Journal of General Microbiology (1985), **131**, 165–173. *Printed in Great Britain*

Nucleic Acid Studies on Halophilic Archaebacteria

By H. N. M. ROSS AND W. D. GRANT*
Department of Microbiology, University of Leicester, LE1 7RH, UK

(*Received 13 July 1984; revised 13 September 1984*)

DNA–16S rRNA hybridization studies of archaebacterial halophiles revealed nine major groups. High (>45%) DNA–DNA homologies were found only within DNA–rRNA groups. The DNA–DNA homology between the type strains of *Halobacterium halobium*, *Hb. salinarium* and *Hb. cutirubrum* was >70%. The implications for the taxonomy of the extreme halophiles are discussed.

INTRODUCTION

Archaebacteria exhibit considerable phylogenetic diversity despite the relatively restricted range of archaebacterial phenotypes. The methanogens comprise three orders (Balch *et al.*, 1979; Wildgruber *et al.*, 1982) and the thermoacidophilic bacteria have been shown to include several major groups probably equivalent to orders (Tu *et al.*, 1982). However, until recently only two major groups of extreme halophiles have been recognized, the rod-shaped or pleomorphic isolates (the halobacteria) and the coccoid isolates (the halococci). The thorough taxonomic studies of Kocur & Hodgkiss (1973) indicated that all strains of halococci formed a tightly clustered group comprising a monospecific genus with *Halococcus morrhuae* as the sole species. The halobacteria presently include ten species (Skerman *et al.*, 1980; Soliman & Trüper, 1982; Rodriguez-Valera *et al.*, 1983; Oren, 1983) and in addition Javor *et al.* (1982) have suggested the creation of a new genus, *Haloarcula*, for certain pleomorphic isolates. However, this genus has never been formally proposed and its validity would be in question in view of the similarity of the isolates to certain other halobacteria (Nicholson & Fox, 1983).

A recent indication of considerable diversity within the halophile phenotype has come with the discovery of the haloalkaliphiles (Tindall *et al.*, 1980, 1984; Ross *et al.*, 1981; Soliman & Trüper, 1982). This group constitutes a clear, phenotypically distinct group within the general halophile phenotype in that these isolates have an obligate requirement for high pH, and have a very low Mg^{2+} tolerance (Tindall *et al.*, 1980, 1984; Soliman & Trüper, 1982). All haloalkaliphilic bacteria isolated to date have ether-linked lipids based both on the universal archaebacterial $C_{20}C_{20}$ diether isopranoid core and an asymmetric $C_{20}C_{25}$ diether isopranoid core that is not universally present in 'classical' halobacteria (Ross *et al.*, 1981, 1984; De Rosa *et al.*, 1982; Tindall *et al.*, 1984). One isolate has an additional $C_{25}C_{25}$ diether isopranoid core lipid (De Rosa *et al.*, 1983). The haloalkaliphilic group includes both rod-shaped and coccoid isolates. Nucleic acid hybridization studies and polar lipid analyses have indicated that the rod-shaped isolates are as distinct from the coccoid isolates as 'classical' halobacteria are from ' classical' halococci, leading Tindall *et al.* (1984) to propose the new genera *Natronobacterium* and *Natronococcus* for the rods and cocci, respectively.

In the course of this work it was noted that the 'classical' halobacteria *Hb. volcanii* and *Hb. halobium* appeared as unrelated to each other as each was unrelated to *Hc. morrhuae*. Earlier, 16S rRNA sequence comparisons of these halobacteria had led Fox *et al.* (1980) to the same conclusions. The polar lipids from a small number of halobacterial isolates have been compared and shown to have some value as taxonomic markers (Evans *et al.*, 1980; Kushwaha *et al.*, 1982) and a more comprehensive investigation of the value of polar lipid analyses in halophile

taxonomy (Ross *et al.*, 1984) confirmed the similarity of isolates of halococci and pointed again to the differences between *Hb. halobium* and *Hb. volcanii*. Also revealed by this work were a further six or seven discrete groups of halobacteria equally distinct in terms of their lipid composition, despite, in several instances, identical specific epithets (Ross *et al.*, 1984).

Difficulties in assigning halobacterial isolates to appropriate taxa arise in that 'classical' halobacteria are somewhat biochemically inert and thus phenotypically similar, Although a few isolates are known to utilize certain sugars (Tomlinson & Hochstein, 1976; Gonzalez *et al.*, 1978; Rodriguez-Valera *et al.*, 1983), in general, standard biochemical tests have not proved very useful in the taxonomy of the group, even when quite extensive numerical taxonomic studies have been carried out (Colwell *et al.*, 1979). Thus there are many isolates residing in culture collections under the same names or simply as *Halobacterium* sp.

