Alt. haloplanktis ATCC 19855					66·0	0·15	67·0	0·19	66·5	0·10		
Alt. haloplanktis ATCC 27127							67·0	0·16	65·5	0·10		
Alt. rubra ATCC 29570					67·5	0·14			67·0	0·11		
Alt. citrea ATCC 29719									66·0	0·13		
Alt. hanedai ATCC 33224	67·0		71·0	0·16								
'*Alteromonas* sp.' YK 2031			69·5	0·07								
Alt. vaga ATCC 27119					71·5	0·16	71·5	0·22	70·0	0·09		
Alt. communis ATCC 27118					72·0	0·19	72·0	0·23	70·0	0·10		
Alt. putrefaciens ATCC 8071 t2	68·0	0·15	72·0	0·14								
Alt. putrefaciens ATCC 8073	68·5	0·14	71·5	0·13								
Alt. putrefaciens NCTC 10763	69·5	0·14	72·0	0·13								
Alt. putrefaciens NCTC 10738					65·0	0·12						

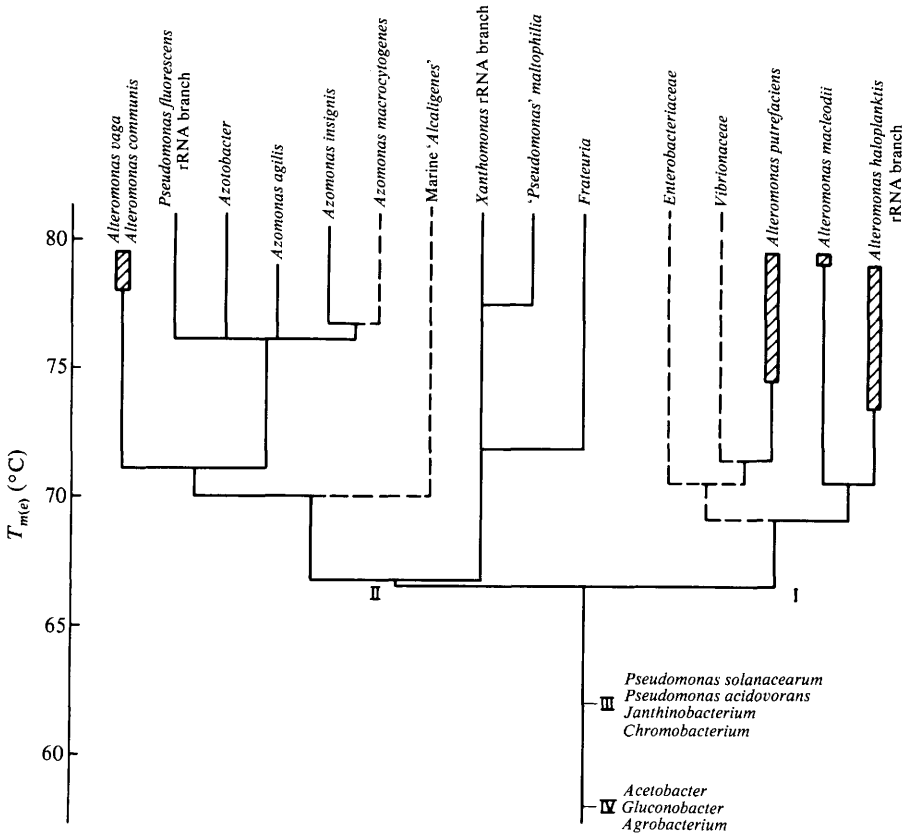

Fig. 6. Position of the four *Alteromonas* rRNA branches in the first and second rRNA superfamilies. The similarities between the rRNA cistrons are expressed as the $T_{m(e)}$ of their DNA/rRNA hybrids. The general structure of the dendrogram is from published data (continuous lines) and unpublished data (dashed lines) from this laboratory. The extent (heterogeneity) of the four *Alteromonas* rRNA branches is represented by the hatched bars. The roman numerals indicate the roots of rRNA superfamilies.

