PHOTOTROPHIC BACTERIA

FROM KENYAN SODA

LAKES

by

B.J. TINDALL, B.Sc.

A thesis submitted for the degree of
Doctor of Philosophy in the Faculty
of Science, at the University of Leicester.

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DECLARATION

The work in this thesis was carried out by the author, unless otherwise stated in the text, during the period September 1976 to September 1979, under the supervision of Dr.W.D.Grant, in the Department of Microbiology, University of Leicester. This thesis is submitted for the degree of Ph.D. at the University of Leicester, and has not been submitted in full, or in part for any other degree.

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November, 1980

PHOTOTROPHIC BACTERIA FROM KENYAN SODA LAKES.

Ph.D. Thesis

by

B.J.Tindall.

The soda lakes of the Rift Valley, Kenya, are a group of closed-basin, alkaline, saline lakes. The most extreme example in the Rift Valley is Lake Magadi, where semi-solid trona deposits fill the lake, and are mined commercially. At present the lakes are isolated from one another, although this may not have always been the case.

Microbiological investigations of these lakes have shown that characteristic phototrophic bacteria and extremely halophilic archaebacteria occur abundantly under certain conditions. The phototrophic bacteria observed at the lakes include Chloroflexus-like organisms of the hot springs, and organisms resembling members of the genus Ectothiorhodospira. Ectothiorhodospira-like organisms isolated from the lakes were similar to Ectothiorhodospira mobilis and Ectothiorhodospira shaposnikovii, in their physiology and biochemistry, but could be divided into two groups on the basis of the occurrence of gas vacuoles in one group. These organisms, which deposit sulphur outside the cell during the photooxidation of sulphide to sulphate, constitute an ecological and physiological group adapted to life in the soda lakes. The taxonomic relationships of the organisms isolated from the lakes is discussed, including the taxonomic status of the gas vacuolate and extremely halophilic organisms of the Ectothiorhodospira group.

The extremely halophilic archaebacteria, isolated from Lake Magadi were similar to known archaebacterial extreme halophiles, in their possession of ether linked lipids, NaCl requirement, and the lack of muramic acid in the cell wall, but were different in

other respects, including adaption to low Mg^{2+} concentrations and pH 9.0-10.0. The organisms constitute an ecological and physiological group within the extremely halophilic archaebacteria, and their taxonomic relationship is discussed.

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INTRODUCTION

1. GEOLOGY AND GEOCHEMISTRY OF SALINE LAKES IN KENYA.

1.1. Geology of the Eastern African Rift Valley, Kenya.

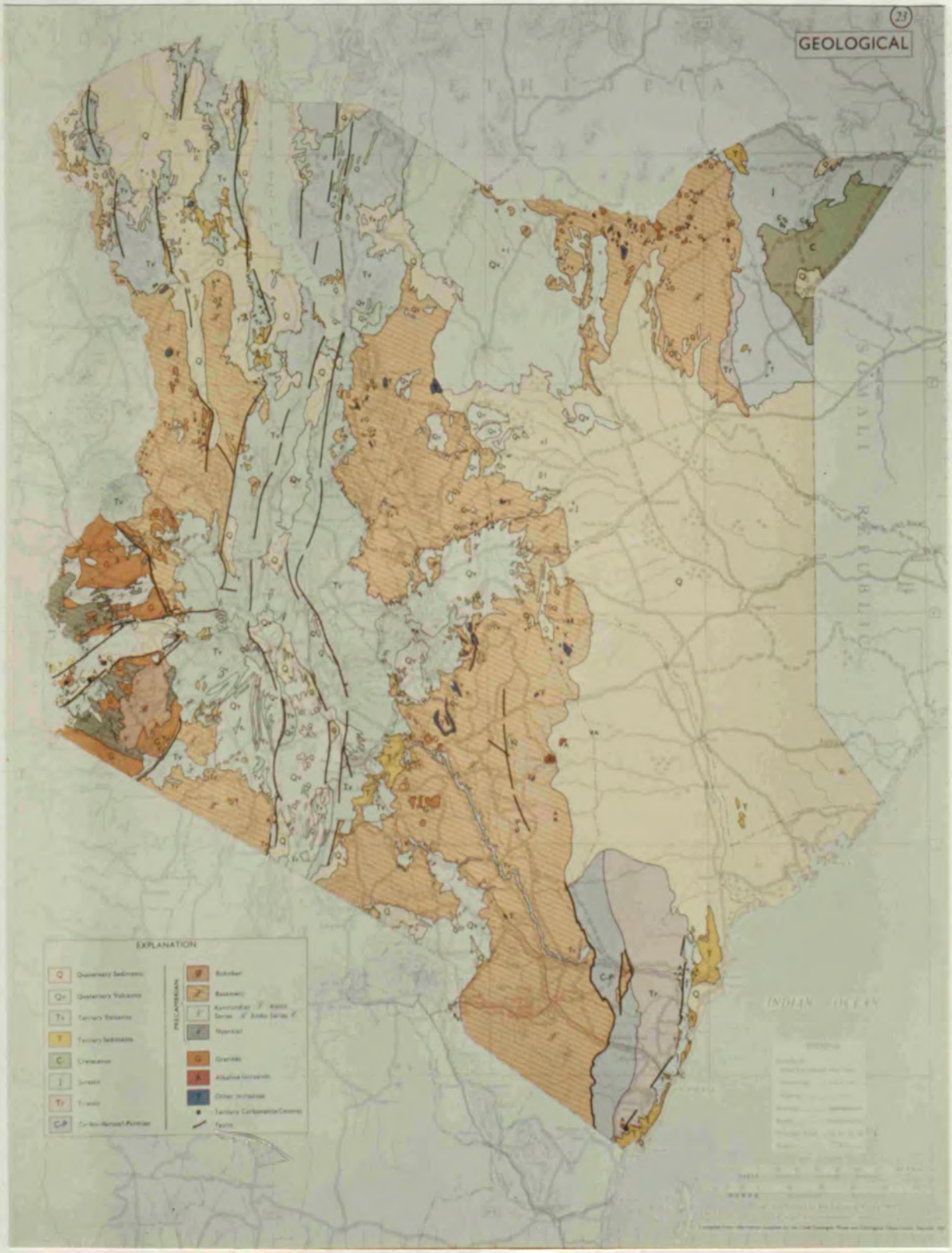
The geological record of Kenya shows that significant topographical changes have taken place (figure 1), due to continental drift and epeirogenic movements. The Rift Valley, being a region of crustal thinning and extension, is an active volcanic, faulting, and earthquake zone (Baker et al., 1972).

The basement, metamorphic, and igneous rocks of Pre-Cambrian and lower Palaeozoic origin formed fold mountains (figure 2a, 3), occupying Eastern Africa for most of the Palaeozoic (Baker, 1958; Baker et al., 1972; McCall, 1967; Thompson & Dodson, 1963). Denudation eventually gave rise to the Eastern African Plateau (figure 2b), which in part covers western and central Kenya. Dome initiation, by uplifting of the plateau, began in the Cretaceous (figures 2c, 3), followed by volcanism, particularly in central Kenya, where the uplift was greatest (Saggerson & Baker, 1965). Erosion and sediment deposition, largely tuffs or of lacustrine origin were also evident before a second upwarping of the dome (figure 2d), which began in the Miocene (Baker et al., 1972; Thompson & Dodson, 1963). At about the same time there was subsidence in the north-west of Kenya, giving way to faulting in north and central Kenya (Baker et al., 1972; Saggerson & Baker, 1965; Thompson & Dodson, 1963). Volcanic eruptions followed from both central and fissure sources, the lavas being largely olivine basalts and phonolites (Thompson & Dodson, 1963). The third and greatest uplift occured near the end of the Tertiary, which caused the margins of the rift to be warped down (figures 2e, 3), forming a true graben for the first time (Baker, 1958; Baker et al., 1972; McCall, 1967; Thompson & Dodson, 1963).

Following initiation of the Rift Valley there was further volcanism along the floor of the graben. The lava flows were

Figure 1. Geological map of Kenya.

The predominant geological features of Kenya are illustrated. The major fault lines (black lines) and the surface rock formations (see inset explanation in map) are shown .



(from Geol. Surv. Kenya, 1978)

Figure 2. Formation of the Rift Valley, Kenya. (Thompson and Dodson, 1963; Baker, 1958; McCall, 1967).

a. Fold mountains form over East Africa during the Palaeozoic.

b. Denudation of the fold mountains results in the formation of the Eastern African Plateau.

c. Domal uplift initiated in the Cretaceous, accompanied by volcanism. Greatest uplift over central Kenya.

d. Second uplift of the dome, begining in the Miocene, and caused minor faulting, particularly in the region of the present Turkana depression. Volcanism gave rise to alkaline larvas.

e. Greatest uplift of dome occurred at the end of the Tertiary, and resulted in the formation of a graben, accompanied by extensive volcanism.

f. Infilling of the central trough by volcanism and lacustrine deposits, interupted by faulting in the Pliocene, further developing the Rift Valley.

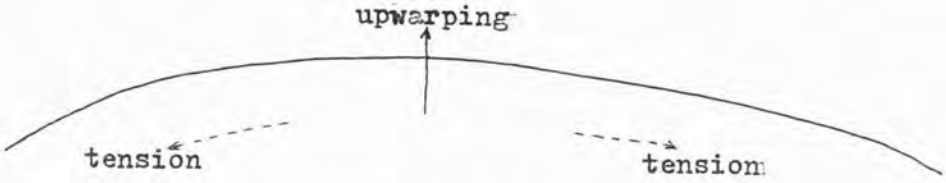
g. Last major period of faulting occurred in the Pleistocene along existing fault lines. In the south grid faulting further fragmented the rift floor. Extensive lacustrine deposits formed in the ensuing period.

h. In the Upper Pleistocene minor faulting caused the floor of the rift, particularly in the central region, to be dropped, creating the present day lake beds, and determining the major topographical features of the present day Rift Valley.

a.

b.

c.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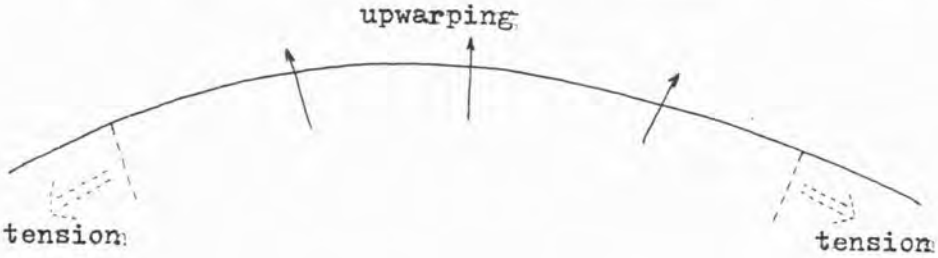
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Age of the Earth	Era	Period	Epoch	Duration
present day		Quaternary	Recent Pleistocene	2 m.y.
dominance of man				
		Tertiary	Pliocene	5 m.y.
	Kainozoic		Miocene	19 m.y.
			Oligocene	12 m.y.
			Eocene	16 m.y.
			Palaeocene	10 m.y.
4,500 m.y.				
extinction of dinosaurs		Cretaceous	Upper	35 m.y.
			Lower	36 m.y.
	Mesozoic	Jurassic		57 m.y.
dominance of dinosaurs		Triassic		33 m.y.
4,350 m.y.				
		Permain		55 m.y.
		Carboniferous	Upper	45 m.y.
			Lower	20 m.y.
	Palaeozoic	Devonian		65 m.y.
		Silurian		30 m.y.
		Ordovician		90 m.y.
appearance of invertebrates		Cambrian		40 m.y.
4,000 m.y.				
evolution of life	Pre-Cambrian			4,000 m.y.
origin of the Earth				

Figure 3. Major divisions of geological time (after Eagar, 1978)

intercalated with sedimentary tuffs and lacustrine deposits. The extent and nature of the lake deposits reflects the geological and climatic changes that were taking place at that time (Baker, 1958; Butzer et al., 1972; McCall, 1967; Richardson & Richardson, 1972; Thompson & Dodson, 1963). A second major faulting period occurred in the Pliocene and further developed the Rift Valley (figures 2f, 3). The ensuing volcanics were largely phonolites and trachytes (Baker, 1958; Baker et al., 1972; McCall, 1967; Thompson & Dodson, 1963). It is the preponderance of these alkaline lavas (i.e. rich in Na^+ and K^+) in the area that has caused the lake waters to show an alkaline trend (Eugster, 1970; Eugster & Hardie, 1978). During this period there was significant and fairly extensive lacustrine sediments formed, such as the Oloronga Lake beds and the chert formations of Lake Magadi (Baker, 1958).

A last major faulting period occurred in the Pleistocene along existing lines, further developing the escarpments, which are a prominant feature (figure 2g). In the south there was grid faulting, which initiated a closer network of faults (Baker, 1958; Baker et al., 1972). Large lacustrine deposits formed in this period and are characterised by the Kariandus diatomite and the Olorgasaille Lake beds (Baker, 1958; Thompson & Dodson, 1963).

Minor faulting in the upper Pleistocene dropped the floor of the Rift Valley along existing fault lines and also created new faults (figures 2h, 3). This closely spaced fracturing helped to develop the existing drainage basins, lowering the beds of the existing lakes, as may be seen by the exposed lake terraces associated with the present day lakes (Baker, 1958; Butzer et al., 1972; McCall, 1967; Thompson & Dodson, 1963). Minor faulting and volcanism has continued to the present day (Baker et al., 1972). This combination of volcanism and faulting has divided the Rift Valley into a series of closed-basin

drainage areas. The major topographical features delineating the drainage basins are fault lines, lava flows, or caldera volcanoes (figures 1, 5) (Baker et al., 1972).

1.2. Formation of the lake brines.

A typical freshwater lake has an inflow equal to its outflow, evaporation being an insignificant part of the outflow. When the rate of evaporation increases to become a significant part of the outflow, or becomes the major outflow, the solute load increases and the lake becomes saline (Eugster & Hardie, 1978). The final composition of the brine is determined by chemical, climatic, and geological parameters (Hardie & Eugster, 1971; Eugster & Hardie, 1978).

The formation of drainage basins with restricted outflow, other than evaporation, may be brought about by block faulting or thrusting, volcanic cratering, or damning by sedimentary processes such as land slide, lava flow, or vegetable detritus (Eugster & Hardie, 1978). These conditions often lead to the formation of closed-basin drainage systems, where under suitable conditions saline lakes form. The principal climatic influences are temperature and rainfall. A saline lake usually forms in desert or semi-arid regions, where the rate of evaporation is high. The geochemistry of the drainage basin influences the chemical composition of the leaching water, determining the relative composition of solutes. Once the water begins to evaporate and the solutes concentrate, the more insoluble compounds are precipitated. Continued evaporation eventually leads to saturation of the brine with the most soluble compounds. The nature of such precipitations is one of the major controlling factors in the final nature of the brine (Eugster & Hardie, 1978; Hardie & Eugster, 1971). The chemical parameters which affect the brine composition of saline lakes have been investigated in a series of computer-modelling studies based upon field data, which although failing to cover all aspects, generally

accounts for the relative composition of the brines examined (Garrels & MacKenzie, 1967; Hardie & Eugster, 1971).

Rock formations are largely, although not exclusively, complex silicates containing Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}, Al^{3+}, and Fe^{3+} (Gilluly et al., 1968). The action of water charged with CO_2 brings about the weathering of the rock in a number of ways, including:

$$2NaAlSi_3O_8 + 2CO_2 + 11H_2O \rightleftharpoons Al_2Si_3O_5(OH)_4 + 2Na^+ + 2HCO_3^- + 4H_4SiO_4$$

albite kaolinite

$$2Mg_2SiO_4 + 3H_2O + 2CO_2 \rightleftharpoons Mg_3Si_2O_5(OH)_4 + Mg^{2+} + 2HCO_3^-$$

forsterite chrysotile

$$Ca.Mg(CO_3)_2 + 2H_2O + CO_2 \rightleftharpoons 4HCO_3^- + Mg^{2+} + Ca^{2+}$$

dolomite

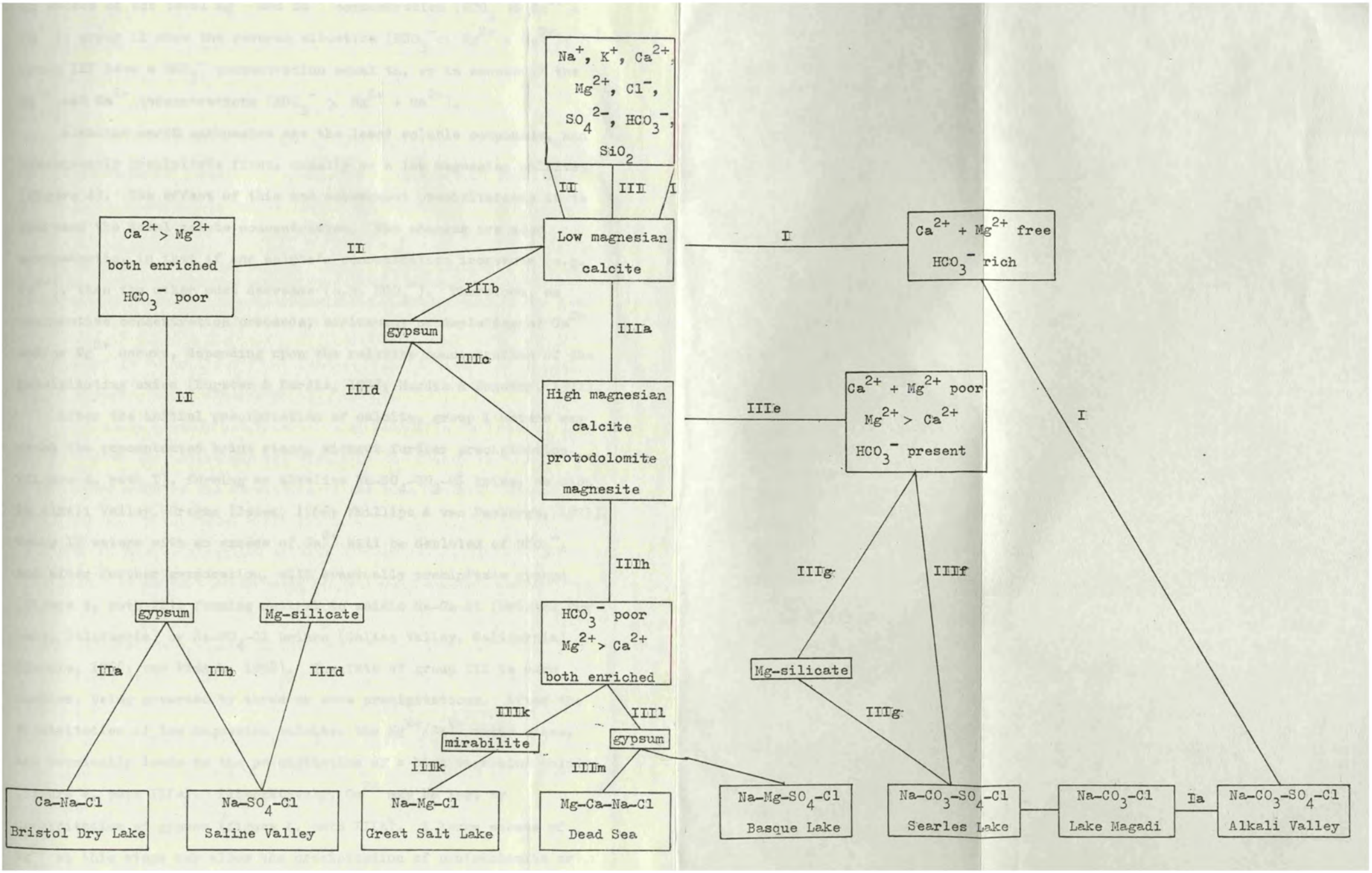
Free ions may be leached from the rocks as a result of the weathering action, the major ions being Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, HCO_3^-, SO_4^{2-}, and SiO_2. Relative concentrations are determined by the nature of the rock, weathering properties of the rock, weathering reactions, and the drainage conditions. HCO_3^- is the major anion in dilute groundwaters and is formed by solution of CO_2 from the atmosphere and respiration. Cl^- and SO_4^{2-} may also be derived from rainwater, although halite and gypsum dissolution are other possible sources (Eugster & Hardie, 1978).