From the preliminary findings of Tindall *et al.* (1984) and Ross *et al.* (1984) a considerable diversity may be expected to emerge when more detailed chemotaxonomic analyses are applied to members of this group of phenotypically similar archaebacteria. We present here the results of such a survey and discuss the implications for the taxonomy of the group.

METHODS

Strains and culture conditions. Details of the test strains and their sources are shown in Table 1. Members of the genera *Halobacterium* and *Halococcus* were grown at 37 °C in the light (Gallenkamp illuminated incubator) in liquid shake culture in the medium of Payne *et al.* (1960), with the exceptions of *Halobacterium saccharovorum*, which was grown at 45 °C, *Hb. volcanii*, which was grown in the medium of Mullakhanbhai & Larsen (1975) and *Hb. sodomense* which was grown in the medium of Oren (1983). Members of the genera *Natronobacterium* and *Natronococcus* were grown in the light in the medium of Tindall *et al.* (1980).

Agar was added to these cultures to a final concentration of 1·8% (w/v) for plate cultures. Agar cultures were sealed in Petri dish bags and incubated in the light.

Nucleic acid analyses. (*a*) DNA–16S rRNA hybridizations were performed using a modification of the procedure of De Ley and De Smedt (1975), as described by Tindall *et al.* (1984). Hybridizations were carried out overnight in 2·0 × SSC (NaCl, 8·7 g l^{-1}, sodium citrate, 4·41 g l^{-1}; pH 7·4, in 20% formamide) at 50 °C. Hybrid stability was measured in 1·5 × SSC (in 20% formamide) in water baths from 55 to 90 °C in 5 °C steps. The hybrid melting temperature was determined as the point of 50% release of label (De Ley & De Smedt, 1975). Total cellular DNA was used. (*b*) DNA–DNA reassociations were performed using the S1 nuclease method of Crosa *et al.*

Table 1. *Origins and distinctive features of strains within 16S rRNA homology groups*

Polar lipid/ rRNA group*	Strain	Distinctive features	Origin	Reference
1	*Hb. cutirubrum* CCM 2088† *Hb. halobium* CCM 2090† *Hb. salinarium* CCM 2084† *Hb. salinarium* CCM 2148 *Hb. salinarium* NCMB 764 *Hb. halobium* NCMB 736	Pleomorphic rods; some strains gas vacuolate; cannot metabolize carbohydrates; Gram-negative; polar lipids: PGP, PGS, PG, GLS.	Salted fish	Petter (1931); Lochhead (1934); Ross *et al.* (1984).
2	*Hb. halobium* NCMB 777 *Hb. salinarium* NCMB 786 *Hb. trapanicum* NCMB 784	Rods, some pleomorphic; metabolize certain carbohydrates; non-gas vacuolate; Gram-negative; possess $C_{20}C_{25}$ diethers; polar lipids: PGP, PG, PGS, GL.	Salted hides	Formisano (1962); Ross *et al.* (1981, 1984).
3	*Hb. volcanii* NCMB 2012† *Hb. mediterranei* CCM 3361†	Coccoid or pleomorphic cells, some strains gas vacuolate; high Mg^{2+} optima, low NaCl optima. Some strains grow on single C sources; Gram-negative; polar lipids: PGP, PG, S-DGD, DGD.	Salt ponds; Dead Sea	Mullakhanbhai & Larsen (1975); Rodriguez-Valera *et al.* (1983); Kushwaha *et al.* (1982).

Table 1. (*continued*)