largest *Alteromonas* group. In addition to the reference species, it also contains the following named *Alteromonas* species: the violacein-producing *Alt. luteoviolacea* (Gauthier, 1976*a*, 1982), the prodigiosin-producing *Alt. rubra* (Gauthier, 1976*b*), the lemon-yellow-pigmented *Alt. citrea* (Gauthier, 1977), the orange-pigmented *Alt. aurantia* (Gauthier & Breittmayer, 1979), *Alt. espejiana* (Chan *et al.*, 1978) and *Alt. undina* (Chan *et al.*, 1978). Striking phenotypic similarities between these seven species have already been reported (Chan *et al.*, 1978; Gauthier, 1976*a*, 1976*b*, 1977; Gauthier & Breittmayer, 1979).

We discovered that a number of misnamed *Pseudomonas* species and some unnamed alginolytic marine bacteria also belong to this group.

'Pseudomonas atlantica' (Humm, 1946; Yaphe, 1957) ATCC 19262 (= NCMB 301) and CIP 59.31 (= NCIB 8959), were examined. Both strains are agarolytic. They were isolated from seawater or marine algae in the vicinity of Halifax, Nova Scotia, Canada (Yaphe, 1957). Strain ATCC 19262 is a member of the *Alt. haloplanktis* group, with a $T_{m(e)}$ of 77·5 °C. Its mol % G + C is 42·3 (our data) or 43·5 (Mandel, 1966). This *'Ps. atlantica'* strain has been generically misnamed and belongs in the genus *Alteromonas*. The other *'Ps. atlantica'* strain is probably quite different from strain ATCC 19262 (see below). Strain CCEB 506 (= CIP 63.28) is not identical with strain ATCC 19262. It was sent many years ago by O. Lysenko as CCEB 506 to the senior author (J.D.L.) and to the Collection de l' Institut Pasteur (CIP 63.28). The mol % G + C of the CCEB strain is 66·4 (De Ley & Friedman, 1965).