The most important stages in the formation of a concentrated brine are the precipitations of insoluble carbonates, sulphates, and silicates through evaporative concentration. The relative concentration of ions, the solubility of the products formed, and the concentration of the insoluble precipitates formed have considerable importance in determining the final composition of the brine (Eugster, 1970; Eugster & Hardie, 1978; Hardie & Eugster, 1971).

Dilute waters may be divided into three groups (Eugster & Hardie, 1978). Group I waters (figure 4) have a HCO_3^- concentration greatly

Figure 4. Formation of saline lake waters. (Hardie and Eugster, 1970; Eugster and Hardie, 1978).

The physio-chemical parameters involved in the formation of saline lakes are illustrated. The major ions leached from rocks, and present in the inflowing waters, are Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, SO_4^{2-}, and SiO_2. Group I waters are enriched for HCO_3^- over Ca^{2+} and Mg^{2+}, group II waters are enriched for Ca^{2+} and Mg^{2+} over HCO_3^-, and group III waters have HCO_3^- slightly in excess or in equal concentration to Ca^{2+} and Mg^{2+}. Evaporative concentration further concentrates the ions, and selective precipitations occur, which determine the final nature of the lake water. Lake brines resulting from the various pathways are exemplified by the lakes given at the end of the relavent pathway.


Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, HCO_3^-, SiO_2
II
III
I
$Ca^{2+} > Mg^{2+}$ both enriched HCO_3^- poor
II
Low magnesian calcite
I
$Ca^{2+} + Mg^{2+}$ free HCO_3^- rich
IIIb
gypsum
IIIc
IIIa
II
IIId
High magnesian calcite protodolomite magnesite
IIIe
$Ca^{2+} + Mg^{2+}$ poor $Mg^{2+} > Ca^{2+}$ HCO_3^- present
I
IIIh
IIIg
IIIf
gypsum
Mg-silicate
HCO_3^- poor $Mg^{2+} > Ca^{2+}$ both enriched
Mg-silicate
IIa
IIb
IIId
IIIk
IIIl
IIIg
mirabilite
gypsum
IIIk
IIIm
Ca-Na-Cl Bristol Dry Lake
$Na-SO_4-Cl$ Saline Valley
Na-Mg-Cl Great Salt Lake
Mg-Ca-Na-Cl Dead Sea
$Na-Mg-SO_4-Cl$ Basque Lake
$Na-CO_3-SO_4-Cl$ Searles Lake
$Na-CO_3-Cl$ Lake Magadi
Ia
$Na-CO_3-SO_4-Cl$ Alkali Valley

in excess of the total Mg^{2+} and Ca^{2+} concentration ($HCO_3^- \gg Mg^{2+} + Ca^{2+}$); group II show the reverse situation ($HCO_3^- \ll Mg^{2+} + Ca^{2+}$); group III have a HCO_3^- concentration equal to, or in excess of the Mg^{2+} and Ca^{2+} concentrations ($HCO_3^- \geqslant Mg^{2+} + Ca^{2+}$).

Alkaline earth carbonates are the least soluble compounds, and consequently precipitate first, usually as a low magnesian calcite (figure 4). The effect of this and subsequent precipitations is to decrease the total solute concentration, The changes are also antipathetic, in that if one solute's concentration increases (e.g. Ca^{2+}), then the other must decrease (e.g. HCO_3^-). Therefore, as evaporative concentration proceeds, enrichment or depletion of Ca^{2+} and/or Mg^{2+} occurs, depending upon the relative concentration of the precipitating anion (Eugster & Hardie, 1978; Hardie & Eugster, 1971).

After the initial precipitation of calcite, group I waters may reach the concentrated brine stage, without further precipitation (figure 4, path I), forming an alkaline Na-SO_4-CO_3-Cl brine, as seen in Alkali Valley, Oregon (Jones, 1966; Phillips & van Denburgh, 1971). Group II waters with an excess of Ca^{2+} will be depleted of HCO_3^-, and after further evaporation, will eventually precipitate gypsum (figure 4, path II), forming neutral to acidic Na-Ca-Cl (Bristol Dry Lake, California) or Na-SO_4-Cl brines (Saline Valley, California) (Hardie, 1968; ver Planck, 1958). The fate of group III is more complex, being governed by three or more precipitations. After the precipitation of low magnesian calcite, the Mg^{2+}/Ca^{2+} ratio rises, and eventually leads to the precipitation of a high magnesian calcite (figure 4, path IIIa). Alternatively, Ca^{2+} may be lost by precipitation of gypsum (figure 4, path IIIb). A large excess of Mg^{2+} at this stage may allow the precipitation of protodolomite or magnesite. The resulting brines will be either HCO_3^- or Mg^{2+}/Ca^{2+} enriched. HCO_3^- enriched waters result in the formation of alkaline

$Na-CO_3-SO_4-Cl$ brines (figure 4, path IIIe, f), often via the precipitation of magnesium silicate (figure 4, path IIIg). The precipitation of sulphates from Mg^{2+}/Ca^{2+} rich water as gypsum (figure 4, path IIIl), or the freezing out of mirabilite ($Na_2SO_4.10H_2O$) from Mg^{2+}/Ca^{2+} poor water (figure 4, path IIIk) may produce neutral to acidic Na-Mg-Cl (Great Salt Lake, Utah), Mg-Ca-Na-Cl (Dead Sea, Israel), or $Mg-Na-SO_4-Cl$ brines (Hot Lake, Washington) (Livingstone, 1963; Neev & Emery, 1967; Whitehead & Feth, 1961). Alkaline $Na-CO_3-Cl$ brines such as those found at Lake Magadi, Kenya may arise from the modification of $Na-CO_3-SO_4-Cl$ brines derived from either group I or III waters by removal of the sulphate by bacterial sulphate reduction or by sorption to soil particles (Abd-el-Malek & Rizk, 1963a; 1963b; 1963c; Eugster, 1970; Jones B et al., 1977).

Once these primary precipitations of insoluble carbonates, sulphates, and silicates has determined the nature of the brine, further evaporation leads to the deposition of the more soluble compounds, which may modify the brine still further.

1.3. The chemical composition of East African lake waters.

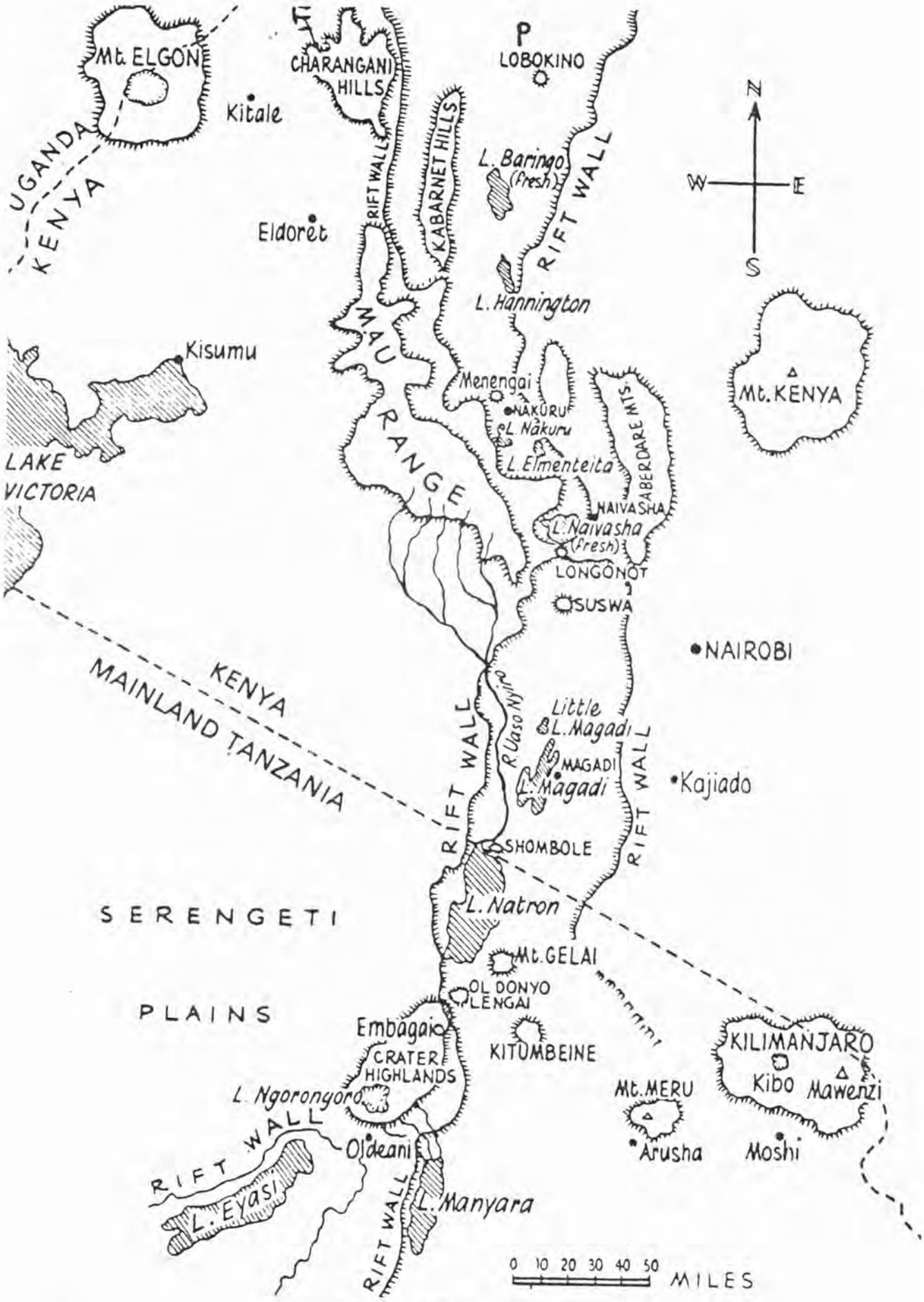
East African lake waters show an extremely varied chemical composition. They may arise from volcanic cratering, the formation of grabens or shallow tectonic basins, or by damning by lava flow (Hutchinson, 1957). The degree of outflow strongly influences the chemical composition of the lakes, those with the largest outflow being the most dilute (Hutchinson, 1957; Talling & Talling, 1965). The lakes have been described according to their method of formation and their chemical composition (Hutchinson, 1957; Talling & Talling, 1965). Both methods are useful for describing a lake, but it is ultimately the chemical composition which influences the biology. A convenient classification of the lake waters has been based on the conductivity, a similar method to that employed for temperate

freshwater lakes (Ratcliffe, 1977; Talling & Talling, 1965). This method has the advantage of being related to both ionic composition and the total alkalinity.

East African Lakes (figure 5), which most probably belong to Eugster and Hardie's group I (Eugster & Hardie, 1978), are arbitarily divided into three classes. Class I consists of low conductivity waters, less than 600mho, and includes lakes Victoria, Baringo, and Naivasha (figure 5) (Beadle, 1932; Jenkin, 1932; Talling & Talling, 1965). All these lakes have some form of outflow which is large enough to prevent the formation of a saline lake. Their total alkalinity is not high and the pHs are between 7.0 and 9.0. Class II contains those lakes with a more restricted outflow and higher conductivity, 600mho to 6,000mho. The lakes are typically saline and have a moderate alkalinity, with pHs between 9.0 and 10.0. Lake Turkana (figure 1) is an example of this class, but is atypical in that it is a closed-basin lake (Beadle, 1932). The very saline lakes of class III have conductivities in excess of 6,000mho, and are described as alkaline, saline, or soda lakes, due to the preponderance of bicarbonate and carbonate among the anions. In extreme cases the lakes may form solid deposits, which is mainly trona ($NaHCO_3.Na_2CO_3.2H_2O$, sodium sesquicarbonate) (Baker, 1958; Eugster & Hardie, 1978). Lakes Bogoria, Elmentieta, Magadi, and Naivasha Crater Lake (figure 5) belong to this class (Baker, 1958; Beadle, 1932; Jenkin, 1932; McCall, 1967; Thompson & Dodson, 1963). The pH of these lakes is usually in excess of 10.0 and the upper limit is greater than 11.0. The total salinity varies from 5% to in excess of 30%. One of the most extreme examples, which is actively depositing trona and common salt, is Lake Magadi, Kenya, which is similar to the lakes of the Wadi Natrun, Egypt (Baker, 1958; Imhoff et al., 1978b).

Figure 5. Map of the Rift Valley, Kenya.

The map of the Rift Valley shows the position of the lakes in relation to the main topographical features of the Rift. All lakes in the Rift Valley are soda lakes, unless otherwise marked (fresh). The major geological features of the area, fault lines and volcanos, are also illustrated. Lake Hannington is now called Lake Bogoria.



(from Brown, 1973)

1.4. Lake Magadi as an example of a soda lake.

Lake Magadi is an extreme example of a highly saline, alkaline (soda) lake. It has been studied largely because of the vast trona deposits which are mined commercially (Baker, 1958); there are also some similarities to the Eocene Green River formation of Colorado, Utah, and Wyoming (Eugster & Hardie, 1978). Although the brines of Lake Magadi can be distinguished from those of other soda lakes in the Rift Valley, Kenya, the factors which affect the brine composition are fairly typical of this region (Baker, 1958; Eugster, 1970; Jones B et al., 1977).

Lake Magadi lies in the floor of the Rift Valley 2° south of the Equator at an elevation of 600m above sea level, and covers an area of 40 sq miles (figure 5). To the west the Loita Hills and the Mau Escarpment create a rain shadow over the lake, which recieves a total of 10-20 inches of rainfall in the two rainy seasons. Evaporation is intense during the dry season, the average extremes of temperature being between 22°C and 34°C (Geol. Surv. Kenya, 1978).

The lake occupies a trough created in the Pleistocene plateau lavas by faulting, and is the lowest point in the southern part of the Rift Valley. The predominant rocks are of volcanic origin and consist mainly of alkaline trachyte type lavas (Baker, 1958). 100Km south of the Magadi trough is the active volcano Oldoinyo Lengai, which produces lavas rich in sodium carbonate, and has been assumed by some to be a major source of the trona deposits in the lake. The presence of the soda volcano can, however, be shown to be insignificant in the formation of the trona, although wind-blown ash may form a minor contribution (Eugster, 1970).

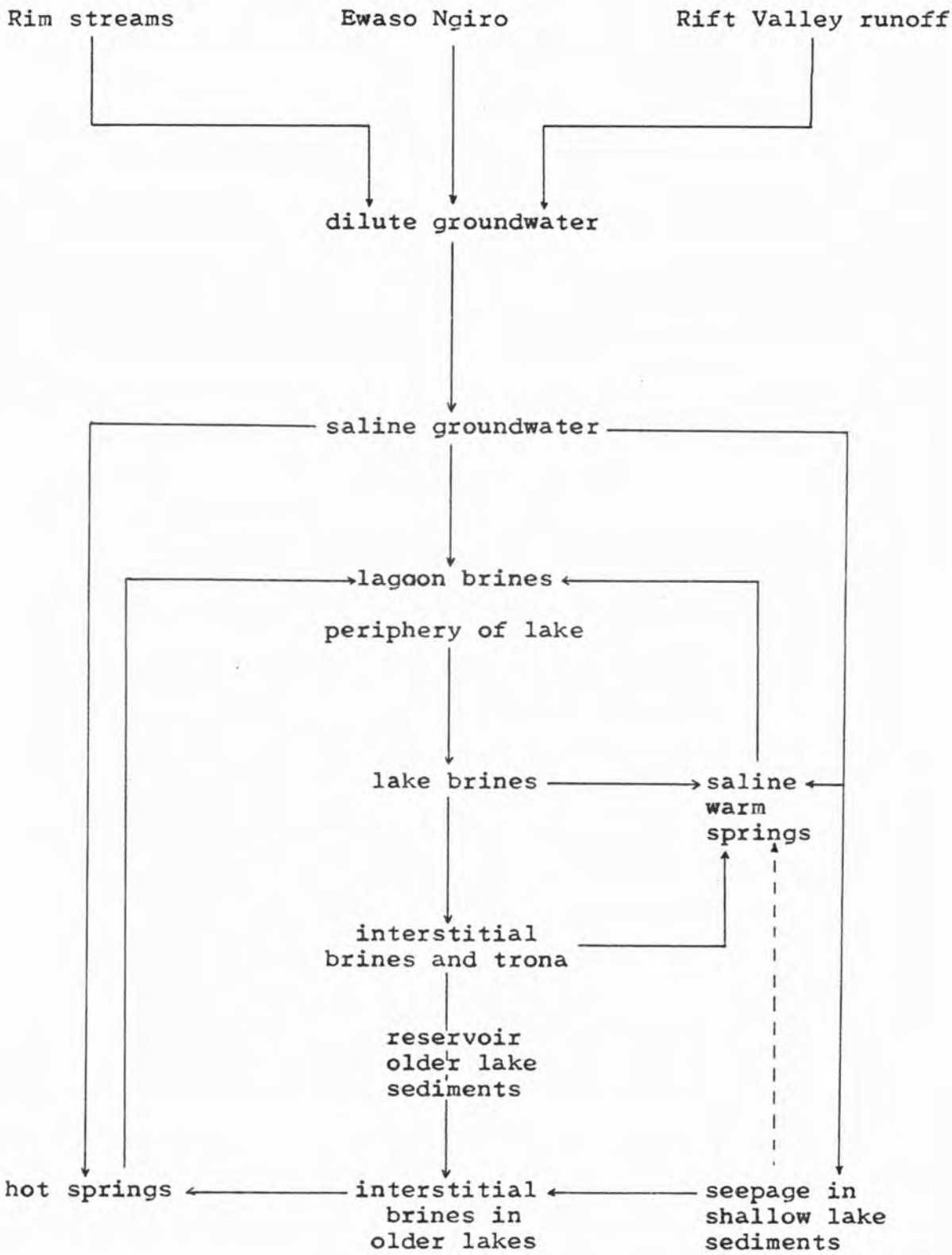
The catchment area for Lake Magadi is principally the Loita Hills and the Mau Escarpment (figure 5). It has also been postulated that subterranean outflow form Lake Naivasha and, therefore, indirectly

the Aberdare Range may also be involved (Baker, 1958; Eugster, 1970; Jones B et al., 1977). Direct stream or river flow is ephemeral, occuring in the rainy seasons, and most of the rivers which drain the Loita Hills and the Mau Escarpment are lost in the alluvial fans along the rift floor. Only the Ewaso Ngiro River is aseasonal, but it flows into Lake Natron, although water may be lost to the Magadi water table (Eugster, 1970; Eugster & Hardie, 1978; Jones B et al., 1977).

Drainage water, which leaches the bed rock, is low in Mg^{2+} and Ca^{2+} due to the preponderance of potassium and sodium lavas, and the absence of sedimentary calcium deposits (Baker, 1958; Eugster, 1970). The evolution of the brines has been followed in the inflowing waters (Eugster, 1970; Jones B et al., 1977). As in most dilute streamflows the surface water becomes rapidly saturated with calcite, and Ca^{2+} and Mg^{2+} are depleted as the dilute groundwaters are formed. Silica and sulphate are also lost in the first concentration stage. Almost complete removal of Ca^{2+} and Mg^{2+} occurs by the second evaporative stage, the formation of the saline groundwater. K^{+} is also lost rapidly at first through ion exchange with either the trachyte lava or Na-Al-silicates (Eugster & Jones, 1977). Solute bearing groundwater near the surface is drawn upwards by capillarity as surface water evaporates. An efflorescent crust forms on the ground if evaporation is complete, or the sub-surface water may increase its solute load if the rate of evaporation is slow. The efflorescent crust is composed of the more readily soluble compounds, and is, therefore, washed back into the groundwater by rainfall. This method of raising the solute load of sub-surface waters appears to be a significant process in the formation of the brines at Lake Magadi and elsewhere (Hsu & Siegenthaler, 1969; Jones B et al., 1977). Apart from the saline groundwater and ephemeral runoff which reaches the lake, hot

Figure 6. Formation of Lake Magadi brines (Jones B. et al., 1977).

The most dilute run-off leaches solutes from the rocks, and becomes more concentrated as it travels through the more arid parts of the Magadi water shed. The method by which the lake water becomes an alkaline brine is outlined in figure 4. The present model for the formation of the lake brines incorporates previous theories of recirculation (Baker, 1958) and subterranean reservoir (Parkinson, 1914). Water recirculates in the lake brines and saline warm, or hot spirings, the interstitial brines in the trona and older lake sediments acting as a reservoir.