Polar lipid/ rRNA group*	Strain	Distinctive features	Origin	Reference
4	*Nb. gregoryi* NCMB 2189† *Nb. magadii* NCMB 2190† *Nb. pharaonis* DSM 2160† *Nb. pharaonis* NCMB 2191	Rods in liquid, cocci on solid media; high pH optima (9·5), low Mg^{2+} tolerance; Gram-negative; one strain metabolizes carbohydrates; possess $C_{20}C_{25}$ diethers but no glycolipids; polar lipids: PGP, PG.	Saline soda lakes	Tindall *et al.* (1980, 1984); Soliman & Trüper (1982); Ross *et al.* (1981, 1984).
5	*Nc. occultus* NCMB 2192†	Cocci; metabolizes carbohydrates; high pH optimum (9·5), low Mg^{2+} tolerance; colonies pale brown; Gram-positive or negative; refractile, non-motile; possess $C_{20}C_{25}$ diethers but no glycolipids; polar lipids: PGP, PG.	Saline soda lakes	Tindall *et al.* (1984); Ross (1982); Ross *et al.* (1984).
6	*Hc. morrhuae* NCMB 787† *Hc. morrhuae* NCMB 761	Cocci; cannot metabolize carbohydrates; cells stable in hypotonic solutions; non-gas vacuolate, refractile, non-motile; Gram-positive; possess $C_{20}C_{25}$ diethers; polar lipids: PGP, PG, TGD-1, S-DGD.	Salted fish	Kocur & Hodgkiss (1973); De Rosa *et al.* (1982); Ross *et al.* (1984).
7	*Hb. saccharovorum* NCMB 2081† *Hb. sodomense* ATCC 33755† *Hb. trapanicum* NRC 34021†	Pleomorphic rods; metabolize numerous carbohydrates; one strain has very high Mg^{2+} optimum (0·6–1·2 M); motile; Gram-negative; polar lipids: PGP, PG, PGS, S-DGD.	Salt pans; Dead Sea	Tomlinson & Hochstein (1976); Ross (1982); Oren (1983).
8	*Hb. marismortui*‡ *Hb. vallismortis* ATCC 29715†	Highly pleomorphic or plate-shaped; some rods; some strains facultatively anaerobic; carbohydrates metabolized; Gram-negative; polar lipids: PGP, PG, PGS, TGD-1.	Salt pools; Dead Sea	Gonzalez *et al.* (1978); Ross (1982); Evans *et al.* (1980).
9	*Hb. cutirubrum* NCMB 763	Rods, highly variable in length; some carbohydrates metabolized; coccoid cells in old cultures; Gram-negative; polar lipids: PGP, PG, PGS, TGD-2, S-DGD.	Salted fish	Ross (1982); J. Shewan (personal communciation); Ross *et al.* (1984).

* Polar lipid groups described by Ross & Grant (1984); rRNA homology groups described in this paper – see Table 2.

† Type strain of currently recognized species.

‡ This strain resembles the lost strain of *Hb. marismortui* (Elazari-Volcani, 1957) and was kindly donated by Dr. M. Ginzburg, Jerusalem, Israel.

Abbreviations: *Hb., Halobacterum*; *Hc., Halococcus*; *Nb., Natronobacterium*; *Nc., Natronococcus*. ATCC, American Type Culture Collection (Maryland USA); CCM, Czechoslovakian Collection of Microorganisms (Brno, Czechoslovakia); DSM, Deutsche Sammlung von Mikroorganismen (Munich, FRG); NCMB, National Collection of Marine Bacteria (Aberdeen, UK).

PG, phosphatidylglycerol; PGP, phosphatidylglycerophosphate; PGS, phosphatidylglycero-sulphate; GLS, glycolipid sulphate; DGD, glycodiosyl diether; S-DGD, sulphated DGD; TGD-1, TGD-2, triglycosyl diethers with different end sugars (Kushwaha *et al.* 1982); GL, unknown glycolipid.

(1979). Reassociations were done in 200 mM-NaCl at 68 °C (Tjndall *et al.*, 1984). Total cellular DNA was used. (*c*) Plasmids were detected using the alkaline denaturation method of Kado & Liu (1981). Samples were analysed by agarose gel electrophoresis (0·8%, w/v). (*d*) G + C content was determined by the buoyant density method of Mandel *et al.* (1968).

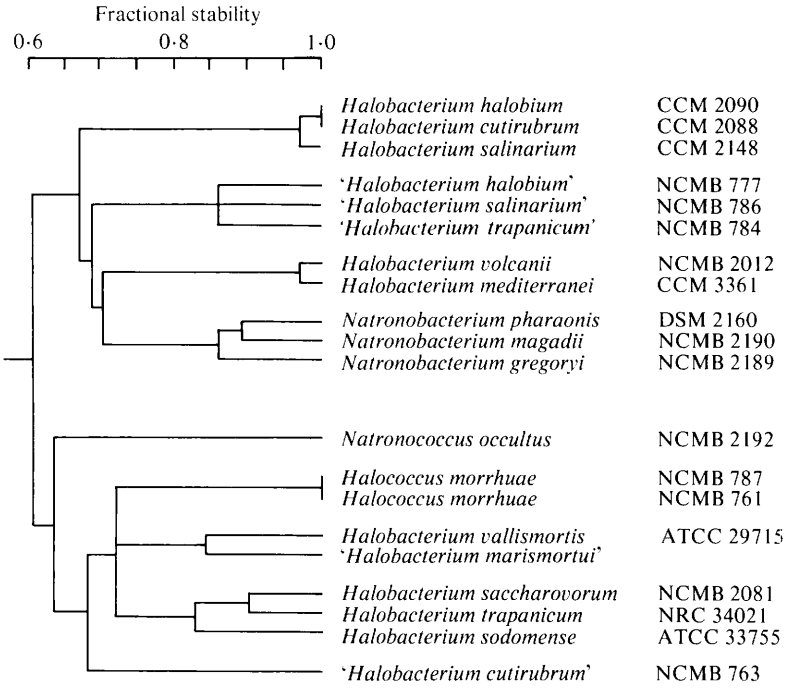

Fig. 1. Relationships amongst extreme halophiles based on DNA/16S rRNA hybrids after Tu *et al.* (1982).