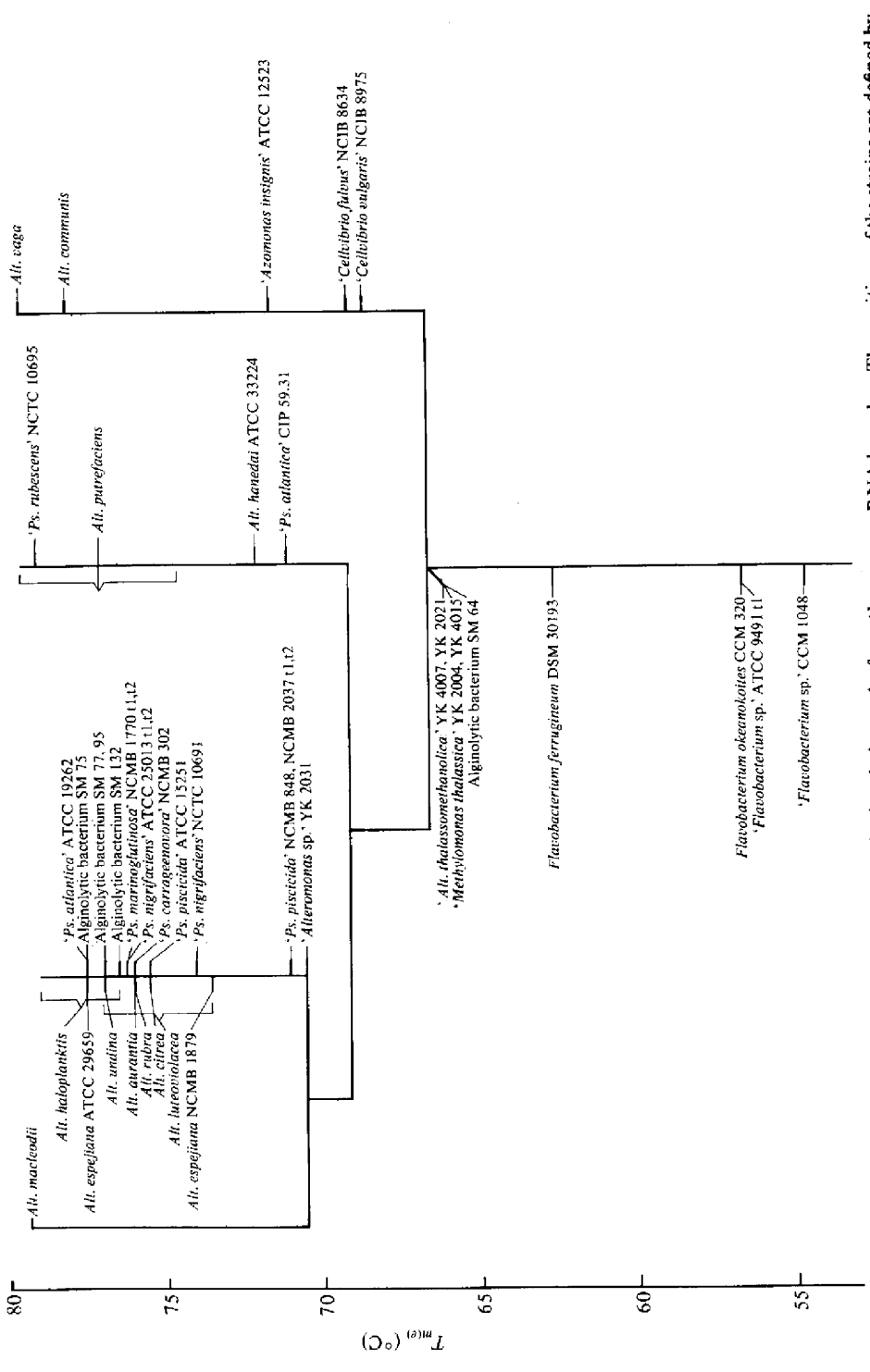

Fig. 7. Position of some unnamed, misnamed or unidentified strains in relation to the four *Alteromonas* rRNA branches. The positions of the strains are defined by the $T_{m(e)}$ values of their DNA/rRNA hybrids.

'Pseudomonas marinoglutinosa' (ZoBell & Allen, 1935; ZoBell & Upham, 1944) was isolated from slides submerged in the sea for one to seven days at La Jolla, Cal., U.S.A. We used strain NCMB 1770, which displayed two agarolytic colony types: t1 (white) and t2 (beige, transparent). They are probably two variants of the same strain as they fell close together (Fig. 3) with $T_{m(e)}$ values of 76 °C and 76·5 °C. Their mol % G + C values are almost the same: 42·1 and 42·2. Strain NCMB 1770 has been misnamed and belongs to the *Alt. haloplanktis* group.

'Pseudomonas nigrifaciens' was isolated by White (1940) as a Gram-negative, polarly flagellated, strictly aerobic rod from salted butter. It produces a black pigment. Baumann *et al.* (1972) suspected that strain NCTC 10691 belongs in *Alteromonas* because of its mol % G + C of 42·9 and its similar phenotypic characters, but they did not make a formal taxonomic assignment. We examined two strains, NCTC 10691 and ATCC 25013 (which consisted of two stable colony types, t1 and t2). The latter strain was isolated from seawater. Hybridizations show clearly that both strains belong in the *Alt. haloplanktis* group ($T_{m(e)}$ 74·0 to 76·5 °C and mol % G + C 40·4 and 41·0) and should thus be renamed.