Rim streams
Ewaso Ngiro
Rift Valley runoff
dilute groundwater
saline groundwater
lagoon brines
periphery of lake
lake brines
saline warm springs
interstitial brines and trona
reservoir older lake sediments
hot springs
interstitial brines in older lakes
seepage in shallow lake sediments

springs contribute to the brines, and are comparable in solute load to the saline groundwater (Eugster, 1970; Jones B et al., 1977). Further evaporation leads to the formation of the brines, which eventually become saturated with trona, accumulating at the rate of 1-3cm per year.

However, the mechanisms described above fails to account for the present extent of the trona, and a deep, saline subterranean reservoir has been postulated to be the main source of the trona (Parkinson, 1914). This theory too has its limitations, and the most recent studies suggest a recirculation theory (Baker, 1958; Eugster, 1970; Jones B et al., 1977). In this theory, it is postulated that the inflowing water contributes to the waters of the lake either directly or through mixing with the brines (figure 6). The surface waters of the lake are usually peripherally located lagoons, which concentrate by evaporation and mix with the lake brines. The lagoon brines are maintained by direct groundwater inflow, or by spring water. As the circulating brines pass through the deeper and older lake deposits they become modified through contact with inflowing groundwater and by precipitation. The lower brines are returned to the surface by the hot and saline warm springs. The brines found in the trona and older lake deposits, therefore, act as a reservoir within the lake's own sediments, rather than having a separate subterranean reservoir (figure 6).

2. MICROBIAL LIFE IN THE ALKALINE ENVIRONMENT.

A number of reviews have dealt with life in extreme environments and have included extremes of pH (Baas-Becking et al., 1960; Brock & Darland, 1970; Hewitt, 1957; Kushner, 1978a; Meadows, 1969; Vallentyne, 1963). Compared to other extreme environments there is relatively little known about life in the alkaline milieu (Langworthy, 1978). Much of the work on the extremes of pH has been restricted to the acidic environment, and much of the knowledge about the ecology of acid environments is the result of Brock's work (Brock, 1978) in Yellowstone National Park. Acidophiles are of economic importance in industry, since they can cause spoilage in some preserved foods, and have, therefore, received attention. There has been no extensive study of organisms living in the alkaline environment, although naturally occuring alkaline environments occur on most continents (Te-Pang, 1942). The terms alkalophile, alkalinophile, and alkaliphile have been used to describe organisms which are dependant upon an alkaline pH for growth and survival. The term alkalophile is the most commonly used, but Trüper (personal communication) has suggested that, because of its Arabic derivation, the term alkaliphile is correct.

A number of accounts of organisms living in a variety of alkaline environments have appeared, including rayon waste (Ikura & Horikoshi, 1977), indigo balls (Ohta et al., 1975), simulated Jovian environments (Deal et al., 1975), and alkaline springs (Schmidt & Cole, 1976; Souza et al., 1974; Souza & Deal, 1977). Most accounts have concentrated on the genus Bacillus. A large number of Bacillus strains have been isolated and maintained at high pH, while other strains are capable of adapting to optimal growth at an elevated pH (Langworthy, 1978). Bacillus pasteurii, B.alcalophilus, B.sphaericus, B.pantothenticus, and B.rotans are all obligate alkaliphiles, and Bacillus circulans and B.cereus are capable of adapting to the alkaliphilic way of life

(Chislett & Kushner, 1961; Kushner & Lisson, 1959; Langworthy, 1978). A large number of alkaliphilic or alkali-tolerant species of Bacillus have been isolated by Japanese workers, but they have not been fully characterised (Horikoshi, 1971a; 1971b; 1972; Horikoshi & Atsukawa, 1975; Ikura & Horikoshi, 1977; Kurono & Horikoshi, 1973; Nakamura & Horikoshi, 1976; Yamamoto et al., 1975; Boyer et al., 1973).

Apart from Bacillus spp., a number of other alkaliphilic or alkali-tolerant organisms have been isolated from alkaline environments, including Rhizobium spp. (Thimann, 1963; Allen & Holding, 1974), Thiobacillus spp. (Rupela & Tauro, 1973a; 1973b), Flavobacterium spp. (Souza et al., 1974), an unidentified Gram-variable, strictly anaerobic, spore forming rod (Souza et al., 1974), and a group of Gram-positive, non-spore forming, facultatively anaerobic rods (Gee et al., 1980). The most unusual organism isolated from the alkaline environment is the so called "living fossil", Kakabekia umbellata (Siegel & Giumarro, 1966), which grows in a semi-defined medium with a very high ammonia content. Some members of the enterococci and enterobacteria are characterised by their alkali-tolerance, but they do not seem to be alkaliphiles (Langworthy, 1978). Tolerance of high pH is not restricted to bacteria; and fish, protozoa, algae, and fungi have also been described from alkaline waters (Beadle, 1932; Hecky & Kilham, 1973; Jenkin, 1932, 1936; Melack & Kilham, 1974; Rich, 1932; 1933; see also vol 38, J. Linn. Soc. Lond., 1932/1933).

The examination of the physiological dependance of alkaliphiles on high pH has been undertaken in a few cases. Wiley and Stokes (1962; 1963) showed that Bacillus pasteurii had a specific requirement for ammonia, but this was lost when the cells were disrupted, indicating that ammonia was involved in transport of substrates across the cell membrane. Although other species of Bacillus are alkaliphilic, they do not appear to be dependant on the presence of ammonia for the

transport of substrates across the cell membrane. In these organisms ammonia may be highly toxic (Deal et al., 1975). They do, however, appear to be dependant on high pH for the transport of charged substances across the cell membrane (Guffanti et al., 1978; Ohta et al., 1975). Transmembrane pH gradients appear to be important in the transport of substrates across the membrane in many organisms, including alkaliphiles (Harold et al., 1970; Guffanti et al., 1978; Koyama et al., 1976; Krulwich et al., 1979; Ohta et al., 1975; Riebling et al., 1975). Studies on the enzymes of alkaliphilic and alkali-tolerant bacteria have shown that the extra-cellular enzymes are alkaliphilic or alkali-tolerant, but those from the cytoplasm function optimally at pHs near neutrality, suggesting that the cytoplasm is close to neutrality (Boyer & Ingle, 1972; Horikoshi, 1971a; 1971b; 1972; Horikoshi & Atsukawa, 1975; Ikeda et al., 1974; Kurono & Horikoshi, 1973; Nakanishi & Yamamoto, 1974; Nakamura & Horikoshi, 1976; Ohta et al., 1975; Tsuru et al., 1966; Yamaguchi & Gocho, 1973; Yamamoto et al., 1972). However, the pH of the cytoplasm may rise as the external pH is raised, and may become the growth limiting factor (Riebeling et al., 1975; Guffanti et al., 1978). Some authors have shown that the pH of the growth medium influences the alkaliphilicity of the organism in subsequent tests (Chislett & Kushner, 1961; Koyama & Nosoh, 1976). Brock and Darland (1970) also observed that the tolerance of high temperature and extremes of pH were connected. Examining thermal springs they found that the upper temperature limit of growth increased as the pH was raised .

Soda lakes are practically undescribed bacteriologically. The relative lack of bacteriological data on the soda lakes is not due to the lack of life in these alkaline waters, since the highest primary productivity rates have been recorded in these waters, which are rich in algae (Melack & Kilham, 1974; Talling et al., 1973).

These high rates of primary productivity allow these lakes to support vast numbers of flamingos in the Eastern African Rift Valley. The most comprehensive studies of life in soda lakes were carried out by the Percy Sladen and Cambridge expeditions to the lakes of the Eastern African Rift Valley (Beadle, 1932; Jenkin, 1932; 1936; Worthington, 1932; see also vol 38 J. Linn. Soc. Lond., 1932/1933). They did not, however, investigate the microflora below the level of algae and cyanobacteria. An early investigation of the lakes of the Kulunda Steppe by Isachenko (1934; 1951) suggested that such lakes harboured large numbers of phototrophic bacteria. Recent investigations have shown that the soda lakes may contain a characteristic population of phototrophic bacteria (Imhoff et al., 1978a; 1978b; Imhoff & Trüper, 1977; 1978; Jannasch, 1957; Grant et al., 1979), which are present in large numbers.

3. PHOTOTROPHIC BACTERIA.

3.1. Taxonomy of the green and purple bacteria.

Phototrophic bacteria comprise three major groups, the prochlorophyta, the cyanobacteria, and the purple and green bacteria (Lewin, 1976; 1977; Murray, 1974; Pfennig, 1977; Stanier, 1974), differentiated from one another on the basis of their photopigments and the nature of their photosynthetic metabolism. In addition to carotenoids, possessed by all phototrophic bacteria, the cyanobacteria contain chlorophyll a and phycobiloproteins, the prochlorophyta contain chlorophylls a and b, whereas the purple and green bacteria contain a variety of bacteriochlorophylls. The cyanobacteria and prochlorophyta are capable of oxygenic photosynthesis, like higher plants, utilising two linked photosystems and water as an electron donor, whereas the phototrophic purple and green bacteria, which belong to the order Rhodospirillales, do not have two photosystems, and are not capable of oxygenic photosynthesis. Such bacteria depend upon anaerobic conditions for phototrophic growth, utilising reduced sulphur compounds, molecular hydrogen, and simple organic compounds as electron donors. Recently it has been shown that some cyanobacteria are capable of anoxygenic photosynthesis in the presence of sulphide (Castenholz, 1977; Cohen et al., 1975). Under these conditions photosystem II is inhibited and sulphide acts as an electron donor for photosystem I, the process being unaffected by inhibitors of photosystem II, like 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU).

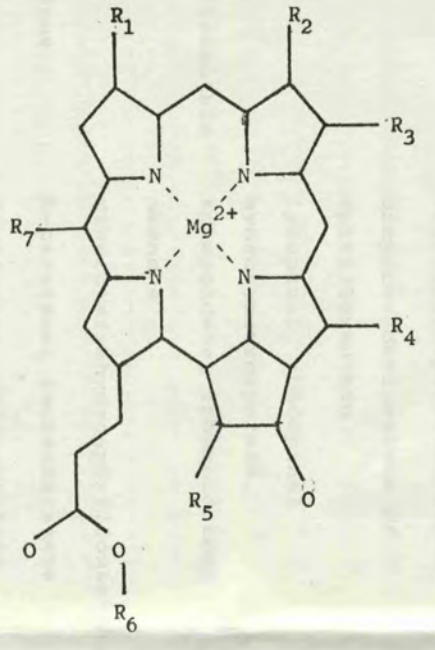
The higher taxa of the phototrophic purple and green bacteria have remained little changed since they were first described. Originally the purple bacteria were divided into the "Thiorhodaceae" and the "Athiorhodaceae", on the basis of the inability of the latter to accumulate sulphur globules inside the cell (Molisch, 1907; van Niel, 1944; 1957).

As a modification of this Baas-Becking (1925) proposed that purple sulphur bacteria storing sulphur inside the cell be placed in the "Endothiorhodaceae", and later Pelsh (1937) created a subfamily of the "Thiorhodaceae", the "Ectothiorhodaceae" for an organism depositing sulphur outside the cell, "Ectothiorhodospira mobile". The green sulphur bacteria were placed in the "Chlorobacteriaceae" (Lauterborn, 1915; van Niel, 1957). The currently accepted taxa are not markedly different from these groupings, and at present the order Rhodospirillales contains all bacteria possessing bacteriochlorophylls, of which there are seven known types (figure 7), bacteriochlorophyll a_p being common to all species as a component of the reaction centre (Gloe & Pfennig, 1974; Gloe & Risch, 1978; Gloe et al., 1975; Kunzler & Pfennig, 1973). A large number of carotenoids have been described and these are divided into five groups (figure 8), which are characteristic of various taxa (Pfennig, 1978b; Pfennig & Trüper, 1974; Schmidt, 1978). The photopigments are located in intracytoplasmic membrane systems, of which two basic types have been described, each characteristic of one of the suborders Rhodospirillineae (purple) and Chlorobiineae (green) (Pfennig and Trüper, 1974; Trüper, 1976; Trüper & Pfennig, 1978).

The members of the Chlorobiineae have their photopigments located in vesicles known as chlorosomes or chlorobium vesicles, which are formed from a non-unit membrane (Cohen-Bazire et al., 1964; Schmidt, 1980; Staehelin et al., 1978). Although the chlorosomes are closely associated with the cell membrane it has not been possible to demonstrate continuity between the cell membrane and the chlorosome (Remsen, 1978; Staehelin et al., 1978). Members of the Chlorobiineae are also characterised by the possession of bacteriochlorophylls c, d, or e, and carotenoids of the isorenieratene series (Pfennig, 1977; 1978b; Pfennig & Trüper, 1974; Trüper, 1976; Trüper & Pfennig, 1978).

Figure 7. The structure of chlorophylls and bacteriochlorophylls

Generalised structure of chlorophylls, including bacteriochlorophylls.



pigment[a]	structure of the R groups						
	R_1	R_2	R_3	R_4	R_5	R_6[b]	R_7
chl.a	$-CH-CH_3$	$-CH_3$	$-CH_2-CH_3$	$-CH_3$	$-CO-CH_3$ (=O)	-P	-H
chl.b	$-CH-CH_3$	-CH=O	$-CH_2-CH_3$	$-CH_3$	$-CO-CH_3$ (=O)	-P	-H
bchl.a_p	$-CO-CH_3$	$-CH_3$	$-CH_2-CH_3$	$-CH_3$	$-CO-CH_3$ (=O)	-P	-H
bchl.a_{Gg}	$-CO-CH_3$	$-CH_3$	$-CH_2-CH_3$	$-CH_3$	$-CO-CH_3$ (=O)	-Gg	-H
bchl.b	$-CO-CH_3$	$-CH_3$	$-CH-CH_3$	$-CH_3$	$-CO-CH_3$ (=O)	-P	-H
bchl.c	$-CHOH\ CH_3$	$-CH_3$	$-CH_2-CH_3$ $-CH_2-CH_2-CH_3$ $-CH_2-CH_2-CH_2-CH_3$	$-CH_2-CH_3$	-H	-F	$-CH_3$
bchl.c_s	$-CHOH-CH_3$	$-CH_3$	$-CH_2-CH_3$ $-CH_2-CH_2-CH_3$ $-CH_2-CH_2-CH_2-CH_3$	$-CH_2-CH_3$	-H	-S	$-CH_3$
bchl.d.	$-CHOH-CH_3$	$-CH_3$	$-CH_2-CH_3$ $-CH_2-CH_2-CH_3$ $-CH_2-CH_2-CH_2-CH_3$	$-CH_2-CH_3$	-H	-F	-H
bchl.e	$-CHOH-CH_3$	-CH=O	CH_2-CH_3 $-CH_2-CH_2-CH_3$ $-CH_2-CH_2-CH_2-CH_3$	$-CH_2-CH_3$	-H	-F	$-CH_3$

a, chl. = chlorophyll, bchl. = bacteriochlorophyll; b, P = phytol, F = farnesol, Gg = geranylgeraniol, S = sterol.

Figure 8. Carotenoid groups of the purple and green phototrophic bacteria.

group	name of series	major components
1a	normal spirilloxanthin	lycopene, rhodopin, spirilloxanthin
1b		tetrahydrospirilloxanthin
1c		dihydro- derivatives of spirilloxanthin
2	rhodopinal	lycopenal, rhodopinal
3	alternative spirilloxanthin	hydroxyneurosporene, spheroidene, spheroidenone
4a	okenone	okenone
4b		diketotetrahydrospirilloxanthin
5a	isorenieratene	β-carotene, isorenieratene
5b		γ-carotene, chlorobactene
5c		β-carotene, γ-carotene γ-carotene-glucoside

(Data from Pfennig, 1978b; Pfennig & Trüper, 1974; Schmidt, 1978).

None of the members of the Chlorobiineae possess flagella (Trüper & Pfennig, 1978). The suborder Chlorobiineae is further divided into two families (table 1), the Chlorobiaceae and Chloroflexaceae on the basis of physiology and morphology (Pfennig, 1977; Trüper, 1976; Trüper & Pfennig, 1978).

Members of the Chlorobiaceae (tables 1, 2) are anaerobes, utilising sulphide as an electron donor, depositing sulphur globules outside the cell, and further oxidising sulphur to sulphate. Some species are capable of using simple organic compounds, but only in the presence of sulphide and bicarbonate, indicating a purely anabolic intermediary carbon metabolism (Kelly, 1974; Sadler & Stanier, 1960). All genera are non-motile, some possessing gas vacuoles. Cells divide by binary fission, and members of some genera are capable of ternary fission, forming a three dimensional network (Pfennig & Trüper, 1974; Trüper & Pfennig, 1978). Although usually referred to as the green bacteria, the Chlorobiaceae have both brown and green members. The separation of the green and brown species into different genera is indicated by their characteristic bacteriochlorophyll and carotenoid content (Pfennig, 1977). Members of the green species have bacteriochlorophyll c or d and carotenoids chlorobactene and hydroxychlorobactene, whereas members of the brown species have as their major photopigments bacteriochlorophyll e and the carotenoids isorenieratene and β-isorenieratene.

Members of the Chloroflexaceae (table 1, 3) do not have flagella, but have a flexible cell wall and are capable of gliding motility, like some cyanobacteria and flexibacteria (Trüper, 1976). This family contains the thermophilic Chloroflexus aurantiacus (Pierson & Castenholz, 1974a) as the only currently accepted species (Trüper & Pfennig, 1978), but recently new genera, Chloronema (Dubinina & Gorlenko, 1975) and Oscillochloris (Gorlenko & Pivovarova, 1977) have

Table 1. Higher taxa of the purple and green phototrophic bacteria.

Order I

Rhodospirillales

organisms containing bacteriochlorophyll and carotenoid photopigments, capable of anoxygenic photosynthetic growth.

Suborder

Rhodospirillineae

organisms containing bacteriochlorophyll a or b and carotenoids of groups 1 to 4. Cells may be motile by flagella, and have intracytoplasmic membranes which are continuous with the cell membrane.

Suborder

Chlorobiineae

organisms containing bacteriochlorophyll c, d, or e and carotenoids of group 5. Are not capable of flagellate motility, and have chlorosomes as the photosynthetically active intracytoplasmic cell membranes.

Family I

Rhodospirillaceae

Most species not capable of photolithotrophic growth. Multiply by budding or by binary fission. Some species capable of aerobic growth in the dark.

Family III

Chlorobiaceae.

Cells are non-motile, multiply by binary fission. Capable of photolithotrophic growth, are strictly anaerobic.

Family II

Chromatiaceae

Capable of photolithotrophic grwoth. In the presence of sulphide, sulphur globules are stored intracellularly. Multiply by binary fission.

Family IV

Chloroflexaceae

Cells show gliding motility, multiply by hormogonia, and have a flexible cell wall. Capable of aerobic growth in the dark.

Table 2. Taxonomic features of members of the Chlorobiaceae.

genus/species	photopigments[a]	GC content[b]	shape	size	gas vacuoles
Chlorobium limicola	bchl.c or d/5b	51.0-58.1	rod	0.7-1.0 x 0.9-1.5µm	-
vibrioforme	bchl.d or c/5b	52.0-57.1	vibrio	0.5-0.7 x 1.0-2.0µm	-
chlorovibriodes	bchl.c/5b	?	vibrio	0.3-0.4 x 0.7-1.2µm	-
phaeobacteriodes	bchl.e/5a	49.0-50.0	rod	0.6-0.8 x 1.3-2.7µm	-
phaeovibriodes	bchl.e/5a	52.0-53.0	vibrio	0.3-0.4 x 0.7-1.4µm	-
Prosthecochloris aestuarii[c]	bchl.c/5b	50.0-56.0	sphere	0.5-0.7 x 1.0-1.2µm	-
phaeoasteroidea	bchl.e/5a	52.2	sphere	0.3-0.6 x 0.5-0.8µm	-
Pelodictyon luteolum	bchl.c or d/5b	53.5-58.1	oval	0.6-0.9 x 1.2-2.0µm	+
clathratiforme	bchl.c or d/5b	48.5	rod	0.7-1.2 x 1.5-2.5µm	+
phaeum	bchl.e?/5a	?	vibrio	0.6-0.9 x 1.0-2.0µm	+
Clathrochloris sulfrica	bchl.?/?	?	sphere	0.5-1.5µm	+
Anachloris perfilievii[c]	bchl.?/?	?	sphere	0.5-1.0µm	+

a, see figures 7 & 8; b, GC content in mol%; c, members of these genera produce prostheca.