RESULTS

DNA–16S RNA hybridizations. Thermal stabilities of DNA–16S rRNA hybrids between different strains and percentage bindings are shown in Table 2. Homologous hybrids had melting temperatures of between 78 °C and 80 °C. Stable hybrids were not formed between archaebacterial rRNA and eubacterial DNA under the conditions used. From Table 2 it is possible to construct a dendrogram showing relatedness of strains based on melting temperatures as originally devised by Tu *et al.* (1982) (Fig. 1). Strains fall into nine distinct groups which follow closely the groups generated from polar lipid pattern analysis (Ross *et al.*, 1984), with the exception of the alkaliphiles, which show very similar polar lipid patterns but split into two separate, unrelated groups on rRNA data (Tindall *et al.*, 1984). Distinctive phenotypic features of strains in each group are shown in Table 1.

DNA–DNA homologies. Homologies between strains are shown in Table 3. High homologies (>45%) were seen only between strains within the same 16S rRNA/polar lipid group. Homologies within a 16S rRNA group were never lower than 32%, whilst homologies between groups were never higher than 37% and dropped as low as 12%. Particularly high homologies have already been reported between *Natronobacterium pharaonis* strains SP1 (NCMB 2191) and DSM 2160 (96%) (Tindall *et al.*, 1984). High homologies were also detected between the type strain *Hb. halobium* CCM 2090, the type strains of *Hb. cutirubrum* and *Hb. salinarium* and various strains named *Hb. halobium*, *Hb. cutirubrum* and *Hb. salinarium* (>60%), in agreement with early studies on a limited number of strains by Moore & McCarthy (1969). The two *Halococcus morrhuae* strains tested showed a similar high degree of homology with each other.

G + C and plasmid contents. G + C contents of strains are given in Table 4. It can be seen that the majority of the strains possess both main band DNA and substantial amounts of satellite DNA. Those strains which showed satellite bands in CsCl gradients also possessed plasmids when the analytical procedure of Kado & Liu (1981) was applied, with the exception of *Hb.*

Table 2. *DNA–16S rRNA hybridization data: melting points (T_m, °C) and percentage binding*

Hybridization values within boxes indicate strain homology groups

Unlabelled DNA	^{32}P-labelled 16S rRNA: *Hb. halobium* CCM 2090		*Hb. volcanii* NCMB 2012		*Hb. salinarium* NCMB 786		*Nb. pharaonis* NCMB 2191		*Nc. occultus* NCMB 2192		*Hc. morrhuae* NCMB 787		*Hb. saccharovorum* NCMB 2081		*Hb. vallismortis* ATCC 29715		*Hb. cutirubrum* NCMB 763	
	T_m	% Bound	T_m	% Bound	T_m	% Bound	T_m	% Bound	T_m	% Bound	T_m	% Bound	T_m	% Bound	T_m	% Bound	T_m	% Bound
Hb. cutirubrum CCM 2088	79·0	0·15	—	—	—	—	67·5	0·05	—	—	—	—	—	—	—	—	—	—
Hb. halobium CCM 2090	79·0	0·15	—	—	67·0	0·09	68·0	0·03	65·0	0·12	65·5	0·07	—	—	66·0	0·03	—	—
Hb. salinarium CCM 2084	78·0	0·14	70·0	0·09	67·0	0·11	65·0	0·03	—	—	66·0	0·11	66·5	0·13	—	—	66·5	0·10
Hb. volcanii NCMB 2012	—	—	80·0	0·11	68·0	0·10	—	—	65·0	0·08	68·5	0·07	—	—	66·0	0·04	64·5	0·15
Hb. mediterranei CCM 3361	—	—	79·0	0·10	68·0	0·08	68·0	0·07	—	—	67·0	0·06	—	—	—	—	—	—
Hb. halobium NCMB 777	65·5	0·07	69·5	0·12	75·0	0·13	69·0	0·07	—	—	—	—	—	—	—	—	—	—
Hb. salinarium NCMB 786	—	—	—	—	79·0	0·17	—	—	—	—	—	—	65·5	0·07	67·5	0·03	—	—
Hb. trapanicum NCMB 784	—	—	—	—	75·0	0·10	—	—	—	—	—	—	65·0	0·09	—	—	—	—
Nb. pharaonis NCMB 2191	—	—	71·0	0·07	68·0	0·07	78·0	0·12	66·0	0·09	66·0	0·08	66·5	0·06	66·5	0·04	65·0	0·14
Nc. occultus NCMB 2192	68·0	0·04	65·0	0·03	66·0	0·10	65·0	0·04	78·5	0·16	67·0	0·12	67·0	0·11	66·5	0·03	68·0	0·10
Hc. morrhuae NCMB 787	66·0	0·05	65·0	0·03	—	—	—	—	65·5	0·11	78·0	0·13	68·0	0·12	70·5	0·04	64·5	0·11
Hc. morrhuae NCMB 761	66·0	0·05	—	—	66·5	0·09	67·0	0·06	—	—	78·0	0·12	—	—	—	—	—	—
Hb. saccharovorum NCMB 2081	67·0	0·12	—	—	—	—	69·5	0·07	—	—	—	—	80·0	0·16	—	—	66·0	0·05
Hb. sodomense ATCC 33755	—	—	—	—	—	—	—	—	66·5	0·12	—	—	75·0	0·12	70·5	0·05	—	—
Hb. trapanicum NRC 34021	66·0	0·03	67·0	0·02	—	—	—	—	—	—	—	—	77·0	0·09	—	—	66·0	0·05
'*Hb. marismortui*'	66·0	0·05	—	—	66·0	0·09	—	—	—	—	69·0	0·07	—	—	74·0	0·09	—	—
Hb. vallismortis ATCC 29715	—	—	67·0	0·04	—	—	66·0	0·05	65·0	0·10	—	—	—	—	78·5	0·10	—	—
Hb. cutirubrum NCMB 763	64·5	0·05	67·0	0·03	67·0	0·05	64·5	0·05	68·0	0·11	66·0	0·09	66·0	0·08	68·0	0·01	78·0	0·20