'Pseudomonas carrageenovora' (Yaphe & Baxter, 1955) was isolated by these authors from seawater and various marine algae collected in the vicinity of Halifax, Nova Scotia, Canada. The organisms decompose the polysaccharide carrageenin. The species was described, but not named, by Yaphe & Baxter (1955). The name *'Ps. carrageenovora'* was used in later papers (Yaphe, 1959, 1962). We used strain NCMB 302, isolated from red algae, Cow Bay, Nova Scotia, Canada in September 1953. This strain falls clearly in the *Alt. haloplanktis* group with 76·0 °C $T_{m(e)}$ and 40·0% G + C and should be renamed as *Alteromonas*.

'Pseudomonas piscicida' (Bein) Buck, Meyers & Leifson, 1963 was originally isolated from red tide waters off the south-west coast of Florida, U.S.A. It produces a yellow-orange pigment and is toxic for fish (Bein, 1954). Other strains have since been isolated and studied numerically (Hansen *et al.*, 1965). The mol % G + C is low (44·5 ± 1) (Mandel *et al.*, 1966), which excludes it from the genus *Pseudomonas*. Four strains were examined (strain NCMB 2037 contained two colony types: t1, beige, and t2, orange). Their mol % G + C values ranged from 41·9 to 44·5. Strain ATCC 15251 (an original Bein strain) is located in the *Alteromonas* area. The three other strains gave $T_{m(e)}$ values a few °C lower (see below).

Of Meland's marine alginolytic strains SM 75, 77, 95, 132 and 64, the first four were isolated from seawater at the surface and at depths of up to 60 m in the vicinity of Tromsø, northern Norway, between November 1956 and October 1963. Strain 64 was isolated from a floating fragment of *Laminaria* species in the same general area in September 1955. All strains were isolated by Mrs S. Meland. Strains 75 and 77 were described and named *'Alginovibrio aquatilis'*, strain 95 was attributed to *'Alginovibrio norvegicus'*, and strain 64 to *'Alginovibrio immotus'* (Meland, 1963). We are not aware of a published description of strain 132; however, the following is known: the cells are small rods, motile with one or two polar flagella; colonies on nutrient agar or fish agar become greyish and later blackish (we did not observe the latter pigment); the organism decomposes agar and alginic acid, and produces acid from carbohydrates (S. Meland, personal communication).

Hybridizations showed that the marine alginolytic strains SM 75, 77, 95 and 132 belong to the *Alt. haloplanktis* rRNA branch. Strain SM 64 is discussed below.

(3) *The Alt. putrefaciens group*

'Pseudomonas' putrefaciens (Derby & Hammer) Long & Hammer, 1941 has been briefly reviewed by Bergan (1981). The cells are aerobic Gram-negative rods with one polar flagellum; they produce a typical pinkish, reddish or apricot-coloured water-soluble pigment and H_2S. The organism has been isolated from clinical sources but also from soil and water, oil brines, etc. *'Pseudomonas' putrefaciens* seems to be one of the most important spoilers of protein food kept at chill temperatures (2 to 4 °C). It seems to be rather heterogeneous phenotypically (Debois *et al.*, 1975; Levin, 1972; Riley *et al.*, 1972) and genotypically (43 to 53% G + C; four DNA homology groups, Owen *et al.*, 1978). These organisms cannot belong to *Pseudomonas* because the mol % G + C is too low. Lee *et al.* (1977) proposed on phenotypic grounds to rename this taxon as *Alt. putrefaciens*. Our DNA/rRNA hybridizations (Table 1; Figs 5 and 7) showed that nine *Alt.*

putrefaciens strains, with mol % G + C ranging from 44·4 to 53·3, and from very diverse origins (haddock, cuttlefish, butter, faeces, oil brine, cerebrospinal fluid, bottled blood) all belonged in one rRNA branch separate from all others. Although the resolution of the DNA/rRNA hybridization method is limited within a species or a small genus, we could nevertheless distinguish the four DNA homology groups of Owen *et al.* (1978). In addition, by plotting the DNA homology data from Owen *et al.* (1978) against our $T_{m(e)}$ values for the same pairs of strains, it is found that the top 6 or 7 °C of the $T_{m(e)}$ range corresponds to the entire 0 to 100% DNA homology scale, a conclusion agreeing very well with previous calculations on *Pseudomonas* (De Vos & De Ley, 1983).