(Data from Pfennig and Trüper, 1974; Trüper and Pfennig, 1978; Gorlenko et al.,1973; 1974)

Table 3. Taxonomic features of members of the Chloroflexaceae.

genus/species	photopigments[a]	GC content[b]	shape	size	gas vacuoles
Chloroflexus aurantiacus	bchl.c_s/5c	53.0-55.0	filament	0.6-0.7 x 2.0-6.0µm	-
Chloronema giganteum	bchl.c/?	?	filament	2.0-2.5 x 3.5-4.5µm	+
spiroideum	bchl.c/?	?	filament	1.5-2.0 x 2.5-3.0µm	+
Oscillochloris chrysea[c]	bchl.c/?	?	filament	4.5-5.5 x 3.5-7.0µm	+

a, see figures 7 & 8 ; b, GC content in mol%; c, this species is the only know Gram-positive member of the Rhodospirillales.

(data from; Pierson & Castenholz, 1974a; 1974b; Halfen et al.,1972; Gloe & Risch, 1978; Gorlenko & Pivovarova, 1977; Pivovarova & Gorlenko, 1977)

been described, which may also belong to this family (Pfennig, 1977; Gorlenko & Pivovarova, 1977). A mesophilic strain of Chloroflexus has been isolated by Gorlenko (1975), which appears to be very similar to Chloroflexus aurantiacus. In the only well studied species, Chloroflexus aurantiacus, the major photopigments are the carotenoids β-carotene, and a stenol esterified analogue of bacteriochlorophyll c (Pierson & Castenholz, 1971; 1974b; Halfem et al., 1972; Gloe & Risch, 1978). Other genera may contain bacteriochlorophyll c or d (Dubinina & Gorlenko, 1975; Gorlenko & Pivovarova, 1977). Unlike the Chlorobiaceae, members of the Chloroflexaceae are not fastidious and are capable of growth under anaerobic and aerobic conditions. Anoxygenic photosynthesis occurs under anaerobic conditions with simple carbon compounds, or reduced sulphur compounds as an electron donor (Pierson & Castenholz, 1974a; Dubinina & Gorlenko, 1975). In the presence of sulphide, globules of sulphur are deposited outside the cells of Chloroflexus aurantiacus, but are not further oxidised to sulphate (Castenholz, 1973; Madigan & Brock, 1975).

The suborder Rhodospirillineae may also be divided into two families (table 1), the family Rhodospirillaceae, which corresponds to Molisch's "Athiorhodaceae" (Molisch, 1907), and the Chromatiaceae, corresponds to the "Thiorhodaceae". Unfortunately Molisch's original criterion relating to sulphur deposition has proven to be no longer entirely valid, and currently the Chromatiaceae contains the genus Ectothiorhodospira (Trüper, 1968), which deposits sulphur outside the cell, and additionally certain members of the Rhodospirillaceae are now known to be capable of dissimilatory sulphur metabolism, depositing sulphur globules outside the cell (Hansen & van Gemerden, 1972; Hansen & Veldkamp, 1973; Keppen & Gorlenko, 1975).

The Rhodospirillaceae is a very diverse family (table 4), showing the greatest degree of variation within the Rhodospirillales.

Table 4. Taxonomic features of members of the Rhodospirillaceae.

genus/species		photopigments[a]	GC content[b]	shape	size	membrane[c]
Rhodospirillum	fulvum	bchl.a/1a	64.3-65.3	spirilla	0.5-0.7 x 3.5µm	lamellar
	molischianum	bchl.a/1a	61.7-64.8	spirilla	0.7-1.0 x 5.0-8.0µm	lamellar
	photometricum	bchl.a/1a	65.8	spirilla	1.2-1.5 x 7.0-10.0µm	lamellar
	rubrum	bchl.a/1a	63.8-65.8	spirilla	0.8-1.0 x 7.0-10.0µm	vesicular
	tenue	bchl.a/1a,2	64.8	spirilla	0.3-0.5 x 3.0-6.0µm	tubular
Rhodopseudomonas	acidophila[d]	bchl.a/1a	62.2-66.8	rod	1.0-1.3 x 2.0-5.0µm	lamellar
	palustris[d]	bchl.a/1a	64.8-66.3	rod	0.6-0.9 x 1.2-2.0µm	lamellar
	sulfoviridis[d]	bchl.b/3	67.8-68.4	rod	0.5-0.9 x 1.2-2.0µm	lamellar
	viridis[d]	bchl.b/1c	66.3-71.4	rod	0.6-0.9 x 1.2-2.0µm	lamellar
	gelatinosa	bchl.a/3	70.5-72.4	rod	0.4-0.5 x 1.0-2.0µm	tubular
	capsulata	bchl.a/3	65.5-66.8	rod	0.5-1.2 x 2.0-2.5µm	vesicular
	globiformis	bchl.a/4b	66.3	sphere	1.6-1.8µm	vesicular
	sphaeroides	bchl.a/3	68.4-69.9	sphere	0.7 x 2.0-2.5µm	vesicular
	sulfidophila	bchl.a/3	67.0-71.0	rod	0.6-0.9 x 0.9-2.0µm	vesicular
Rhodocyclus purpureus[e]		bchl.a/2	65.3	half-circle	0.6-0.7 x 2.7-5.0µm	tubular
Rhodomicrobium vannielii		bchl.a/1a	61.8-63.8	ovoid	1.0-1.2 x 2.0-2.8µm	lamellar

a, see figures 7 & 8; b, GC content in mol%; c, form of the intracytoplasmic membranes; d, these species multiply by budding, the parent cells being non-motile, and forming motile daughter cells; e, non-motile. (Data from Pfennig, 1978a; 1974; Pfennig and Trüper, 1974; Trüper and Pfennig, 1978; Schmidt, 1978; Whittenbury and McClee, 1967; Dow and Whittenbury, 1978).

All the genera contain species which are capable of microaerophilic or aerobic growth in the dark. Under anaerobic conditions all species are capable of anoxygenic phototrophic growth (Pfennig & Trüper, 1974; Trüper & Pfennig, 1978). With the exception of the genus Rhodocyclus, all genera are motile, usually by polar flagella, although Rhodomicrobium is peritrichously flagellate (Pfennig, 1978a; Pfennig & Trüper, 1974). Multiplication is by binary fission in the genera Rhodospirillum, Rhodocyclus, and some members of the genus Rhodopseudomonas. Members of the genus Rhodomicrobium and the remainder of the genus Rhodopseudomonas multiply by budding. The major photopigments are bacteriochlorophyll a or b, and a variety of carotenoids (Pfennig & Trüper, 1974; Trüper & Pfennig, 1978). Until fairly recently it was believed that all genera of the Rhodospirillaceae were unable to use sulphide as a photosynthetic electron donor. However, the work of Hansen and van Gemerden (1972) has established that some species can tolerate low concentrations of sulphide, and are capable of photolithotrophic growth in the presence of sulphide or thiosulphate. New species have since been discovered, which can tolerate higher concentrations of sulphide (Hansen & Veldkamp, 1973; Keppen & Gorlenko, 1975), and one species, Rhodopseudomonas globiformis, lacks an assimilatory sulphate reductase, and, therefore, a reduced source of sulphur is required for growth (Pfennig, 1974). Most members of the Rhodospirillaceae are, however, unable to oxidise elemental sulphur, the end product of dissimilatory metabolism being sulphur, which accumulates outside the cell, or sulphate, which is formed without sulphur as an intermediate.

The Chromatiaceae are characterised by their ability to store sulphur globules inside the cell (table 1), the only exception is the members of the genus Ectothiorhodospira, which deposit sulphur outside the cell. However, this genus, like all others in the

Table 5. Taxonomic features of members of the Chromatiaceae.

genus/species.	photopigments[a]	GC content[b]	shape	size	membrane[c]	motile[d]
Chromatium okenii	bchl.a/4a	48.0-50.0	rod	4.5-6.0 x 8.0-15.0µm	vesicular	+
weissei	bchl.a/4a	48.0-50.0	rod	4.5 x 7.0-9.0µm	vesicular	+
warmingii	bchl.a/2	55.1-60.2	rod	3.5-4.0 x 5.0-11.0µm	vesicular	+
buderi	bchl.a/2	62.2-62.8	rod	3.5-4.5 x 4.5-9.0µm	vesicular	+
purpuratum	bchl.a/4a	68.9	rod	1.2-1.7 x 3.0-4.0µm	vesicular	+
minus	bchl.a/4a	52.0-62.2	rod	2.0 x 2.5-6.0µm	vesicular	+
violascens	bchl.a/2	61.8-64.3	rod	2.0 x 2.5-6.0µm	vesicular	+
vinosum	bchl.a/1a	61.3-66.3	rod	2.0 x 2.5-6.0µm	vesicular	+
gracile	bchl.a/1a	68.9-70.4	rod	1.0-1.3 x 2.0-6.0µm	vesicular	+
minutissimum	bchl.a/1a	63.7	rod	1.0-1.2 x 2.0µm	vesicular	+
Thiocystis gelatinosa	bchl.a/4b	69.4-69.9	sphere	3.0µm	vesicular	-
violacea	bchl.a/2	63.3-66.3	sphere	2.5-3.0µm	vesicular	-
Thiosarcina rosea	bchl.a/1a	?	oval	2.0-3.0µm	?	-
Thiospirillum sanguineum	bchl.a/1a	?	spirilla	2.5-4.0 x 10-100µm	vesicular	+
jenense	bchl.a/1a	45.5	spirilla	2.5-4.5 x 30-40µm	vesicular	+
rosenbergii	bchl.a/?	?	spirilla	1.5-2.5 x 4.0-12.0µm	vesicular	+
Thiocapsa roseopercina	bchl.a/1a	63.3-66.3	sphere	1.2-3.0µm	vesicular	-
pfennigiii	bchl.b/1b	69.4-69.9	sphere	1.2-1.5µm	tubular	-
Lamprocystis roseopercina	bchl.a/2	63.8	sphere	3.0-3.5µm	vesicular	+/gv
Thiodictyon elegans	bchl.a/2	66.3	rod	1.5-2.0 x 3.0-8.0µm	vesicular	-/gv
bacillosum	bchl.a/2	65.3	rod	1.5-2.0 x 3.0-6.0µm	vesicular	-/gv
Thiopedia rosea	bchl.a/4a	?	sphere	1.0-2.0 x 1.2-2.5µm	vesicular?	-/gv
Amoebobacter roseus	bchl.a/1a	64.3	sphere	2.0-3.0µm	vesicular	-/gv
pendens	bchl.a/1a	65.3	sphere	1.5-2.5µm	vesicular	-/gv
Ectothiorhodospira mobilis	bchl.a/1a	67.3-69.9	spirilla	0.7-1.0 x 2.0-2.6µm	lamellar	+
shaposnikovii	bchl.a/1a	62.3-64.0	spirilla	0.8-0.9 x 1.5-2.5µm	lamellar	+
halophila	bchl.a/1a	68.4	spirilla	0.8 x 5.0µm	lamellar	++
halochloris	bchl.b/1a	52.9	spirilla	0.5-0.6 x 2.5-8.0µm	lamellar	++
abd-el-malekii	bchl.b/1a	63.0	spirilla	0.5-0.6 x 4.0-15.0µm	lamellar	++

a, see figures 7 & 8; b, GC content in mol%; c, form of the intracytoplasmic membranes; d, + polarly flagellate, ++ bipolarly flagellate, - non-motile, gv cells contain gas vacuoles.

(Data from Trüper and Pfennig, 1978; Schmidt, 1978; Imhoff and Trüper, 1977; 1980; in preparation)

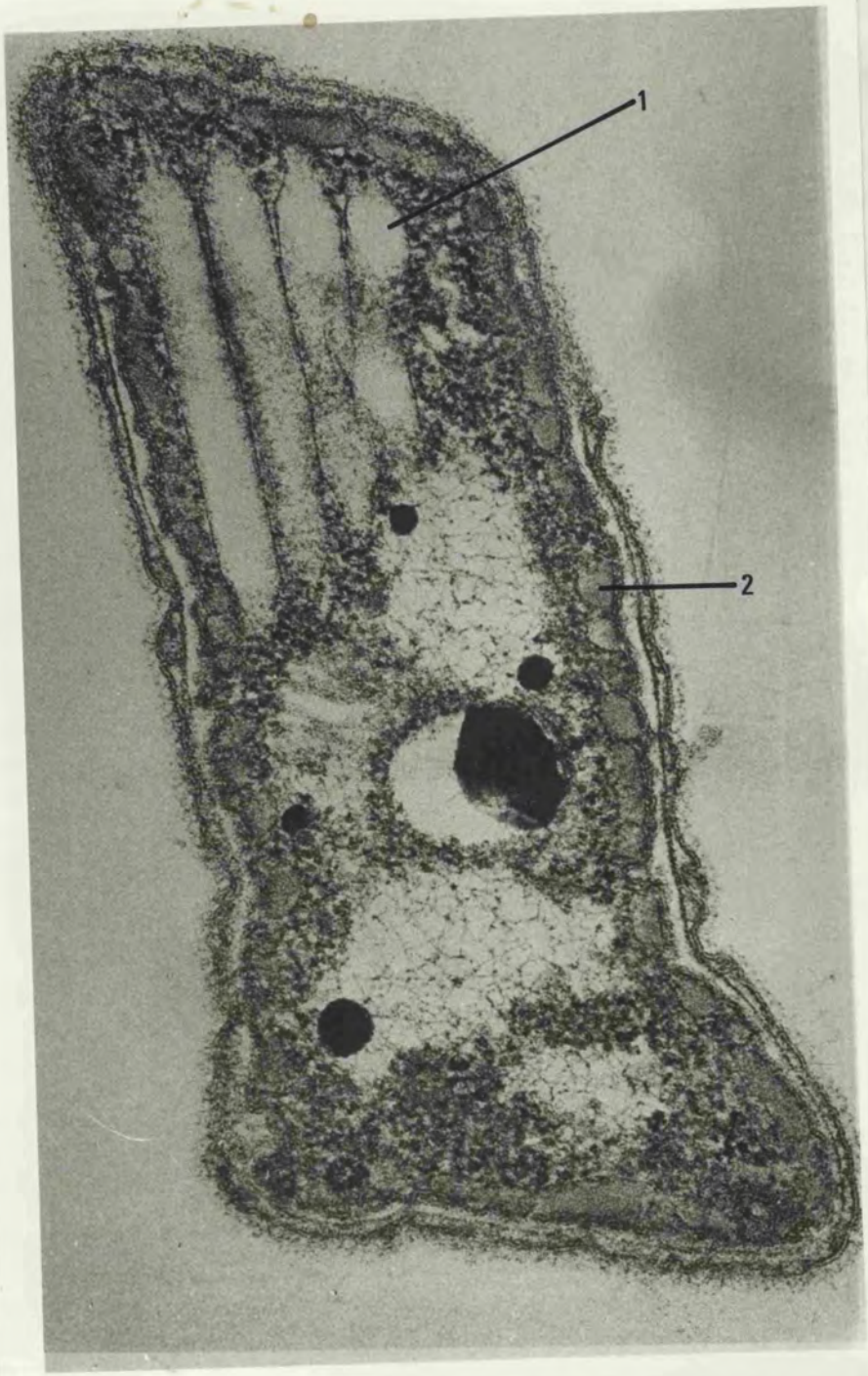
Chromatiaceae, is capable of oxidising sulphide to sulphate via elemental sulphur, which in part justifies its present taxonomic position (Trüper & Pfennig, 1978). The position of this genus still confuses taxonomists, and its present status is far from settled (Trüper, personal communication). At present included in the family Chromatiaceae, Pelsh (1937) originally proposed that members of the genus Ectothiorhodospira be placed in a subfamily, the "Ectothiorhodaceae", while Pfennig (1977) has recently proposed that this genus be placed in the family Rhodospirillaceae. Under suitable conditions most of the Chromatiaceae are capable of microaerophilic growth in the dark, the large Chromatium species being a notable exception (Trüper, personal communication). All genera grow photolithotrophically and photomixotrophically in the presence of sulphide. Some species lack assimilatory sulphate reduction, and, therefore, require reduced sulphur compounds for growth (Pfennig & Trüper, 1974; Trüper & Pfennig; 1978). Most species are capable of photoorganotrophic growth in the presence of simple organic compounds. All species multiply by binary fission, budding species are not known. Cells may be motile by polar flagella (Table 5). Non-motile forms may contain centrally located gas vacuoles; one motile gas vacuolate genus is known (table 5).

3.2. Structure and biochemistry of the Rhodospirillales.

Whereas the chlorosomes of the Chlorobiineae are characteristic of this suborder (figures 9, 10), and there appears to be little variation in the fine structure, the photosynthetic membranes of the Rhodospirillineae show a greater variety in their structural organisation. According to the classification systems used by Pfennig (1967), Oelze and Drews (1972), Trüper and Pfennig (1978), and Remsen (1978), it is possible to distinguish three basic structures in the Rhodospirillineae, although there are several variations. The intracytoplasmic membrane arises as a simple sack

Figure 9. Thin section of a green phototrophic bacterium.

Thin section of Pelodictyon clathratiforme showing the location of gas vacuoles (arrowed 1) and chlorosomes (arrowed 2). The chlorosomes of the members of the Chlorobiineae are closely associated with the cell membrane, but it has not been possible to demonstrate continuity between the cell membrane and the chlorosomes.



(from Bergey's Manual, 1974)

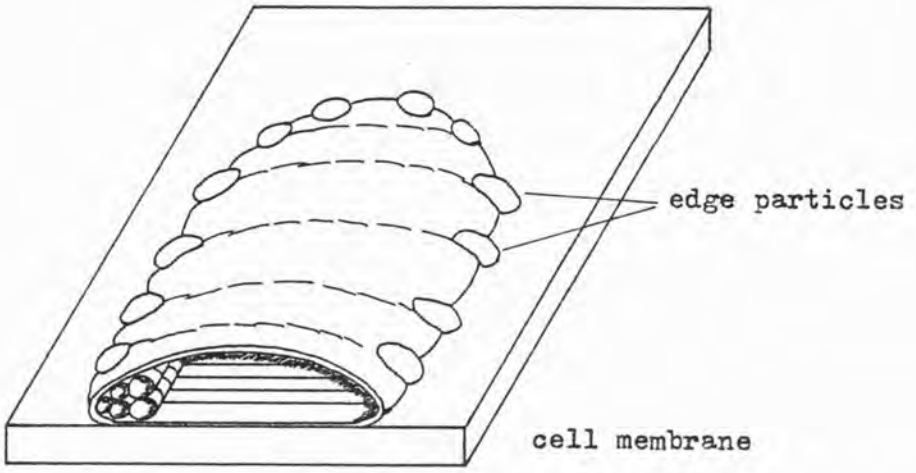


Figure 10. Visualisation of the chlorosomes of green bacteria.

The chlorosome consists of a non-unit membrane, which forms a closed sack. The internal space of the chlorosome contains numerous rod shaped units, arranged longitudinally. The function of the 'edge particles' is not known. The cell membrane associated layer of the chlorosome differs structurally from the cytoplasm associated layer, however, the significance of these findings are unclear, but may indicate different functions of the two layers. The cell membrane associated layer of the chlorosome is known to be closely linked to the cell membrane, but the exact nature of the association has not been determined.