—, Values not measured.

Table 3. *DNA homologies amongst archaebacterial halophiles*

Unlabelled DNA	^{3}H-labelled DNA: *Hb. halobium* CCM 2090	*Hb. volcanii* NCMB 2012	*Hb. salinarium* NCMB 786	*Nb. pharaonis* NCMB 2191	*Hb. saccharovorum* NCMB 2081	'*Hb. marismortui*'	*Hc. morrhuae* NCMB 787
Hb. cutirubrum CCM 2088	81	31	—	34	29	—	34
Hb. halobium CCM 2090	100*	33*	—	35*	30	—	32*
Hb. halobium NCMB 736†	76	25	—	35	25	—	34
Hb. salinarium CCM 2084	72	31	—	33	—	—	—
Hb. salinarium CCM 2148†	60	—	—	—	24	—	32
Hb. salinarium NCMB 764†	77	31	—	34	33	—	22
Hb. mediterranei CCM 3361	31	38	—	30	27	—	25
Hb. volcanii NCMB 2012	35*	100*	—	32*	31	—	21*
Hb. halobium NCMB 777	33	35	50	24	33	—	32
Hb. salinarium NCMB 786	31	26	100	32	31	—	26
Hb. trapanicum NCMB 784	23	13	52	31	20	—	27
Nb. pharaonis NCMB 2191	35*	32*	—	100*	30	—	35*
Nc. occultus NCMB 2192	22	12*	—	16*	22	—	25
Hb. saccharovorum NCMB 2081	36	22	—	33	100	—	30
Hb. sodomense ATCC 33755	—	—	—	—	45	—	—
Hb. trapanicum NRC 34021	—	—	—	—	52	—	—
'*Hb. marismortui*'	38	35	—	33	30	100	24
Hb. vallismortis ATCC 29715	31	31	—	35	23	39	36
Hc. morrhuae NCMB 787	34*	32*	—	31*	12	—	100*
Hc. morrhuae NCMB 761	26	—	—	30	—	—	76
Hb. cutirubrum NCMB 763	29	34	—	34	25	—	25

—, Values not determined.
* Values quoted by Tindall *et al.* (1984).
† Strains not included in DNBA–16S rRNA hybridization data.

Table 4. *Plasmid and G + C content of halophilic archaebacteria*

Strain	Approx. size of plasmid (kbp)	G + C content (%): Major component	Minor component
Hb. cutirubrum NCMB 763	144	67·1	57·8
Hb. halobium CCM 2090*	144†	70·9	59·2
'*Hb. marismortui*'	—	61·9	54·7
Hb. mediterranei CCM 3361	—	62·2	—
Hb. saccharovorum NCMB 2081	—	71·2	—
Hb. salinarium NCMB 786	144	69·9	60·0
Hb. sodomense ATCC 33755	—	67·4	—
Hb. vallismortis ATCC 29715	—	64·7	—
Hb. volcanii NCMB 2012*	90,6†	66·5	55·3
Hb. trapanicum NRC 34021	90,6†	64·3	—
Hc. morrhuae NCMB 787	ND	64·6	53·4
Nb. pharaonis NCMB 2191*	144	62·1	51·4
Nc. occultus NCMB 2192*	144	64·0	55·7

ND, Not detectable by method of DNA extraction.
* Strains and values quoted by Tindall *et al.* (1984).
† Values quoted by Pfeifer *et al.* (1981).

marismortui, for which no plasmid was detected. This correlates well with data published by Tindall *et al.* (1984) for a smaller number of strains.