'Pseudomonas rubescens' (Pivnick, 1955) was originally isolated from oil emulsions from factories. It is a pink, aerobic, Gram-negative rod with one polar flagellum, producing H_2S. Pivnick (1955) recognized the close similarity with *'Ps.' putrefaciens*. Recently the identity between both species has been repeatedly stressed: phenotypically (Lee *et al.*, 1977), by DNA/DNA hybridizations (Owen *et al.*, 1978), and by lipid analysis (Wilkinson & Caudwell, 1980). Our rRNA hybridizations confirm this: *'Ps. rubescens'* NCTC 10695, isolated by Pivnick from an oil emulsion from a machine shop, resembles most closely the type strain of *Alt. putrefaciens*.

(4) *The Alt. vaga–Alt. communis group*

Unfortunately, only the type strain of each species was available. They are very similar (Figs 4 and 7) to each other but very different from all other alteromonads (see below). No other organisms tested belonged to the *Alt. vaga–Alt. communis* rRNA branch: they all remained below 72·5 °C $T_{m(e)}$.

Relationships among the different Alteromonas rRNA branches and their position among the Gram-negative bacteria

The genus *Alteromonas* is extremely heterogeneous. All four rRNA branches are 9 to 13 °C $\Delta T_{m(e)}$ removed from each other. This is greater than, for example, the differences between *Pseudomonas* and *Azotobacter* (De Smedt *et al.*, 1980), between *Xanthomonas* and *Frateuria* (Swings *et al.*, 1980) or between *Janthinobacterium, Alcaligenes* and *Bordetella* (De Ley *et al.*, 1978; J. De Ley & P. Segers, unpublished). At least two of these four *Alteromonas* groups are thus different at and above the genus level.

The *Alt. haloplanktis* and *Alt. macleodii* rRNA branches are both members of the first rRNA superfamily *sensu* De Ley (1978). They are closest to each other, at 9 °C $\Delta T_{m(e)}$. Some noticeable phenotypic differences might be expected. However, phenotypically *Alt. macleodii* is not clearly separated from the species of the *Alt. haloplanktis* group; it differs in 6 out of about 200 features listed: growth on salicin, D-gluconate, DL-glycerate, and glycerol; synthesis of antibiotics and resistance to thiophenicol. *Alteromonas espejiana*, which belongs to the *Alt. haloplanktis* rRNA branch, has a slightly greater phenotypic similarity to *Alt. macleodii* than to *Alt. haloplanktis*. At present there seem to be no convincing phenotypical arguments to separate the two rRNA branches. The genus *Alteromonas* can temporarily be delineated above about 70 °C $T_{m(e)}$ with an atypical *Alt. macleodii* as type species. In this case one should also admit three more low $T_{m(e)}$ strains to this genus: *'Ps piscicida'* NCMB 2037 and NCMB 848, and *'Alteromonas* sp.' YK 2031, a facultative methylotroph of marine origin. The genus description by Baumann *et al.* (1972) should be extended with the features in Table 4.

The *Alt. putrefaciens* rRNA cluster (including *'Ps. rubescens'*) is closer to the family of the *Vibrionaceae* (average $T_{m(e)}$ 71·4 °C) than to the *Enterobacteriaceae* (average $T_{m(e)}$ 69·3 °C) or the *Alt. macleodii* and *Alt. haloplanktis* branches (average $T_{m(e)}$ 68·6 °C). Our results suggest that *Alt. putrefaciens* is not an *Alteromonas*, but deserves separate genus status. Recently Wilkinson & Caudwell (1980) found significant differences between the lipids and fatty acids of the *putrefaciens* organisms and those reported for a strain of *Alt. haloplanktis* (DiRienzo & MacLeod, 1978), thus opposing the inclusion of the *putrefaciens* organisms in the genus *Alteromonas*. One would expect that there are several other phenotypic differences. Unfortunately the individual strains of *Alt. putrefaciens* have been incompletely described so far. More phenotypic data on *Alt. putrefaciens* would be useful.