(after Staehelin et al., 1978)

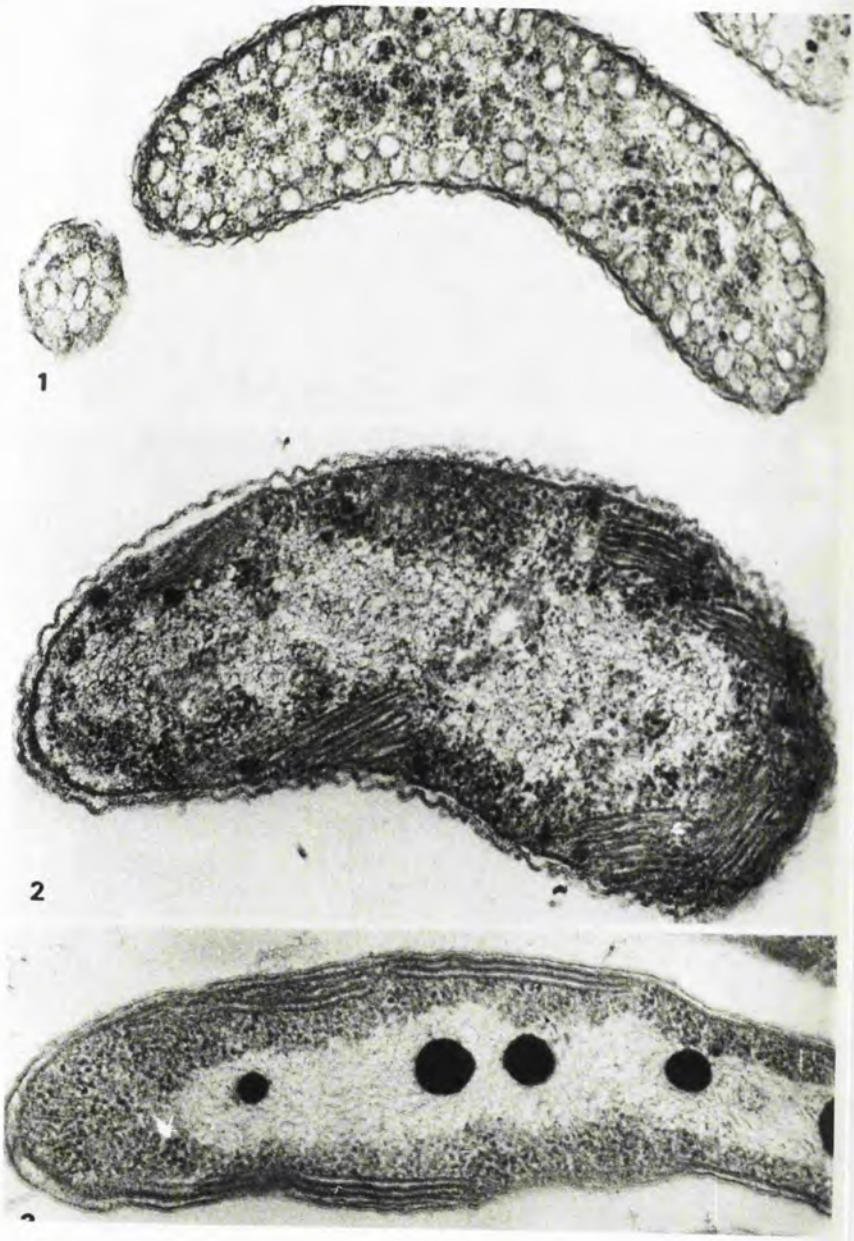
in most cases, the primary invagination of the cytoplasmic membrane subsequently differentiating into tubular, vesicular, or lamellar membrane systems (Remsen, 1978). Continuity between the cytoplasmic and intracytoplasmic membrane is maintained (Cohen-Bazire & Kunisawa, 1963; Boatman, 1964; Holt & Marr, 1965; Peters & Cellarius; 1972), although there is now some doubt in the vesicular membrane containing members of the family Chromatiaceae (Remsen, 1978). Up to two forms of tubular intracytoplasmic membrane are recognised. Elongated bundles of parallel tubes are characteristic of Thiocapsa pfennigii(figures 13.2, 14.2), and are clearly visible in electron micrographs of ruptured cells (Eimhjellan, 1970; Eimhjellan et al., 1967). Tubular membranes have also been described in old cultures of Thiocystis violacea and members of the genera Rhodocyclus, Rhodospirillum, and Rhodopseudomonas (de Boer, 1969; Pfennig, 1978a; 1978b; Remsen, 1978; Weckesser et al., 1969). In these organisms the tubes are often irregular in size and shape, and are classified by others as poorly developed invaginations, or as a vesicular form. The lamellar type of membrane may be divided into as many as five distinct structures. In the brown, microaerophilic species Rhodospirillum fulvum, Rhsp.molischianum, and Rhsp.photometricum (figures 11.2, 12.2), a series of adjacent invaginations are appressed together forming a group of flattened discs, and constitute a lamellar stack (Hickman & Frenkel, 1965; Oelze & Drews, 1972; Pfennig, 1967). In the above mentioned species the stacks lie at a sharp angle to the cytoplasmic membrane. The genus Ectothiorhodospira is also characterised by lamellar stacks (Cherni et al., 1969; Imhoff & Trüper, 1977; Raymond & Sistrom, 1967; 1969; Trüper, 1968), but these lie parallel to the cytoplasmic membrane and may arise in a different manner (Holt et al., 1968; Oyewole & Holt, 1976; Remsen et al., 1968). The budding species of the Rhodospirillaceae

Figure 11. Thin sections of purple non-sulphur bacteria.

1. Thin section of Rhodospirillum rubrum containing vesicular membranes. These type of membranes are found in members of the purple sulphur and purple non-sulphur bacteria. The vesicles form from an invagination of the cell membrane, which subsequently branches and proliferates into a network, largely remaining continuous with the cell membrane.

2. Thin section of Rhodospirillum molischianum, showing lamellar membranes. The lamellar stacks may be seen to lie at an acute angle to the cell membrane, and each of the constituent lamellae in the stack arise from a separate invagination. Such lamellar membranes are characteristic of the brown species of the genus Rhodospirillum.

3. Thin section of Rhodopseudomonas palustris showing lamellar membranes. The lamellae overlie one another along the periphery of the cell, except at the poles of the cell. Although they appear as a lamellar stack, the lamellae each arise as separate invaginations, except where branching occurs, and may encircle the cell in a series of concentric layers. This type of membrane system is characteristic of the budding species of the purple non-sulphur bacteria.



(from Bergey's Manual, 1974)

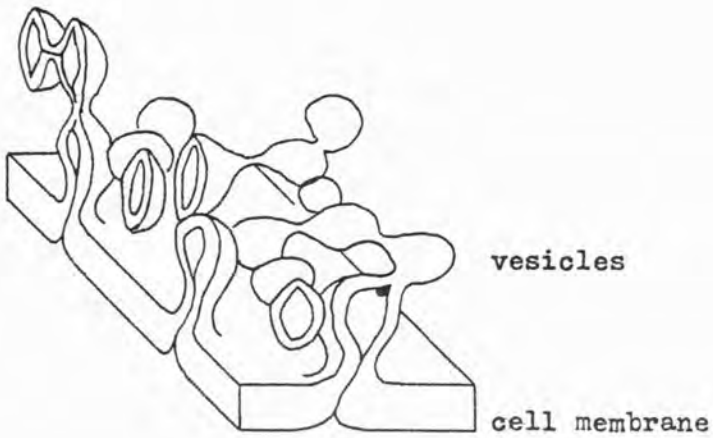


Figure 12.1. Visualisation of the vesicular intracytoplasmic membranes.

The vesicular intracytoplasmic membrane system is found in purple sulphur and purple non-sulphur phototrophic bacteria. The vesicles form by invagination of the cell membrane, followed by branching into a network of vesicles. The entire network of vesicles maintains continuity with the cell membrane, with the exception of purple sulphur phototrophic bacteria, when vesicles may become detached from the network and contribute to the formation of sulphur globules within the cell.

(after Remsen, 1978)

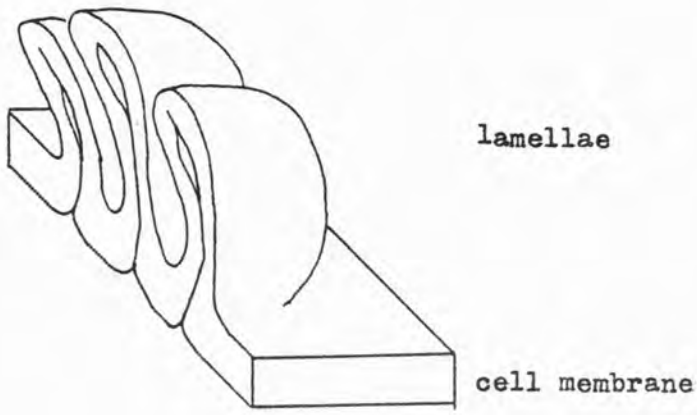


Figure 12.2. Visualisation of the lamellar membranes found in *Rhodospirillum molischianum*.

This type of lamellar intracytoplasmic membrane system is characteristic of the brown species of the genus *Rhodospirillum*. The lamellae arise as separate invaginations of the cell membrane, become appressed together, and form a group of flattened lamellae. The lamellae typically lie at an acute angle to the cell membrane. Upon dissociation of the cell membrane the vesicles may be observed as separate lamellae in electron micrographs, indicating their individual origins.

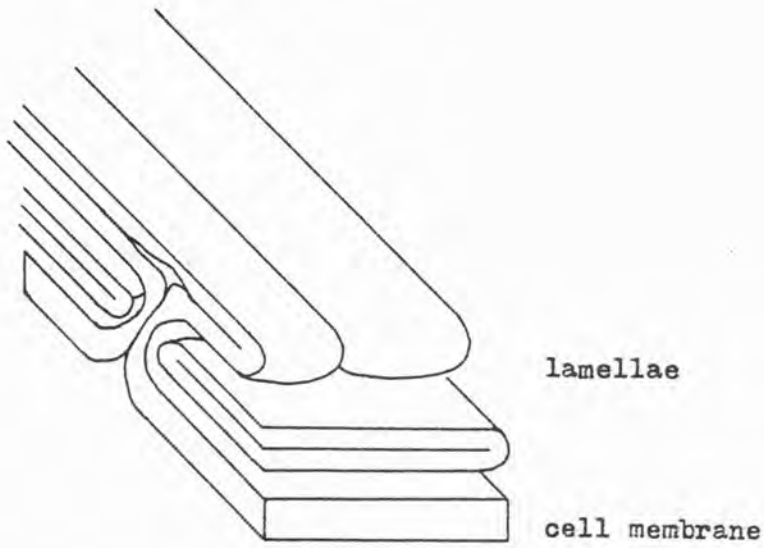


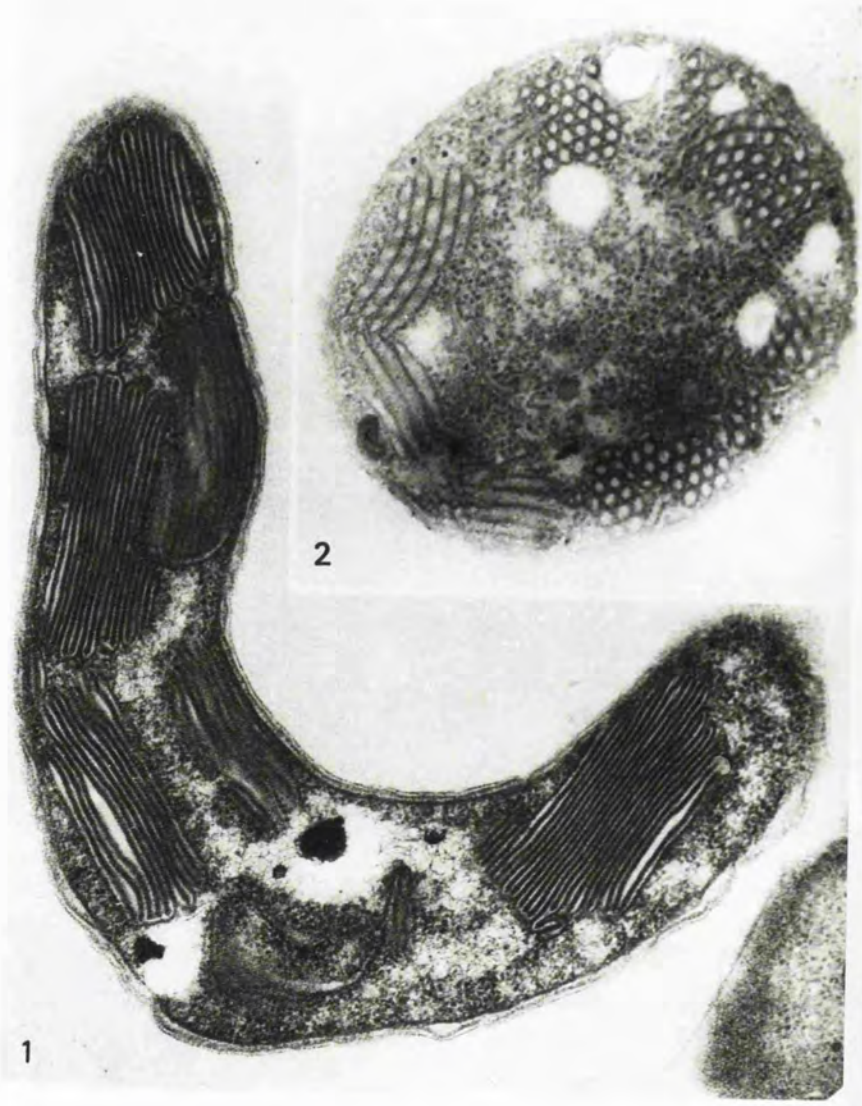
Figure 12.3. Visualisation of the lamellar membranes of budding species of Rhodospirillaceae.

The budding species of the Rhodospirillaceae are characterised by this type of lamellar intracytoplasmic membrane system, in which the lamellae may form concentric layers around the periphery of the cell. The poles of the cell are usually free of intracytoplasmic membranes, and the daughter cells which form during budding are devoid of intracytoplasmic membranes. Branching of the lamellae may occur in some species, although the lamellae are usually unbranched, and arise from a number of invaginations of the cell membrane, which are not interconnected.

Figure 13. Thin sections of purple sulphur bacteria.

1. Thin section of Ectothiorhodospira mobilis showing the lamellar membrane stacks, which lie parallel to the cell membrane. The entire stack is continuous with the cell membrane, and apparently arises as a single invagination. This type of membrane system is characteristic of those members of the purple sulphur bacteria which deposit sulphur outside the cell during the photo-oxidation of sulphide to sulphate.

2. Thin section of Thiocapsa pfennigii showing the tubular membrane system characteristic of this species. Tubular membranes may occur in other species, but they are poorly developed and less extensive, and may be regarded as structurally different. The tubes are found in groups of parallel membranes around the periphery of the cytoplasm.



(from Bergey's Manual, 1974)

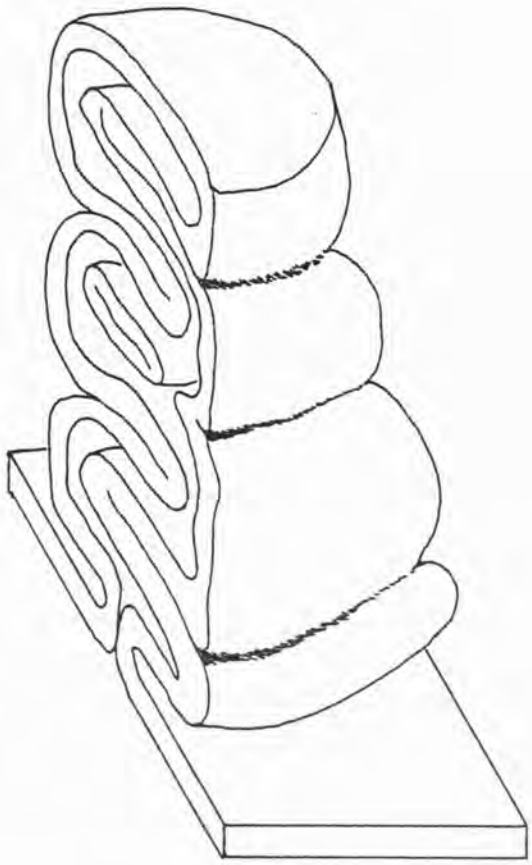


Figure 14.1. Visualisation of the lamellar membranes found in members of the genus Ectothiorhodospira.

The lamellar membrane system found in the members of the genus Ectothiorhodospira are characteristic of this genus, and are structurally distinctive. The membranes may be seen in the negatively stained cells as dark areas within the cell, due to the penetration of the stain into the space within the intracytoplasmic membranes. Although it has been postulated that the intracytoplasmic membranes in this genus arise as a simple sack, which develop secondary folds, thus maintaining continuity between the lamellae within the stack, such differentiation of the developing invagination has not been observed. The integrity of the stack is maintained during disruption of the cell, unlike other types of lamellar intracytoplasmic membranes.

(after Remsen et al., 1968)

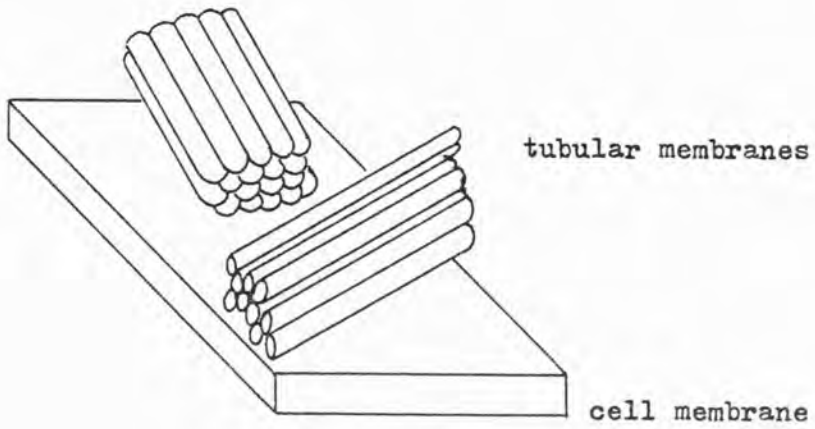


Figure 14.2. Visualisation of the tubular membranes of *Thiocapsa pfennigii*.

The tubular membranes of *Thiocapsa pfennigii* are characteristic of this species. Although tubular membranes have been described in some members of the *Rhodospirillaceae*, they are not as extensive, nor as well developed. The tubular intracytoplasmic membranes of *Thiocapsa pfennigii* may be regarded as a development of the vesicular type of intracytoplasmic membranes, in which constriction of the developing invagination does not occur, thus preventing the formation of a vesicle.

have a lamellar membrane system, which has been described in up to three forms. In Rhodopseudomonas viridis the lamellae are described as being formed into slightly curved double membranes, lying parallel to the cytoplasmic membrane as a grana-like stack (Oelze & Drews, 1972; Giesbrecht & Drews, 1966). The remaining budding species of this genus have a concentric series of partly branched membranes, which overlie one another and are parallel to the cytoplasmic membrane (figures 11.3, 12.3) (Tauschel & Drews, 1967; Tauschel & Hoeniger, 1974). Rhodomicrobium vannielii has a similar membrane system, which Oelze and Drews (1972) distinguish from the Rhodopseudomonas type as being unbranched. The vesicular membrane system is found both in the Rhodospirillaceae and the Chromatiaceae (figures 11.1, 12.1), giving rise to a network of interconnected branched vesicles (Remsen, 1978). Initially it was thought that these vesicular structures were not connected to the cytoplasmic membrane, and were free cytoplasmic organelles (Vatter & Wolfe, 1958). It has been postulated that in the Chromatiaceae vesicles may become free in the cytoplasm and coalesce to form the limiting membrane of the sulphur globules (Remsen, 1978).

Little work has been carried out on the origin of the sulphur globules within the cells of the Chromatiaceae. It is, however, known that the limiting membrane is not a unit membrane, and consists of a protein, the molecular weight of which may vary with species (Goyne et al., in Remsen, 1978; Nicholson & Schmidt, 1971; Schmidt et al., 1971). Similarities to the non-unit membranes of the chlorosomes and the gas vesicles are indicated. Remsen (1978) has suggested that vesicles become detached from the intracytoplasmic network and oxidise sulphide contained within them to sulphur. The small sulphur globules formed coalesce to form a larger globule, and selective degradation of the vesicle membrane occurs, leaving a mainly

proteinaceous limiting membrane. There appears to be little information regarding the nature of the extracellular sulphur globules formed in the Rhodospirillaceae, Chlorobiineae, or the genus Ectothiorhodospira.