DISCUSSION

It is clear from these studies that the genetic diversity of the extremely halophilic archaebacteria is considerable, despite relatively narrow phenotypic variation. The nine groups defined by DNA–16S rRNA hybridization data are likely to be at supra-specific level (De Smedt & De Ley, 1977; Stackebrandt *et al.*, 1981). The results presented here support the idea of *Halococcus* as a monospecific genus. Within the genus *Halobacterium*, the type strains *Hb. salinarium* CCM 2084, *Hb. halobium* CCM 2090 and *Hb. cutirubrum* CCM 2088 show high DNA homologies with each other (Table 2) and probably should be considered different isolates of the same species. These type strains have at various other times been considered identical (Gibbons, 1974; Colwell *et al.*, 1979). Most other strains examined and originally described as *Halobacterium* sp. fall into other quite distinct rRNA homology groupings. In all cases the groupings have distinct polar lipid patterns (Ross *et al.*, 1984), with the exception of the haloalkaliphilic genera *Natronobacterium* and *Natronococcus*, which show very similar patterns and yet appear only distantly related to each other and the other halophiles (Tindall *et al.*, 1984; Ross *et al.*, 1984) (Fig. 1). The diether core lipid composition of all strains within each group is identical (Ross *et al.*, 1984).

Phenotypic differences between DNA–rRNA homology groups are in most cases not clear cut (Table 1). Environmental pressures in the extreme conditions of salt pans and soda lakes may limit variety in biochemical and physiological processes, reflected in relative biochemical inactivity in most strains examined (Colwell *et al.*, 1979; Gibbons, 1974). Some phenotypic differences do exist between the RNA homology groups, as can be seen in Table 1, although the number of biochemical traits is small and a greater divergence may emerge as more biochemical data is accumulated.

DNA homologies between strains within rRNA homology groups are $>32\%$ but high DNA homologies also exist between strains from different rRNA homology groups (Table 3). Sapienza & Doolittle (1982) have shown the presence of large numbers of repeat sequences in the genomes of *Hb. halobium* and *Hb. volcanii*, some of which are common to both species. This would significantly increase the homology between strains even though they lie in different rRNA homology groups. If these repeat sequences are present in all extreme halophiles, overall DNA homologies may be higher than expected. Additionally, the reassociations were performed at the optimal hybridization temperature which allows partial sequence homologies to be measured giving higher overall values (Garvie *et al.*, 1981). The presence of similar-sized plasmids in most strains may also produce further sequence homology between apparently unrelated strains. Furthermore, Pfeifer *et al.* (1981) have shown that extensive plasmid sequences can be found in the chromosomal DNA of strains that do not possess plasmids. The interpretation of DNA–DNA homology studies, whether the cross-matching of plasmid-bearing strains, non plasmid-bearing strains or plasmid and non plasmid-bearing strains, must thus be treated with caution. DNA–rRNA hybridization studies performed with total DNA are not subject to the same problems of interpretation. We believe that it is significant that high homologies ($>45\%$) are to be seen only within DNA–rRNA groups.

Given the proposal of the two genera *Natronococcus* and *Natronobacterium* (Tindall *et al.*, 1984), then the other rRNA groups shown here are probably of equivalent taxonomic rank. The results presented here agree well with groups obtained by 5S rRNA sequence comparisons of these strains (G. E. Fox, personal communication). Groupings at suprageneric level are uncertain using DNA–rRNA hybridization data and detailed 16S rRNA oligonucleotide cataloguing will be necessary for such definition. A new taxonomy for the archaebacterial halophiles comparable in depth to those taxonomies currently accepted for the other archaebacteria is overdue.

We are grateful to Dr G. E. Fox for invaluable advice during the compilation of this paper. H. N. M. R. acknowledges with thanks the award of an SERC research studentship.

REFERENCES

BALCH, W. E., FOX, G. E., MAGRUM, L. J., WOESE, C. R. & WOLFE, R. S. (1979). Methanogens: re-evaluation of a unique biological group. *Microbiological Reviews* **43(2)**, 260–296.

COLWELL, R. R., LITCHFIELD, C. D., VREELAND, R. H., KIEFER, L. A. & GIBBONS, N. E. (1979). Taxonomic studies of red halophilic bacteria. *International Journal of Systematic Bacteriology* **29**, 379–399.