Table 4. *Features differentiating between the proposed genus Marinomonas and the remaining members of the genus Alteromonas*

The data were taken from the literature: *a*, Baumann *et al.* (1972); *b*, Chan *et al.* (1978); *c*, Reichelt & Baumann (1973); *d*, Gauthier (1976*a*); *e*, Gauthier (1982); *f*, Gauthier (1976*b*); *g*, Gauthier (1977); *h*, Gauthier & Breittmayer (1979).

The symbols + and − are used to indicate that all strains of the species were positive or negative, respectively, with respect to the property tested. The numbers in the table indicate the number of positive strains. ND, Not determined; m, meta cleavage.

	Marinomonas		*Alteromonas*							
Property	*communis* (*a*)	*vaga* (*a*)	*macleodii* (*a*)	*haloplanktis* (*a,b,c*)	*luteoviolacea* (*d,e*)	*rubra* (*f*)	*citrea* (*g*)	*espejiana* (*b*)	*undina* (*b*)	*aurantia* (*h*)
No. of strains . . .	33	17	21	25	16	3	3	18	8	4
Ring cleavage	m	m	−	−	ND	−	−	−	−	−
Gelatinase	−	−	20	+	+	+	+	+	+	+
Lipase	−	−	+	+	+	+	+	+	+	+
Saccharate	+	+	−	−	ND	ND	ND	−	−	ND
DL-Malate	+	+	−	−	−	−	−	−	−	−
2-Oxoglutarate	+	+	−	−	−	−	−	−	−	−
Sorbitol	+	+	−	−	−	−	−	−	−	−
meso-Inositol	+	+	−	−	−	−	−	−	−	−
m-Hydroxybenzoate	+	+	−	−	−	−	−	−	−	−
p-Hydroxybenzoate	+	+	−	−	−	ND	−	−	−	−
Quinate	+	+	−	−	−	ND	ND	−	−	ND
D-α-Alanine	+	+	−	−	−	ND	−	−	−	−
L-Ornithine	+	+	−	1	−	−	−	ND	ND	−
γ-Aminobutyrate	+	16	−	−	ND	ND	ND	−	−	−
Betaine	+	16	−	−	ND	ND	ND	ND	ND	−
Sarcosine	+	+	−	−	ND	ND	ND	−	−	−
Putrescine	+	15	−	−	ND	ND	ND	−	−	ND
G + C range (mol %)	46–48	46·5–49·5	44·5–46·5	42–44	41–42	47	41·5–44·5	43–44	43–44	39–42

The *Alt. communis–Alt. vaga* branch is quite far away from the three other *Alteromonas* branches and from the entire first rRNA superfamily, at 66·7 °C $T_{m(e)}$. The *Alt. communis–Alt. vaga* rRNA branch is a member of the second rRNA superfamily. It is close to, but still distinctly different from, the *Pseudomonas* section 1–*Azotobacter–Azomonas* cluster, at 71·5 °C $T_{m(e)}$. From our extensive experience with this method (De Ley & De Smedt, 1975; De Ley *et al.*, 1978; De Smedt *et al.*, 1980; De Smedt & De Ley, 1977; Gillis & De Ley, 1980) we conclude that the *Alt. vaga–Alt. communis* group is certainly not a member of *Alteromonas*; it constitutes a new genus, which is part of a different family. This is supported by the fact that data in the literature reveal considerable phenotypic differences between the *Alt. vaga–Alt. communis* rRNA branch on the one hand, and the *Alt. macleodii* and *Alt. haloplanktis* branches on the other hand (Table 4). Therefore we propose *Marinomonas* (L. adj. *marinus*, pertaining to the sea; Gr. n. *monas*, a unit, monad; M.L. *Marinomonas*, sea monad) as a new genus for both species, with *M. communis* as the type species and ATCC 27118 as the type strain. The description of the new genus is the same as that given for the *Alt. communis–Alt. vaga* group by Baumann *et al.* (1972).