The division of the Rhodospirillales on the basis of their sulphur metabolism seems to be justified at present. Organisms within genera belonging to the families Chlorobiaceae and Chromatiaceae are capable of oxidising sulphide, via elemental sulphur, to sulphate, and most strains so far studied contain the enzyme adenyl sulphate (APS) reductase. Members of genera in the families Rhodospirillaceae and Chloroflexaceae have not been shown to contain APS reductase and have not been shown to be capable of oxidising extracellularly deposited sulphur to sulphate (Trüper, 1978). Kondrat'eva and Krasil'nikova (1979) and Kondrat'eva (1976) were unable to detect APS reductase in E.shaposnikovii, although this species is capable oxidising extracellularly deposited sulphur to sulphate, like other members of the genus Ectothiorhodospira. Differences exist between the sulphur metabolism of members of the Chlorobiaceae and Chromatiaceae. In the Chlorobiaceae the oxidation of sulphide to sulphur, and sulphur to sulphate occurs in two strictly separate phases, and this has also been observed in one genus of the Chromatiaceae, Ectothiorhodospira, which deposits sulphur outside the cell like members of the Chlorobiaceae (Trüper, 1978). The majority of the Chromatiaceae do not show this phasic sulphur metabolism, and the two reactions may occur similtaneously. Whether this is connected to the deposition of sulphur inside the cell is unclear. The genus Ectothiorhodospira is the only known genus capable of forming polysulphide as an intermediate in dissimilatory sulphur metabolism (Trüper, 1978), possibly due to the alkaline pH optimum of this group. Other members of the Chromatiaceae are not

capable of utilising polysulphides supplied in the growth medium.

The gas vacuoles found in the Rhodospirillales resemble those found in the cyanobacteria and halobacteria (Cohen-Bazire et al., 1964; Pfennig & Cohen-Bazire, 1967). The gas vacuoles consist of a group of gas vesicles, which are usually cylindrical with conical ends. The wall of the vesicle is a non-unit proteinaceous membrane, and has a distinctive ribbed appearance in freeze fracture preparations (Walsby, 1978). The function of the gas vacuole is to provide buoyancy, and allow an otherwise non-motile organism to migrate in the water column (Pfennig, 1967; Walsby, 1972; 1974; 1978). The gas vacuoles may be distinguished from other cellular inclusions by their collapse under pressure (Walsby, 1972; 1974; 1978).

Investigations of the cell envelope of members of the Rhodospirillales indicate that there may be significant differences in the lipid content of the families (Kenyon, 1978). The Rhodospirillaceae and Chlorobiaceae are the most extensively studied groups. Species of the Chlorobiaceae examined so far contain phosphatidyl glycerol (PG) and diphosphatidyl glycerol (DPG) as the main phospholipids. The glycolipids, monogalactosyldiglyceride (MGDG), 1,2-diacyl-sn-glycero-3-(6-sulpho-α-D-quinovopyranoside) (SQDG), and glycolipid II of Constantopoulos and Bloch (1967) are also present. In contrast, members of the Rhodospirillaceae have a large variety of phospholipids and only traces of glycolipid. In all strains examined phosphatidyl ethanolamine (PE), phosphatidyl glycerol, and phosphatidyl choline (PC) are present. An ornithine phospholipid is also present, while diphosphatidyl glycerol is nearly always present, and phosphatidic acid (PA) occurs in some species. Of the Chloroflexaceae only Chloroflexus aurantiacus has been examined (Kenyon & Gray, 1974), and is distinctive in that it has the phospholipids phosphatidyl inositiol (PI) and phosphatidyl glycerol, and the glycolipids MGDG,

SQDG, and digalactosyldiglyceride (DGDG). The thermophilic nature of this species may, however, influence the lipid composition, and until members of the genera Chlornema and Oscillochloris, or Gorlenko's mesophilic Chloroflexus aurantiacus f. mesophilus (Gorlenko, 1975; Pivovarova & Gorlenko, 1977) are examined care should be taken in interpreting the results. The paucity of data on the lipid composition of members of the Chromatiaceae make it difficult to draw conclusions. To date only the species Chromatium vinosum, Thiocapsa roseopercina, Ectothiorhodospira halophila, and E.mobilis have been studied (Kenyon, 1978; Oyewole & Holt, 1976; Steiner et al., 1969; Takacs & Holt, 1971). Although the results show that the lipid composition of Chr.vinosum and Th.roseopercina are very similar, the halophilic E.halophila has a distinctive lipid composition. The lipid analysis of E.mobilis is not sufficiently comprehensive to determine whether the lipid composition of the genus Ectothiorhodospira is distinctive, or the lipids of E.halophila a peculiar feature of the halophilic existence. In Chr.vinosum, Th.roseopercina, and E.mobilis the major phospholipids are phosphatidyl ethanolamine and phosphatidyl glycerol (Kenyon, 1978; Oyewole & Holt, 1971; Steiner et al., 1969; Takacs & Holt, 1976). Diphosphatidyl glycerol is also present in Chr.vinosum and Th.roseopercina, but not in E.mobilis. The glycosyl analogues of MGDG and DGDG are found in Chr.vinosum and Th.roseopercina, but have not been looked for in E.mobilis and E.halophila. The major phospholipids of the halophilic E.halophila are not like those of the members of the Halobacteriaceae (Kates, 1978; Raymond, unpublished, in Kenyon, 1978), and are diacyl forms of phosphatidyl glycerol, phosphatidyl ethanolamine, and an ornithine phospholipid. Possible differences between the lipids of members of the Chromatiaceae and other families are suggested, but the relationship of the genus Ectothiorhodospira to the Chromatiaceae requires further investigation.

3.3. Ecological aspects.

A number of environmental parameters play an important role in influencing which species of the Rhodospirillales predominate. The importance of sulphide concentration and pH on the growth of the purple and green bacteria was first comprehensively described by van Niel (1931). On the basis of their tolerance and optimal growth in varying pH and sulphide concentrations van Niel divided the sulphur purple and green bacteria into three groups. The purple non-sulphur bacteria (Rhodospirillaceae) were distinguished by their intolerance of low sulphide concentrations, irrespective of the pH, and constituted a fourth group separate from the sulphur purple and green bacteria. Group I comprised the purple sulphur "Pseudomonas type", which deposited sulphur outside the cell, and was probably identical to the genus Ectothiorhodospira. These organisms tolerated pH values between 8.4 and 10.4 over a fairly wide range of sulphide concentrations, up to 0.2-0.3% (w/v). Group II, which contained members of the Chromatiaceae, deposited sulphur inside the cell, and tolerated pHs between 6.5 and 9.5 if the sulphide concentration was low, but raising the sulphide concentration to 0.1-0.2% restricted growth to pH 8.5 to 9.0. The third group (III) contained the Chlorobiaceae, showed optimal growth at pH 6.5 to 8.5, more or less irrespective of the sulphide concentration.

The groupings used by van Niel were based on relatively few strains, but the properties of the groups, with respect to pH optima and sulphide tolerance, may still be used for selective enrichment of the various genera. The medium devised by Pfennig (1965a) is suitable for the maintainance of most members of the Rhodospirillales when adjusted for the peculiarities of some species. Restricting the wavelength of the incident illumination enables enrichment of species absorbing maximally in that region. The use of wavelengths greater

than 900nm allows selective enrichment of bacteriochlorophyll b containing species, and wavelengths below 800nm enrichment of green sulphur bacteria, bacteriochlorophyll a containing species being enriched at intermediate wavelengths (Pfennig, 1965a; Whittenbury, 1971). Gas vacuolate forms are usually selected by growth temperatures of 10-20°C, and float to the surface when the temperature is lowered to 4-10°C (Whittenbury, 1971). Many of the large members of the Chromatiaceae grow preferentially at 15-20°C (Pfennig, 1965a), and some thermophiles may be isolated at temperatures in excess of 35°C (Imhoff & Trüper, 1977; Pierson & Castenholz, 1974a; 1974b; Raymond & Sistrom, 1967). At sulphide concentrations of 0.1-0.2% the majority of the sulphur purple and green bacteria may be cultured, but some forms, in particular the gas vacuolate species, large members of the Chromatiaceae, and the brown Chlorobiaceae are favoured by lowering of the sulphide concentration to 0.01-0.05% (Pfennig, 1965a; 1978b; Whittenbury, 1971). Members of the Rhodospirillaceae are generally intolerant of high sulphide concentrations, but some species may be isolated from sulphide containing media (Hansen & Veldkamp, 1973; Keppen & Gorlenko, 1975). Particular species are usually selectively enriched using appropriate carbon sources (van Niel, 1944; 1971; Whittenbury, 1971). Some species of the Rhodospirillales do not require the addition of vitamins to the medium, but the majority require some growth factors for satisfactory growth. The number and nature of the growth factors added to the medium has some effect in determining which species predominate (Pfennig, 1965a; 1978a; 1978b; van Niel, 1971; Whittenbury, 1971). The various species of Rhodospirillales do not occupy the same position in the water column and are, therefore, subjected to different light intensities (Dubinina & Gorlenko, 1975; Fenchel & Staarup, 1971; Gorlenko & Kusnetsov, 1971). At high light intensities

most of the small members of the Chromatiaceae and some of the Chlorobiaceae have an advantage, while at lower light intensities most of the large members of the Chromatiaceae and gas vacuolate forms have an advantage (Pfennig, 1965a; 1978b; Whittenbury, 1971). This is related to the light saturation levels of the photometabolism of the various species (Pfennig, 1978b).

It is, therefore, possible, by selecting the appropriate conditions to produce an enrichment medium and environment which will favour a particular group. The species which are isolated, however, depend upon the natural population. Little work has been carried out on the isolation of members of the Rhodospirillales from environments other than freshwater and marine environments. A number of early reports of purple or green bacteria in acidic, alkaline, or saline environments were not supported by isolation and identification of the organisms concerned. This situation is changing as people look for them in more extreme environments.

3.4. Rhodospirillales from the alkaline environment.

Phototrophic microorganisms occur in large numbers in soda lakes, colouring the water shades of green, red, orange, and purple (Melack, 1978; Imhoff et al., 1978b). Vast blooms of algae and cyanobacteria were reported in the earliest scientific investigations of alkaline lakes (Beadle, 1932; Isachenko, 1934; Jenkin, 1932; 1936; Rich, 1932; 1933). Although much of the primary productivity is due to the cyanobacteria and algae (Talling et al., 1973) there is relatively little information on the occurence of purple and green bacteria.

Baas-Becking et al. (1960) recorded the pH range of the purple and green bacteria, but made no reference to genera or species. Van Niel (1931) also recognised that some of the purple bacteria grew optimally at pH 9.0-10.0.

Isachenko's (1934; 1951) study of the soda lakes of the Kulunda

Steppe of central Russia was one of the earliest microbiological investigations of an alkaline environment. Isachenko found that the lakes contained cyanobacteria and algae in such large numbers that they turned the water green. These soda lakes, which were refered to as Tanatar I, II, and III, had pHs of 9.6, 11.6, and 11.06 respectively. Unfortunately no analysis of the water chemistry appears to have been made. By microscopic observation Isachenko was able to describe several species of purple sulphur bacteria, although they were apparently not isolated, so their description was based entirely on morphological characteristics. In Tanatar I the species Chr.gracile, Chr.vinosum, and Thiopedia rosea were observed, but in Tanatar III only the species Thiospirillum rosenbergii was found.

Baas-Becking (1928) was able to isolate a halophilic purple bacterium for Searles Lake and Owens Lake, California, which he named "Rhodospirillum halophilum", indicating it did not deposit sulphur inside the cell, and that it was morphologically similar to the non-sulphur spirilla rather than the pseudomonads. Although van Niel (1931) had the opportunity to examine the same lakes and isolated a similar organism it was lost before a thorough study of it could be made.

Tew's (1966) study of Owens Lake specifically examined the effect of water limitation on the growth of an anaerobic photosynthetic bacterium, which was identified as "a member of the group of small Chromatium species promoting sulphur formation in the medium." This feature of the isolate was unusual, but the organism was not fully characterised to determine the taxonomic relationship of the intracellular sulphur depositing species to this extracellular depositing species, which was probably a member of the genus Ectothiorhodospira.

Some years ago, in the alkaline highly saline lakes of the Wadi Natrun, Egypt, Jannasch (1957) observed a number of purple and green bacteria, which he was able to enrich in culture, but not isolate. The predominant organisms were members of the genera Chromatium and Thiospirillum. Recently this group of alkaline, saline lakes have been the subject of an ecological and microbiological study (Imhoff et al., 1978a; 1978b). The salinity of the lakes varies with the season and among themselves (Imhoff et al., 1978b). The waters were strongly coloured according to the predominant organisms. Algae, cyanobacteria, purple and green bacteria, and halobacteria were the organisms which occured in mass developments in the water and overlying the mud. Imhoff et al. (1978a; 1978b) reported that both green and purple bacteria were present, and described members of the large Chromatium species, which could not be isolated, and spirilla. The spirilla were isolated and found to belong to the genus Ectothiorhodospira.

The species described from the Wadi Natrun were, Ectothiorhodospira halophila (Imhoff et al., 1978 ; 1978b), E.halochloris (Imhoff & Trüper, 1977), E.abd-el-malekii (Imoff & Trüper, 1978; in preparation), and E.mobilis (Imhoff et al., 1978a). With the exception of E.mobilis, which is moderately halotolerant, the species from the Wadi Natrun are extremely halophilic, requiring 15-20% NaCl for optimal growth.

Members of the genus Ectothiorhodospira have been isolated from freshwater and saline environments as well as from highly alkaline environments, although the pH of the environment has always been on the alkaline side of neutrality.

The genus Ectothiorhodospira was first described by Pelsh (1937), when an organism capable of sulphur deposition outside the cell was isolated from a gypsum lake in the Crimea. It was not a green

sulphur bacterium, so it was placed in the Thiorhodaceae, with the suggestion that a new taxon be created for it, the Ectothiorhodaceae being a counterpart of Baas-Becking's Endothiorhodaceae. A number of workers studied the organism (Chesnokov & Shaposnikov, 1936a; 1936b; Shaposnikov, 1937a; 1937b), but the original isolate was lost. Trüper (1968) isolated an organism which deposited sulphur outside the cell and was a member of the Rhodospirillineae from salt flats in the Galapagos Islands. He concluded that his isolate was similar to Pelsh's original, and named it Ectothiorhodospira mobilis. Although Pelsh (1937) was the first to recognise the taxonomic position of this organism other workers have reported spirilla from the aquatic environment, which may have been Ectothiorhodospira species (Onitskaya, 1954; Butlin & Postgate, 1954; Jannasch, 1957; Scardovi, 1950).

A number of Russian scientists worked with "autotrophic Rhodopseudomonas" species, which were later named Ectothiorhodospira shaposnikovii (Kondrat'eva, 1956; Kondrat'eva & Malofeeva, 1964; Moshentseva & Kondrat'eva, 1962; Upsenkaya & Kondrat'eva, 1962; Yang Hui-Fang, 1962). Other autotrophic species of Rhodopseudomonas, "Rhps.issatchenkoi" (Onitskaya, 1954), and "Rhps.vanmielii" (Scardovi, 1950) may also have been Ectothiorhodospira species (Trüper, 1968). Butlin & Postgate (1954) also observed spirilla in mass accumulations of phototrophic bacteria from a gypsum lake in Cyrenaica, which may have been Ectothiorhodospira species. Whether the Thiospirillum rosenbergii reported by Isachenko in Tanatar III (Isachenko, 1931; 1954) was in fact an Ectothiorhodospira species has not been determined. There is a large size difference between them, but it may have been possible to confuse the two. Imhoff and Trüper (1976) isolated E.mobilis and a number of other purple and green bacteria from marine sponges. Matheron and Bauliague (1972)

Herbert and Tanner (1977), and Trüper (1970) found Ectothiorhodospira species in the sea. Members of the genus Ectothiorhodospira are, however, most commonly found in alkaline environments (Imhoff et al., 1978a; 1978b; Grant et al., 1979)

The members of the genus Ectothiorhodospira are motile non-gas vacuolate organisms (table 5). They deposit sulphur outside the cell, never inside the cell, and have a stack-like intracytoplasmic membrane system, which is visible in negatively stained preparations of the cells. With the exception of E.shaposnikovii they are halophilic, E.mobilis requiring 3% (w/v) NaCl for growth, and the other species 15-25% (w/v) NaCl. The species E.mobilis and E.shaposnikovii are presently distinguished from one another by the ability of E.shaposnikovii to grow in a medium without added NaCl, its possession of a textured cell wall, and different GC base content, although the current data suggest little difference (Trüper, personal communication). The reported differences are somewhat dubious and may be due to ecological influences, cultural conditions, or experimental peculiarities. The two species are likely to be different strains of a single species (Trüper, personal communication).

E.mobilis, E.shaposnikovii, and E.halophila contain bacteriochlorophyll a and carotenoids of the normal spirilloxanthin series (group 1a, figure 8). E.halochloris and E.abd-el-malekii both have bacteriochlorophyll b and glucosides of rhodopin as the major photopigments (Imhoff & Trüper, 1977; personal communication). The morphology of the cells varies under different cultural conditions, but they are all slightly bent to spirilloid under some conditions. E.mobilis and E.shaposnikovii are motile by a polar tuft of flagella, and are 0.7-1.0µm wide by 1.5-2.5µm long, the GC base content being in the range 62-64 mole% (Trüper, personal communication). The exteremly halophilic species are bipolarly flagellate, and are

narrower, 0.5-0.8µm wide, and longer, 2.5-8.0µm long, with a more pronounced spirilloid shape (Imhoff & Trüper, 1977; personal communication; Raymond & Sistrom, 1967; 1969). These species are also moderately thermophilic, having temperature optima of 45-50°C. The GC base content varies from 52.9 mole% (E.halochloris) to 68.4 mole% (E.halophila) (Imhoff & Trüper, 1977; Pfennig & Trüper, 1974).

Although the reported pH optima of the various species range from pH 7.5 to 9.5 growth conditions may affect the pH optima markedly (Chesnokov & Shaposnikov, 1936a; van Niel, 1931). There is little doubt that the genus Ectothiorhodospira appears to have a selective advantage at high pH, since in the presence of organic acids E.mobilis is able to out grow a 20-fold higher density of Chr.vinosum if the pH is adjusted to pH 8.3-8.9 (Trüper, in preparation).

A further example of a phototrophic bacterium growing under alkaline conditions is seen in the alkaline hot springs in Yellowstone National Park (Pierson & Castenholz, 1974a; Bauld & Brock, 1973; Brock, 1978). The thermophilic Chloroflexus aurantiacus is usually found in a stratified association with cyanobacteria as a thick gelatinous mat. The organism has been found in most hot springs examined for its presence (Madigan et al., 1974; Florenzano et al., 1978). The pH optimum is similar to that reported by Trüper (1968) for E.mobilis. A mesophilic form has been described (Gorlenko, 1975; Pivovarova & Gorlenko, 1977), but little is known of the ecology of this genus outside the hot springs. Chloroflexus aurantiacus usually occurs in association with cyanobacteria, but when the sulphide level is too high a mono-specific mat of Chlf. aurantiacus is formed (Castenholz, 1977). In the hot springs examined the water does not evaporate to become more concentrated, so it is unlikely that this species is ever subjected to pHs higher than 8.5 due to bicarbonate saturation. Chloroflexus is also interesting for

its apparent occurrence in the fossil record as bacterial stromatolites (Baarghorn & Tyler, 1965; Doemel & Brock, 1974).

4. THE EXTREMELY HALOPHILIC BACTERIA.

4.1. Taxonomy of the extremely halophilic bacteria.

Extremely halophilic bacteria of the genera Halobacterium and Halococcus impart an orange-red colouration to their habitat (Kushner, 1968; Dundas, 1971; Larsen, 1967), and man has been aware of this property for several thousand years (Baas-Becking, 1931). More than 4,000 years ago in the reign of the ancient Chinese Emperor Huang, Shu-Sha perfected the art of extracting salt from sea water by solar evaporation. The sea water was channeled into artificial lagoons, where it was allowed to evaporate until the liquor became a red colour, when salt began to be precipitated. The old Egyptian cultures appear to have used a similar method to obtain solar salt, and in the time of Pliny there were numerous salterns around the Mediterranean (Jones, 1963). The quality of the salt was often judged by the colour, which Pliny notes could only be removed by oils, and not by washing with water alone. We can be almost certain that the colour of the saltern liquor was caused by pigmented halobacteria and halococci from the studies of salterns which exist in the World today (Dundas, 1978).