CROSA, J. H., WILLIAMS, B. L., JORGENSEN, J. J. & EVANS, C. A. (1979). Comparative study of deoxyribonucleic acid homology and physiological characteristics of *Peptococcus saccharolyticus*. *International Journal of Systematic Bacteriology* **29**, 328–332.

DE LEY, J. & DE SMEDT, J. (1975). Improvements of the membrane filter method for DNA–rRNA hybridization. *Antonie van Leeuwenhoek* **36**, 287–307.

DE ROSA, M., GAMBACORTA, A., NICOLAUS, B., ROSS, H. N. M., GRANT, W. D. & BU'LOCK, J. D. (1982). An asymmetric archaebacterial diether lipid from alkaliphilic halophiles. *Journal of General Microbiology* **128**, 343–348.

DE ROSA, M., GAMBACORTA, A., NICOLAUS, B. & GRANT, W. D. (1983). A $C_{25}C_{25}$ diether core lipid from archaebacterial haloalkaliphiles. *Journal of General Microbiology* **129**, 2333–2337.

DE SMEDT, J. & DE LEY, J. (1977). Intra- and intergeneric similarities of *Agrobacterium* ribosomal ribonucleic acid cistrons. *International Journal of Systematic Bacteriology* **27**, 222–240,

ELAZARI-VOLCANI, B. (1957). The genus *Halobacterium*. In *Bergey's Manual of Determinative Bacteriology*, 7th edn, pp. 207–212. Edited by R. S. Breed, E. G. D. Murray & N. R. Smith. Baltimore: Williams & Wilkins.

EVANS, R. W., KUSHWAHA, S. C. & KATES, M. (1980). The lipids of *Halobacterium marismortui*, and extremely halophilic bacterium from the Dead Sea. *Biochimica et biophysica acta* **619**, 533–544.

FORMISANO, M. (1962). Richerche sul "calore rosso" della pelli salate: II. Isolamento e caratterizzazione degli agent microbici causant l'alterazione. *Bolletini della R. Stazione sperimentale per l'industria delle Pelli e delle materie concianti* **38**, 183–213.

FOX, G. E., STACKEBRANDT, E., HESPELL, R. B., GIBBON, J., MANILOFF, J., DYER, T. A., WOLFE, R. S., BALCH, W. E., TANNER, R. S., MAGRUM, L. J., SABLEN, L. B., BLAKEMORE, R., GUPTA, R., BONEN, L., LEWIS, B. J., STAHL, D. A., LUEHRSEN, K. R., CHEN, K. N. & WOESE, C. R. (1980). The phylogeny of prokaryotes. *Science* **209**, 457–463.

GARVIE, E. I., FARROW, J. A. E. & PHILLIPS, B. A. (1981). A taxonomic study of some strains of streptococci which grow at 10 °C but not at 45 °C including *Streptococcus lactis* and *Streptococcus cremoris*. *Zentralblatt für Bakteriologie und Hygiene* (Abteilung Originale C) **2**, 151–165.

GIBBONS, N. E. (1974). The family Halobacteriaceae. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 269–272. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.

GONZALEZ, C., GUTIERREZ, C. & RAMIREZ, C. (1978). *Halobacterium vallismortis* sp. nov. An amylolytic and carbohydrate-metabolizing, extremely halophilic bacterium. *Canadian Journal of Microbiology* **24**, 710–715.

JAVOR, B., REQUADT, C. & STOECKENIUS, W. (1982). Box-shaped halophilic bacteria. *Journal of Bacteriology* **151**, 1532–1542.

KADO, C. I. & LIU, S.-T. (1981). Rapid procedure for detection and isolation of large and small plasmids. *Journal of Bacteriology* **145**, 1365–1373.

KOCUR, M. & HODGKISS, W. (1973). Taxonomic status of the genus *Halococcus* Schoop. *International Journal of Systematic Bacteriology* **23**, 151–156.

KUSHWAHA, S. C., KATES, M., JUEZ, G., RODRIGUEZ-VALERA, F. & KUSHNER, D. J. (1982). Polar lipids of an extremely halophilic strain (R4) isolated from salt pans in Spain. *Biochimica et biophysica acta* **711**, 19–25.

LOCHHEAD, A. G. (1934). Bacteriological studies on the red discolouration of salted hides. *Canadian Journal of Research* **10**, 275–286.

MANDEL, M., SCHILDKRAUT, C. L. & MARMUR, J. (1968). Use of CsCl density gradient analysis for determining the guanine plus cytosine content of DNA. *Methods in Enzymology* **76**, 184–195.