Organisms excluded from Alteromonas (Table 1 and Fig. 7)

Among the Gram-negative organisms which we examined by DNA/rRNA hybridizations, because of their low mol % G + C and/or their marine origin, the following strains were shown not to belong to *Alteromonas*.

(*a*) Strains at the bottom of the *Alt. putrefaciens* rRNA branch. Marine, luminous rods with one polar flagellum and with a mol % G + C of 45·2 ± 0·8, unable to ferment some sugars, have been named *Alt. hanedai* (Jensen *et al.*, 1980). The type strain ATCC 33224 has a 72 °C $T_{m(e)}$

value against *Alt. putrefaciens* and a 67·5 to 68·5 °C value against the other three reference strains. A numerical analysis of phenotypic properties (Jensen *et al.*, 1980) confirms our conclusion that *Alt. hanedai* is outside the *Alt. macleodii—Alt. haloplanktis* groups and very different from the *Alt. vaga–Alt. communis* cluster. *Alt. hanedai* is separate from both the *putrefaciens* rRNA branch and from the *Vibrionaceae* since the $T_{m(e)}$ values with labelled reference rRNA from both taxa are 71 to 72 °C. Strain *'Ps. atlantica'* CIP 59.31 with 45·4% G + C (see above) groups at 71 °C $T_{m(e)}$ against *Alt. putrefaciens.*

(*b*) Organisms belonging in the second rRNA superfamily (De Ley, 1978). The following three organisms are at about 68·5 to 71·5 °C $T_{m(e)}$ against the reference *M. vaga* rRNA, and at an average of 65 °C $T_{m(e)}$ against the *Alteromonas* rRNAs. Their exact taxonomic position is being examined. *'Cellvibrio fulvus'* and *'Cellvibrio vulgaris'* (48·2% and 51·5% G + C, respectively) were originally isolated as cellulose decomposers from forest humus in Germany, and are small vibrios with a single polar flagellum (Stapp & Bortels, 1934). It has been shown in our laboratory that *'Azomonas insignis'* ATCC 12523 has been misnamed (De Smedt *et al.*, 1980). The present hybridizations did not succeed in helping to identify this strain.

(*c*) Organisms of unknown affiliation. The following organisms did not fall specifically on any of the four rRNA branches, and their exact taxonomic affiliations are unknown: the marine methanol-utilizing rods *'Alteromonas thalassomethanolica'* YK 4007 and YK 2021, and *'Methylomonas thalassica'* YK 2004 and YK 4015 (Yamamoto *et al.*, 1980); and the alginolytic bacterium SM 64 of Meland (1963).

In a previous study of DNA/rRNA hybridizations in *Flavobacterium* (Bauwens & De Ley, 1981, and unpublished), the low mol% G + C organisms *Flavobacterium ferrugineum* DSM 30193, *Flavobacterium okeanokoites* CCM 320, *'Flavobacterium* sp.' CCM 1048 and ATCC 9491 t1 were excluded from this genus. From the present results it follows that they are also excluded from *Alteromonas* and *Marinomonas*. Their exact taxonomic position remains unknown.

J.D.L. is indebted to the Fonds voor Kollektief Fundamenteel Onderzoek for research and personnel grants. We are indebted to Drs M. J. Gauthier, R. J. Owen, K. Kouno, M. L. Lizarraga-Partida, S. Meland and Miss M. S. Hendrie for the gracious gift of strains. We are particularly indebted to Dr S. Meland for valuable unpublished information.

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