Since they were first discovered the extremely halophilic rods and cocci have been a problem to the taxonomist. Variously placed as members of the genera "Pseudomonas" (Harrison & Kennedy, 1922), "Serratia" (Lochhead, 1934), "Bacillus" (Klebhan, 1919), "Bacterium" (Petter, 1931), "Flavobacterium" (Elazari-Volcani, 1940), and "Halobacter" (Anderson, 1954), the Gram-negative, extremely halophilic rods are now classified in the genus Halobacterium (Gibbons, 1974). In the 7th. edition of Bergey's manual (1957) the genus Halobacterium was placed in the family "Pseudomonadaceae", while the Gram-negative halococci were placed in the family "Micrococcaceae" as the species "Micrococcus morrhuae", "Sarcina littoralis", and "Sarcina morrhuae". In

the 8th edition of Bergey's manual, Gibbons (1974) recognised the similarity between the extremely halophilic rods and cocci, and placed them in the newly created family Halobacteriaceae, at the same time reducing the halococci to a single species, Halococcus morrhuae (Kocur & Hodgkiss, 1973), which included the three previously recognised species. At present only two species are recognised in the genus Halobacterium, in the 8th edition of Bergey's manual (1974), H.halobium and H.salinarium, with three other species, H.marismortui, H.trapanicum, and Amoebobacter morrhuae listed as species incertae sedis. The unsatisfactory nature of the taxonomy of the group is illustrated by the fact that in the current literature the species names proposed by Elazari-Volcani (1957), in the 7th edition of Bergey's manual are still in use. In particular there is still debate on whether the species H.cutirubrum, H.halobium, and H.salinarium are separate strains of a single species, or distinct species (Colwell et al., 1979; Dundas, 1978; Gibbons, 1974; Stoeckenius, 1978a). Moore and McCarthy (1969a; 1969b) used DNA hybridisation experiments to try to resolve the taxonomic position of the species H.cutirubrum, H.halobium, and H.salinarium, and they proposed that, although there were differences between the three species they were not very great. Zwilling and coworkers' (1969) investigation of immunological reactions within the Halobacteriaceae failed to find any correlation between species and the immunological groupings. The species H.trapanicum and "Amoebobacter morrhuae" are poorly studied, and the species "H.marismortui" has apparently been reisolated (Ginzburg, 1978). Three new species have recently been described, H.volcanii (Mullakhanbhai & Larsen, 1975), H.saccharovorum (Tomlinson & Hochstein, 1976), and H.vallismortis (Gonzalez et al., 1978). Other species to be found in the literature include "H.gibbonsi", "H.minuteum", and "H.simoncinii" (Colwell et al., 1979; National Collection

of Marine Bacteria Catalogue, 1979). Despite recent advances in our knowledge of the halobacteria and halococci their taxonomic differentiation is still inadequate, since many of the biochemical properties vary from strain to strain (Colwell et al., 1979), and others with the salt concentration (Gibbons, 1957). The few distinguishing features include DNA content, optimal salinity, resistance to some halophilic phages, antibiotic susceptibility, production of acid and gas from carbohydrates, and the metabolism of nitrate and nitrite (Dundas, 1978; Gibbons, 1974; Post, 1977; Torsvik & Dundas, 1978).

Members of the genus Halobacterium are Gram-negative, non-spore forming organisms. The cells may be motile by polar flagella, or are non-motile. Cellular morphology under optimal growth conditions is typically a rod 0.7-1.0µm wide by 3.0-5.0µm long, although H.volcanii is disc or cup-shaped, 1.0-3.0µm by 2.0-3.0µm and 0.4-0.5 µm in thickness (Gibbons, 1974; Mullakhanbhai & Larsen, 1975).

The single species in the genus Halococcus is a coccus 0.8-1.5µm in diameter, and occurs singly, in pairs, or tetrads (Gibbons, 1974; Kocur & Hodgkiss, 1973). The Gram reaction has been described as positive (Brown & Cho, 1970) and negative (Gibbons, 1974; Kocur & Hodgkiss, 1973), reflecting the difficulty in staining members of the Halobacteriaceae. All species of the Halobacteriaceae are aerobes, but a single facultative anaerobe is known, H.vallismortis (Gibbons, 1974; Gonzalez et al., 1978). Although all strains may be cultured in media containing peptone, yeast extract, tryptone, and casamino acids (Gibbons, 1969), some species are capable of utilising carbohydrates with the production of acid (Gonzalez et al., 1978; Gibbons, 1974; Tomlinson & Hochstein, 1976). Most strains produce H_2S from peptone or thiosulphate, and indole from peptone. Most species are able to hydrolyse gelatin and casein. All species

investigated are sensitive to novobiocin, but are insensitive to a large number of antibiotics, including penicillin (Colwell et al., 1979; Gibbons, 1974; Kocur & Hodgkiss, 1973). The ability to reduce nitrate to nitrite, with or without the production of gas, and the production of acid and gas from carbohydrates varies from species to species (Dundas, 1978; Elazari-Volcani, 1957; Hochstein, 1978; Larsen, personal communication; Stoeckenius, 1978a; Tomlinson & Hochstein, 1972; 1976). The halobacteria and halococci are orange-red in colour due to the presence of carotenoids, mainly C_{50} bacterioruberins (Kates, 1978; Kelly & Liaaen-Jensen, 1967; Kelly et al., 1970; Kushwaha et al., 1974). The optimal NaCl concentration for growth is usually 4.0-5.0M, except for H.volcanii, where the optimum varies between 1.7 and 2.5M with temperature (Mullakhanbhai & Larsen, 1975). The cells of the halobacteria, but not the halococci lyse in distilled water.

4.2. Structure and biochemistry of the Halobacteriaceae.

Organisms living under low water potential, caused by the highly saline environment, appear to maintain a water potential of similar magnitude within the cell (Brown, 1978; Bayley & Morton, 1978; Kushner, 1978b). In algae of the genus Dunaliella, and in some yeasts this is achieved by the synthesis of glycerol or other polyols. There is some difficulty in determining the intracellular levels of the ions in these organisms, but it is assumed that the total salinity of the cells is lower than that of the suspending medium, although it is not known if a lowering of the internal salinity is brought about by an active transport process, or by an exclusion mechanism (Brown, 1978; Kushner, 1978b; Wegmann, 1978). In their work with certain halotolerant bacteria Matheson et al. (1976), Shindler et al.,(1977), and Christian and Waltho (1962) found that K^+ was concentrated by a factor of 10-100 times over the concentration

in the growth medium. In the organisms which the above authors examined the internal salinity, with respect to Na^+ and K^+, could be either greater or less than that of the medium, but these determinations have indicated that the Na^+ concentration was always less within the cell than in the medium. Although Kushner (1978b) has postulated that polyols may also be involved in the halotolerance of these bacteria, the existence of polyols has not been confirmed.

In contrast, the Halobacteriaceae actively maintain an internal salt concentration equal to or in excess of that in the external environment. Investigating the ionic composition of the halobacteria's cytoplasm Christian and Waltho (1962) found an unusually high concentration of K^+, 4.6M, and a lower concentration of Na^+, 1.4M. The total Cl^- content was estimated to be 3.6M. These results, which are substantiated by later studies (Ginzburg et al., 1970; Lanyi & Silverman, 1972; Matheson et al., 1978) show that the halobacteria and halococci are capable of maintaining a K^+ gradient across the membrane as high as 1,000:1. This selective uptake of K^+ is not confined to the Halobacteriaceae, but the magnitude of the K^+ concentration is a peculiar feature of the family. The effect of the high K^+ content is reflected in the physiological requirements of some of the enzymes and the ribosomes (Bayley & Morton, 1978; Caplan & Ginzburg, 1978; Kushner, 1978b; Matheson et al., 1978). A large number of enzymes of the halobacteria are extremely halophilic and rapidly denature when the salinity is lowered. In some cases it is possible to use salt-free techniques and renature the enzyme against 25% NaCl, but not by adding salt or a salt solution directly (Holmes & Halvorson, 1965). All enzymes studied show an appreciable activity at the optimal salinity for growth. They are not, however, all extremely halophilic, but even the most salt intolerant enzymes have a greater activity at the optimal salinity for growth, 4.0M NaCl,

than their equivalents from halotolerant or non-halophilic organisms (Bayley & Morton, 1978; Larsen, 1967; Kushner, 1978b). In the halophilic Dunaliella species and yeasts the enzymes are intolerant of NaCl, but are unaffected by high concentrations of glycerol (Kushner, 1978b). In members of the Halobacteriaceae two physiological groups of enzymes are described, those with maximal activity at 2-4M NaCl, and those with maximal activity at 0.5-1.5M NaCl (Bayley & Morton, 1978). The role of ions in the stability of the halobacteria's enzymes is now partly elucidated. It has been proposed that the salt requirement of these enzymes is to screen the charged parts of the protein (Baxter, 1959). Such a screening effect would, however, be complete at mono-valent ion concentrations of 1M or less, and it is proposed that the salt in excess of the screening effect is required to increase the hydrophobic nature of the enzymes (Bayley & Morton, 1978; Lanyi, 1974). The thermophilic nature and cold lability of some enzymes has been interpreted as evidence in support of this theory (Lanyi, 1974; Keradjopoulos & Wulff, 1974). The effectiveness of anions which promote hydrophobic bonding in maintaining the activity of the enzymes, rather than those which help decrease the hydrophobicity of the molecule also indicates the function of the high internal salinity (Higa & Cazzulo, 1975; Keradjopoulos & Wulff, 1974; Lanyi & Stevenson, 1970; Lieberman & Lanyi, 1971).

Studying the ionic requirements of the halobacteria Brown and Gibbons (1955) and Sehgal and Gibbons (1960) found that besides Na^+ and Cl^-, K^+, Mg^{2+}, Fe^{2+}, and Mn^{2+} were also stimulatory to growth. A deficiency of K^+ reduced the cell numbers, but no other effects were observed. The optimal concentration of K^+ was 15-30mM, but concentrations of 3M were not inhibitory. In a medium lacking Mg^{2+} the cells of the halobacteria lost their rod-shaped morphology and

became spherical. In a series of experiments to investigate adaptation to low Mg^{2+}, Brown and Gibbons (1955) found that, although the optimal level of Mg^{2+} was 0.1-0.5M, it was possible to subculture isolates on media of low Mg^{2+} content, 0.01M. Under these conditions the cells were spherical and continued subculturing on Mg^{2+} deficient medium led to adaptation of some of the strains to grow optimally at this concentration. The morphology of these strains was, however, always spherical even after transfer to a medium of higher Mg^{2+} content, 0.1M. High concentrations of Mg^{2+} in excess of 1.0M were found to be toxic, although H.volcanii is tolerant of concentrations approaching 2.0M (Mullakhanbhai & Larsen, 1975).

The requirement for relatively high Mg^{2+} concentrations may be related to ribosome structure and function, since the optimal concentration for ribosomal activity in H.cutirubrum is 3.4M K^+ and 100mM Mg^{2+} (Bayley & Morton, 1978). This is similar to the ionic composition of the cytoplasm. In contrast, the ribosomes of Vibrio costicola, a moderate halophile, require 150mM K^+ or NH_4^+ and 18mM Mg^{2+} for in vitro protein synthesis, and these values are very similar to those found in non-halophiles such as E.coli (Matheson et al., 1978).

When cells of halobacteria are subjected to solutions of low ionic strength they loose their rod-shaped morphology and may eventually lyse, whereas cells of the halococci are stable under these conditions. The change in cell shape and eventual lysis has been demonstrated to involve disruption of the cell envelope of the halobacteria (Steensland & Larsen, 1969; Stoeckenius & Rowen, 1967). The cell envelope consists of two distinguishable units, and are described according to Salton's (1964) criteria. The outer layer of the envelope, the cell wall confers the shape and rigidity of the cell, and the inner layer resembles the cytoplasmic membrane.

In early studies of the cell envelope of the halobacteria peptidoglycan was not detected, and electron microscopy revealed the absence of a cell wall, an observation not inconsistent with the absence of peptidoglycan (Brown & Shorey, 1963; Kushner et al., 1964; Kushner & Bayley, 1963; Houwink, 1956; Mohr & Larsen, 1963). However, improved fiaxtion techniques revealed a largely proteinaceous cell wall devoid of peptidoglycan (Blaurock et al., 1976; Cho et al., 1967; D'Aoust & Kushner, 1968; Kirk & Ginsburg, 1972; Steensland & Larsen, 1969; Stoeckenius & Rowen, 1967). In the halobacteria, lowering of the ionic strength of the suspending medium results in a transformation from rods to spheres (Mohr & Larsen, 1963; Steensland & Larsen, 1969; Stoeckenius & Rowen, 1967). There is no apparent increase in cell size during this transformation, indicating that the morphological change is not caused by uptake of solvent (Mohr & Larsen, 1963). Further lowering of the ionic strength of the medium results in lysis of the cells. The transformation and lysis is too rapid to be caused by enzymic action on the cell envelope (Kushner & Onishi, 1966; Mohr & Larsen, 1963; Onishi & Kushner, 1966). During the morphological transformation the proteinaceous cell wall dissociates as the concentration of NaCl is lowered (Kushner & Onishi, 1966; Kushner et al., 1964; Onishi & Kushner, 1966; Steensland & Larsen, 1969; Stoeckenius & Rowen, 1967). Further lowering of the salinity results in lysis of the spherical cells, with the formation of small membranous sheets (Steensland & Larsen, 1969; Stoeckenius & Kunau, 1968; Stoeckenius & Rowen, 1967), which will reassociate into vesicles when KCl or NaCl is added to the suspending solution (Onishi & Kushner, 1966). The cell wall is most effectively prevented from distintegrating by NaCl, while the cell membrane can be maintained in much lower concentrations of KCl, NH_4Cl, LiCl, $MgCl_2$, or $CaCl_2$ (Kushner & Onishi, 1966; Kushner et al., 1964; Mohr & Larsen, 1963; Stoeckenius & Rowen, 1967). Thus, the outer most layer of the cell shows a high degree of specificity fo NaCl, the major ions

present in the external environment, while many of the cytoplasmic enzymes and ribosomes have a greater specificity for KCl, the major ion present in the cytoplasm.

In the genus Halobacterium the cell wall has been extensively studied by sodium dodecylsulphate (SDS) polyacrylamide gel electrophoresis, largely in H.salinarium (Mescher & Strominger, 1976a; 1976b; 1978; Mescher et al., 1974). 15 to 20 proteins have been detected by Coomassie Brilliant Blue stain, most having a molecular weight of 130,000 daltons or less. A single carbohydrate positive protein band was also detected, with a molecular weight of approximately 200,000 daltons. This glycoprotein accounts for 40-50% of the total envelope protein. The carbohydrate fraction of the glycoprotein amounts to 10-20%, and accounts for the total amino-sugar and non-lipid hexose found in the cell envelope. Tryptic digestion of the glycoprotein releases two glycopeptides, the larger of which contains galactose, uronic acid, di- and trisaccharides of glucose, bound by O-glycosidic bonds to the hydroxyl groups of threonine residues. A long-chain amino sugar is found in the smaller peptide bound to the amide group of asparagine via a N-glucosylamine bond. Similarities to eukaryotic glycoproteins have been recognised in the nature, size, and composition of the carbohydrates units, their localisation to limited regions of the protein, and the nature of the protein-carbohydrate linkages. Evidence for the structural role of this glycoprotein is discussed by Mescher and Strominger (1976b; 1978). Growth in a protease containing medium results in removal of the glycoprotein and a concomitant formation of spherical cells. The viability of the cells is not affected and the spherical forms regain their rod-shaped morphology as the glycoprotein reappears, when transfered to a protease free medium. Growth in the presence of bacitracin, which inhibits glycoprotein glycosylation,

also brings about the change of rods to spheres. Other selective inhibitors have been used, and the results confirm that it is the glycoprotein which determines the cell's morphology and rigidity. A glycoprotein has also been found in H.halobium and H.cutirubrum (Koncewicz, 1972; Meścher, personal communication).

The cell wall of Halococcus morrhuae is quite different and readily apparent in electron micrographs (Kocur et al., 1972; Brown & Cho, 1970; Steensland & Larsen, 1971). Murein was, however, shown to be absent, despite similarities between the fine structure of the cell walls of the micrococci and Halococcus morrhuae (Brown & Cho, 1970; Kocur et al., 1972; Reistad, 1970; Steensland & Larsen, 1971). The treatment of the cell envelope with trypsin removes the proteins, which account for 2% of the dry envelope weight, without affecting the rigidity of the cell wall (Steber & Schleifer, 1975). The major components of the cell wall are known to be carbohydrates, which represent about 60% of the dry cell wall (Brown & Cho, 1970; Reistad, 1972; 1974; 1975; 1977; Steber & Schleifer, 1975). The carbohydrates are apparently present in the form of a sulphated heteropolysaccharide, which functionally replaces murein, and has a similar function to that of the glycoprotein found in the halobacteria. The structure of the heteropolysaccharide is not known, but similarities to chondroitin sulphate and eukaryotic marine algal polysaccharaides are suggested (Steber & Schleifer, 1975) A more recent study of the cell wall of Hc.morrhuae (Steber & Schleifer, 1979) suggests that the major structural component of the cell wall may be either a heteropolysaccharide, or a novel peptidoglycan.

The absence of murein in the cell wall has been regarded as an adaptation to life in extreme environments, in particular high salinity (Dundas, 1977; Kushner, 1968; Larsen, 1967). Novitsky and Kushner (1976) have shown that the chemical composition of the

peptidoglycan in Planococcus halophilus was altered by changes in the salinity of the medium. Weiss (1974) has postulated that murein is unstable in an acidic environment at high temperature, which may account for the absence of muramic acid in Sulfolobus acidocaldarius. However, Kandler and Hippe (1977) have attributed the lack of murein in Methanosarcina barkerii not to the environment, but to its possible separate evolutuionary origin, and indeed a novel view of the Halobacteriaceae has arisen from the comparison of this family with a group of non-halophilic organisms, which include the extremely fastidious anaerobic methanogenic bacteria, and certain thermophilic acidophilic bacteria. A study of the base sequence (oligonucleotide catalogue or pattern) of the 16S ribosomal RNA (rRNA) of the methanogenic bacteria indicated that it was different to the 16S rRNAs of a variety of other prokaryotes, including cyanobacteria, and different to the 18S rRNA of eukaryotic cells (Fox et al., 1977). Similarities to the other prokaryotes were no greater than the similarities between these prokaryotes and eukaryotes, indicating that the methanogenic bacteria constituted a separate and distinct evolutionary line. Thus, the prokaryotes were divided into two groups, the eubacteria and the archaebacteria (Balch et al., 1977; Woese & Fox, 1977). It was suggested that the archaebacteria had properties which may have been suited to the Archaean ecology. Later, thermoacidophiles of the genera Sulfolobus and Thermoplasma were also shown to belong to the archaebacteria (Woese et al., 1978). Magrum et al. (1978) also found that the extreme halophiles of the genera Halobacterium and Halococcus showed similarities to the methanogenic bacteria and, therefore, also placed them in the archaebacteria. A corelation between 16S rRNA base composition and the cell envelope structure was also noticed, in that archaebacteria did not have muramic acid and, therefore, the peptidoglycan, murein, was also

absent (Woese et al., 1978). The lipids of this group were also unusual in that they were branched chain isoprenoids, and the main polar lipids were derivatives of isopranyl side chains linked to glycerol in the L-α,β- or sn-2,3- configuration via ether bonds (De Rosa et al., 1974b; Kates, 1978; Tornabene & Langworthy, 1979). The evidence which has been presented indicates that the archaebacteria show some properties typical of eukaryotes, but they also have typical eubacteria features (Woese et al., 1978). Whether these organisms arose from an evolutionary line separate from the rest of the prokaryotes (eubacteria) and the eukaryotes remains to be answered, and the interpretation of the evidence requires caution.

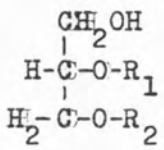
In view of these recent findings it is now necessary to review the interpretation of the halophilic mode of life of the Halobacteriaceae. In the archaebacteria at least three types of cell wall have been described, all lacking muramic acid. In Gram-positive methanogenic bacteria two cell wall types have been described, one consisting of a novel peptidoglycan, which has been named pseudomurein (Kandler & Konig, 1978; Kandler, 1979; Konig & Kandler, 1979a; 1979b), and the other is a heteropolysaccharide, which although not sulphated resembles the polymer found in Hc.morrhuae in chemical composition and its appearance in electron micrographs (Kandler & Hippe, 1977; Zhilina, 1971; Zhilina & Zavarzin, 1979). The Gram-negative methanogenic bacteria have largely proteinaceous cell walls (Jones J et al., 1977; Kandler & Konig, 1978), of which three types are recognised (Balch et al., 1979), and that type found in the genera Methanogenium and Methanococcus may resemble the largely glycoprotein cell wall of H.salinarium (Jones J et al., 1977; Romesser et al., 1979). Thus, it seems that the lack of murein is due to the evolutionary origins of the Halobacteriaceae, and not to the high salinity of their natural habitat.