MOORE, R. L. & MCCARTHY, B. J. (1969). Base sequence homology and renaturation studies of the deoxyribonucleic acid of extremely halophilic bacteria. *Journal of Bacteriology* **99**, 255–262.

MULLAKHANBHAI, M. F. & LARSEN, H. (1975). *Halobacterium volcanii* spec. nov., a Dead Sea *Halobacterium* with a moderate salt requirement. *Archives of Microbiology* **104**, 207–214.

NICHOLSON, D. E. & FOX, G. E. (1983). Molecular evidence for a close phylogenetic relationship between box-shaped halophilic bacteria, *Halobacterium vallismortis* and *Halobacterium marismortui*. *Canadian Journal of Microbiology* **29**, 52–60.

OREN, A. (1983). *Halobacterium sodomense* sp. nov. a Dead Sea *Halobacterium* with an extremely high magnesium requirement. *International Journal of Systematic Bacteriology* **33**, 381–386.

PAYNE, J. I., SEHGAL, S. N. & GIBBONS, N. E. (1960). Immersion refractometry of some halophilic bacteria. *Canadian Journal of Microbiology* **6**, 9–15.

PETTER, H. F. M. (1931). On bacteria of salted fish. *Proceedings of the Academy of Science (Amsterdam)* **34**, 1417–1423.

PFEIFER, F., WEIDINGER, G. & GOEBEL, W. (1981). Characterization of plasmids in halobacteria. *Journal of Bacteriology* **145**, 369–374.

RODRIGUEZ-VALERA, F., JUEZ, G. & KUSHNER, D. J. (1983). *Halobacterium mediterranei* spec. nov., a new carbohydrate-utilizing extreme halophile. *Systematic and Applied Microbiology* **4**, 369–381.

ROSS, H. N. M. (1982). *The extremely halophilic archaebacteria*. PhD thesis, Leicester University, UK.

ROSS, H. N. M., COLLINS, M. D., TINDALL, B. J. & GRANT, W. D. (1981). A rapid procedure for the detection of archaebacterial lipids in halophilic bacteria. *Journal of General Microbiology* **123**, 75–80.

ROSS, H. N. M., GRANT, W. D. & HARRIS, J. E. (1984). *Lipids in Archaebacterial Taxonomy*. In *Chemical Methods in Bacterial Systematics*. Edited by M. Goodfellow & D. E. Minnikin, New York: Academic Press (In the Press).

SAPIENZA, C. & DOOLITTLE, W. F. (1982). Unusual physical organization of the *Halobacterium* genome. *Nature, London* **295**, 384–389.

SKERMAN, V. B. D., MCGOWAN, V. & SNEATH, P. H. A. (eds) (1980). Approved Lists of Bacterial Names. *International Journal of Systematic Bacteriology* **30(1)**, 225–420.

SOLIMAN, G. S. H. & TRÜPER, H. G. (1982). *Halobacterium pharaonis* sp. nov., a new extremely haloalkaliphilic archaebacterium with low magnesium requirement. *Zentralblatt für Bakteriologie und Hygiene* (Abteilung Originale C) **3**, 318–329.

STACKEBRANDT, E., WUNNER-FUSSL, B., FOWLER, V. & SCHLEIFER, K. H. (1981). Deoxyribonucleic acid homologies and ribonucleic acid similarities among spore-forming members of the order Actinomycetales. *International Journal of Systematic Bacteriology* **31**, 420–431.

TINDALL, B. J., MILLS, A. A. & GRANT, W. D. (1980). An alkalophilic red halophilic bacterium with a low magnesium requirement from a Kenyan soda lake. *Journal of General Microbiology* **116**, 257–260.

TINDALL, B. J., ROSS, H. N. M. & GRANT, W. D. (1984). *Natronobacterium* gen. nov. and *Natronococcus* gen. nov., two new genera of haloalkaliphilic archaebacteria. *Systematic and Applied Microbiology* **5**, 41–57.

TOMLINSON, G. A. & HOCHSTEIN, L. I. (1976). *Halobacterium saccharovorum* sp. nov., a carbohydrate-metabolizing, extremely halophilic bacterium. *Canadian Journal of Microbiology* **22**, 587–591.

TU, J., PRANGISHVILLI, D., HUBER, H., WILDGRUBER, G., ZILLIG, W. & STETTER, K. O. (1982). Taxonomic relations between archaebacteria including 6 novel genera examined by cross hybridization of DNAs and 16S RNAs. *Journal of Molecular Evolution* **18**, 109–114.

WILDGRUBER, G., THOMM, M., KONIG, H., OBER, K., RICCHIUTO, T. H. & STETTER, K. O. (1982). *Methanoplanus limicola*, a plate shaped methanogen representing a novel family, the Methanoplanaceae. *Archives of Microbiology* **132**, 31–36.