The lipid composition of members of the Halobacteriaceae has been shown to be characteristic of the family (Kates et al., 1966; Mullakhanbhai & Francis, 1972). The predominant lipids are phospholipids, but they are not diacylglycerol derivatives, and there are no major lipoamino acids. The phospholipids are probably entirely associated with the cell membrane (Kates, 1972a; Kushner et al., 1964). Acid methanolysis of the phospholipids of members of the Halobacteriaceae does not release major amounts of fatty acids, and the major component is 2,3-di-O-phytanyl-sn-glycerol (Kates et al., 1963; 1965a; 1965b). The configuration of the glycerol residue is opposite to that of the diacyl analogues (figure 15), they are mirror images (Kates, 1978; Kates & Kushwaha, 1978). The side chains of these diether lipids is a dihydrophytol, 3R,7R,11R,15-tetramethyl-hexadecyl or phytanyl (Kates, 1972a; 1978; Kates & Kushwaha, 1978), and is structurally related to the esterifying alcohol of some chlorophylls, phytol (Gloe et al., 1975; Kates, 1972b).

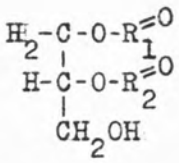
The phospholipids found in members of the Halobacteriaceae are diether analogues (figure 16) of phosphatidyl glycerol (PG), phosphatidyl glycerophosphate (PGP), phosphatidyl glycerosulphate (PGS) (Kates, 1972a; 1978; Kates & Kushwaha, 1978; Kates et al., 1966; Hancock & Kates, 1973). Monophytanyl ethers, a diether glycolipid sulphate (GLS), and a triglycosyl diether (figure 16) have also been identified (Kates & Deroo, 1973; Kates & Hancock, 1976; Kushwaha et al., 1975a; 1976), although there are still a number of unidentified minor lipids (Kates, 1978). The halococci may be distinguished from the halobacteria by the absence of phosphatidyl glycerosulphate and the presence of a novel, unidentified phospholipid (Kates, 1978; Kates et al., 1966).

Originally it was postulated that the possession of diether lipids was an adaptation to the saline environment (Dundas, 1977;

Figure 15. Structural relationship of prokaryote ester and ether lipids.



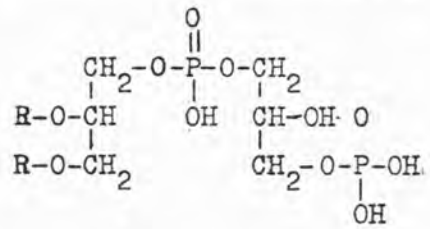
a. Ether lipid: R_1 and R_2 may be identical isopranyl side groups, but R_1 may also be H.



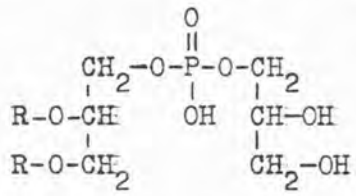
b. Ester lipid: R_1 and R_2 are fatty acids, but R_1 or R_2 may be H.

Figure 16. Structure of the major ether linked lipids in members of the Halobacteriaceae.

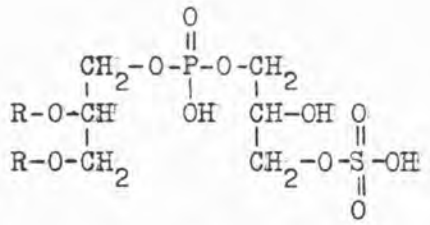
1. diether derivative of phosphatidyl glycerophosphate



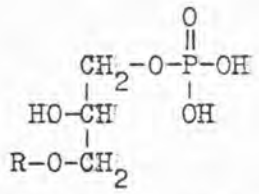
2. diether derivative of phosphatidyl glycerol



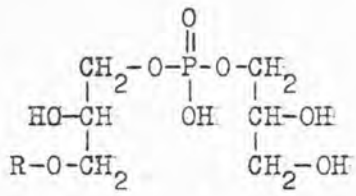
3. diether derivative of phosphatidyl glycerosulphate



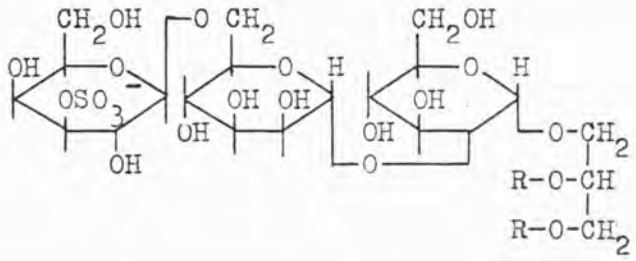
4. monoether derivative of lysophosphatic acid



5. monoether derivative of lysophosphatidyl glycerol



6. diether derivative of glycolipid sulphate



Kushner, 1968; Larsen, 1967). It is known that the salinity of the growth medium can influence the relative composition of the phospholipids and fatty acids in some halotolerant or moderately halophilic bacteria (Hiramatsu et al., 1978), and the halobacteria are also able to synthesise fatty acids via a malonyl-CoA system, which is strongly inhibited at high salinity (Kates & Kushwaha, 1978; Pugh et al., 1971). However, comparison with other archaebacteria, such as the methanogenic or thermoacidophilic bacteria indicates that ether lipids are not primarily synthesised in the saline environment, and are also present in eukaryotic cells (Snyder, 1970; 1972; Woese et al., 1978). Traces of fatty acids have also been detected in some of the methanogenic bacteria (Tornabene et al., 1978). In the methanogenic bacteria the polar lipids are derived from a diphytanyl glycerol diether (figures 17, 18), similar to that found in members of the Halobacteriaceae (Tornabene & Langworthy, 1979; Tornabene et al., 1978), and in most cases also from a dibiphytanyl glycerol tetraether (figures 17, 18), as are some of the polar lipids of the thermoacidophilic bacteria (De Rosa et al., 1974a; 1974b; 1977a; 1977b; Langworthy, 1977a; 1977b; Langworthy et al., 1972; 1974). Identification of the lipids of the genera Thermoplasma and Sulfolobus, including "Ferrolobus" (Brierley & Brierley, 1973), and the "Caldariella" MT strains*(De Rosa et al.,1975)

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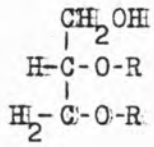
*Footnote:

A recent study of the DNA-dependant RNA polymerase of the thermoacidophiles (Zillig et al., 1980) has suggested that these organisms constitute separate genera, Thermoplasma and Sulfolobus. Ferrolobus is assigned to a new species, Sulfolobus brierleyi, and the Caldierella MT strains to a second species, Sulfolobus sulfataricus, the species Sulfolobus acidocaldarius being retained.

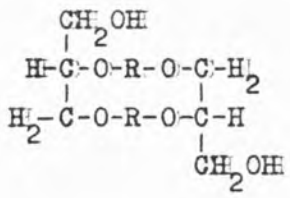
Figure. 17. The generalised structure of ether lipids from archaebacteria.

The major ether lipids found in the archaebacteria are glycerol mono-, di-, and tetraethers (Kates & Hancock, 1976; Kates, 1978; Langworthy, 1977a; 1977b; De Rosa et al., 1977a; 1977b; Balch et al., 1979; Tornabene & Langworthy, 1979). It is not known if the isopranyl side groups in ether type c. are linked to nonitol via ether linkages. The two isopranyl side groups in the di- and tetraethers, represented by the R, are identical in any one molecule.

a. Glycerol diether found in the extremely halophilic and methanogenic members of the archaebacteria.



b. Glycerol tetraether found in the methanogenic and thermoacidophilic members of the archaebacteria.



c. Glycerol tetraether found in the thermoacidophilic members of the archaebacteria.

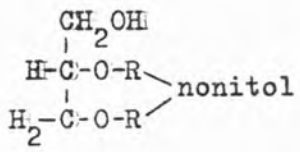
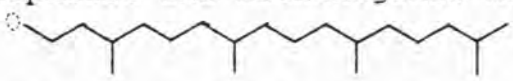


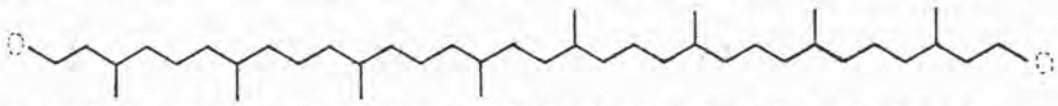
Figure 18. Structure of the isopranyl side chains of the archaebacterial lipids.

The isopranyl side chains found in the archaebacteria are derivatives of the C_{20}, phytanyl, or the C_{40}, biphytanyl, molecules (De Rosa et al., 1977a; 1977b; Kates, 1978; Langworthy, 1977a; 1977b; Tornabene & Langworthy, 1979). The cyclicised forms of the biphytanyl chains are prevalent in some thermoacidophiles, the degree of cyclisation of the molecule increasing with temperature.

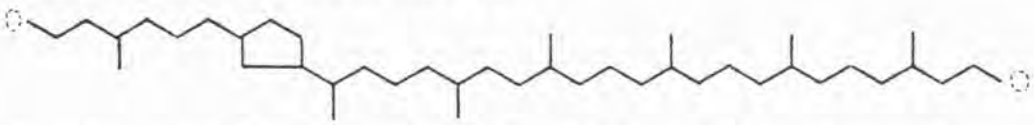
a. 3R,7R,11R,15-tetramethylhexadecyl (phytanyl) side chain of the glycerol diether (figure 17a) found in the extremely halophilic and methanogenic members of the archaebacteria.



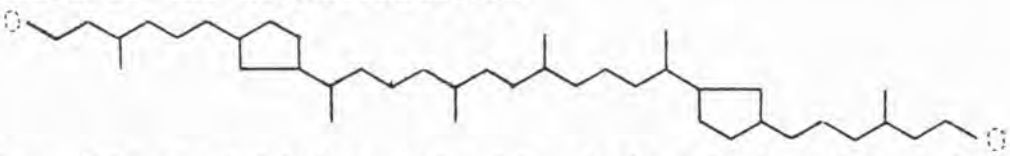
b. w,w'-biphytanyl side chain of the glycerol tetraether (figure 17b,c) found in the methanogenic and thermoacidophilic members of the archaebacteria.



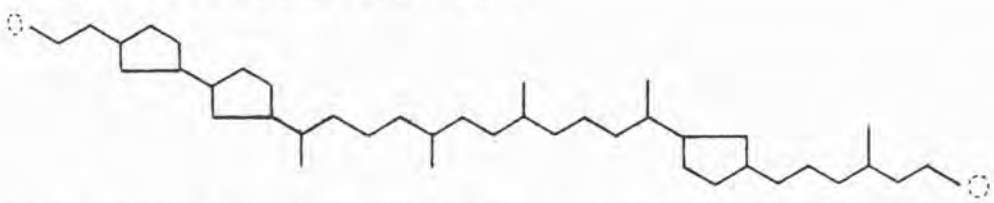
c. mono-cyclic w,w'-biphytanyl side chain of the glycerol tetraether (figure 17b,c) found in the thermoacidophilic members of the archaebacteria.



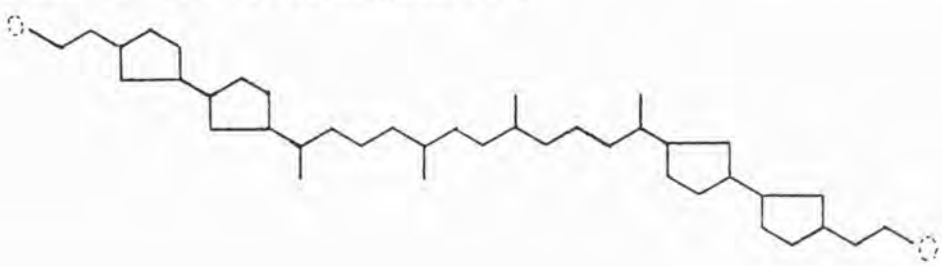
d. bi-cyclic w,w'-biphytanyl side chain of the glycerol tetraether (figure 17b,c) found in the thermoacidophilic members of the archaebacteria.



e. tri-cyclic w,w'-biphytanyl side chain of the glycerol tetraether (figure 17b,c) found in the thermoacidophilic members of the archaebacteria.



f. tetra-cyclic w,w'-biphytanyl side chain of the glycerol tetraether (figure 17b,c) found in the thermoacidophilic members of the archaebacteria.



indicates that, although the major polar lipids are C_{40} tetraethers, there are some differences between the two which may be genus specific (De Rosa et al., 1977a; 1977b; Langworthy, 1977b; Langworthy et al., 1972; 1974). It is possible, therefore, that the particular glycerol diether lipids found in members of the Halobacteriaceae are characteristic of the family.

The neutral lipids found in members of the Halobacteriaceae are mainly unsaturated, branched isoprenoids. The major components are squalenes and C_{50} bacterioruberins (Kelly et al., 1970; Kushwaha et al., 1974; Tornabene et al., 1969), and both have been related to adaptation to the saline environment (Bayley & Morton, 1978; Larsen, 1967). Bacterioruberins are known to have a protective function, shielding the cells from harmful effects of light and associated photooxidation (Hescox & Carlberg, 1972; Krinsky, 1977). This is evident from the work of Dundas and Larsen (1962), who experimented with a colourless mutant of H.salinarium. Using a mixed culture of colourless and pigmented strains, they found that the colourless mutant maintained itself at a constant ratio to the red coloured strain in the dark, but when grown in the light the colourless strain was lost. A number of C_{40} carotenoids other than the C_{50} bacterioruberins occur in members of the Halobacteriaceae (Kushwaha & Kates, 1973; Kushwaha et al., 1972), but because the cultural conditions affect the formation of the carotenoids (Gochnauer et al., 1972; Kushwaha & Kates, 1979a; 1979b), and they are found in other bacteria (Norgard et al., 1970; Schmidt et al., 1965; Weedon, 1971) they cannot be regarded as characteristic of the family Halobacteriaceae.

The discovery of squalenes in members of the Halobacteriaceae (Kushwaha et al., 1972; Tornabene et al., 1969) has led to speculation about their function, since they are also found in

eukaryotic cells and have recently also been found in the genus Thermoplasma, and in the methanogenic bacteria (Tornabene et al., 1978; 1979). Two possible roles have been described for the squalene, dihydrosqualene, tetrahydrosqualene, and dehydrosqualene found in the halobacteria. Lanyi et al. (1974) working with ether lipid vesicles suggested that squalenes have a structural function, and may be important in mediating the formation of Mg^{2+} "bridges" between the lipid cell membrane and the cell wall proteins (Bayley & Morton, 1978). Tornabene et al. (1978) has speculated that squalene was produced in response to an increasingly aerobic environment, since it has been proposed that the halobacteria and other archaebacteria evolved in the primitive anaerobic Archaean atmosphere. In the aerobic genus Halobacterium squalene occurs under aerobic conditions, but as the oxygen tension is lowered the relative proportions of di- and tetrahydrosqualene increase (Tornabene, 1978). In the extremely fastidious anaerobic methanogenic bacteria a much wider range of derivatives from squalene to dodecahydrosqualene are known (Tornabene et al., 1978). The wider range of hydrogenated squalene derivatives seems to be due to the oxygen tension, and Bloch (1976) points out that " oxygen is obligatory for the biological synthesis of the sterol pathway, and any evolution of the sterol pathway (under anoxic conditions) must have ended with squalene." Squalenes also occur in the Micrococcus strain H_5 (National Research Council of Canada, strain 14033), described by Novitsky and Kushner (1976) as Planococcus halophilus, which does not appear to be an archaebacterium, and in other bacteria (Amdur et al., 1978).

Other compounds of the neutral lipid fraction include the isoprenoids geranylgeraniol (C_{20}), also found as the esterifying alcohol in bacteriochlorophyll a_{Gg} (Gloe et al., 1975), 2,3-di-O-phytanyl glycerol (C_{20}), retinal (C_{20}), and menaquinone-8 (MK-8)

(Kates, 1978).

Lysis of halobacteria in media of low salt concentration releases large membranous sheets and a 4S component, which is probably composed of cell wall fragments (Kushner et al., 1964; Stoeckenius & Rowen, 1967). The membranous fraction has been extensively studied in H.halobium and H.cutirubrum (Kushwaha et al., 1975a; 1976; Stoeckenius & Kunau, 1968; Stoeckenius & Rowen, 1967; and reviews see Bayley & Morton, 1978; Henderson, 1977; Stoeckenius, 1978a; Stoeckenius et al., 1979). At least three different membrane types are present in these species, the 'red membrane', the 'purple membrane', and the gas vesicle limiting membrane. The gas vesicles, which collectively form the gas vacuoles are lemon shaped hollow regions of the cytoplasm containing largely nitrogen. The limiting membrane, which is freely permeable to gas is a proteinaceous non-unit membrane resembling the gas vaculoes of other prokaryotic organisms (Blaurock & Woeber, 1976; Cohen-Bazire et al., 1969; Stoeckenius & Kunau, 1968; Stoeckenius & Rowen, 1967; Walsby, 1974; 1978).

The red and purple membranes are part of the cell membrane, and can be separated by density gradient centrifugation using sucrose (Becher & Cassim, 1975; Kushwaha et al., 1975a; Stoeckenius & Kunau, 1968; Oesterhelt & Stoeckenius, 1974). In an initial study of the membranes of H.halobium, Stoeckenius and Rowen (1967) suggested that the purple membranes were produced in older cells by modification of the red membranes, as they doubted that two membranes, which differ in fragment size, could coexist in the cell membrane. Subsequently, the two membranes were found to be present in the cell membrane at the same time under certain conditions (Stoeckenius & Kunau, 1968). It is possible to visually distinguish the two types of membrane by electron microscopy (Henderson, 1977; 1978; Oesterhelt & Stoeckenius, 1974). The red membrane is the usual major

component of the cell membrane, the purple membrane occuring as discrete patches in the cell membrane (Stoeckenius, 1976; 1978a; Stoeckenius et al., 1979). The purple membrane is synthesised under conditions of low oxygen tension, and has an absorption spectrum unlike that of the red membrane (Becher & Cassim, 1975; Kushwaha et al., 1975a; Stoeckenius, 1978a; Stoeckenius et al., 1979; Stoeckenius & Kunau, 1968). Comparison of the two membrane fractions shows that the red membrane contains bacterioruberins and the purple membrane contains a C_{20} isoprenoid, which appears to be identical with the vertabrate isoprenoid, retinal, but little or no bacterioruberins (Kushwaha et al., 1975a; 1976; Oesterhelt & Stoeckenius, 1971). SDS polyacrylamide gel electrophoresis of the membranes also reveals a different protein composition, the purple membrane having a single protein of molecular weight 25,000-26,000 daltons, the red membrane having several proteins of molecular weight 10,000 to 60,000 daltons (Kushwaha et al., 1975a; 1976; Oesterhelt & Stoeckenius, 1971).

The purple membrane is light sensitive, and functions as a primitive photosynthetic membrane (Henderson, 1977; 1978; Lanyi, 1978; Stoeckenius, 1978a; Stoeckenius et al., 1979). The complex, which is responsible for this photosynthetic function, is called bacteriorhodopsin, because of its similarity to visual purple or rhodopsin, the retinal-protein complex of the mammalian eye (Oesterhelt & Stoeckenius, 1971). In both complexes retinal is linked to the protein (opsin, or bacterio-opsin) by a Schiff base from a lysine residue to the terminal (C_{15}) carbon atom of the isoprenoid side chain (Henderson, 1977; Lanyi, 1978; Stoeckenius, 1976; 1978a). Bacteriorhodopsin is involved in the generation of a proton gradient across the cell membrane, which is used to generate ATP. In bacteriorhodopsin, the retinal undergoes a cyclic light-dark photoreaction, which

involves several spectral intermediates (figure 19), and the reversible conformational change of 13-cis retinal to all-trans retinal (figure 20) on illumination (Hurley et al., 1978; Oesterhelt & Hess, 1973; Oesterhelt et al., 1973; Stoeckenius et al., 1979). There is an associated transfer of protons from the cytoplasm to outside the cell (figure 21), resulting in the establishment of a proton gradient across the cell membrane (Bogomolni et al., 1976; Drachev et al., 1974; Renthal & Lanyi, 1976). This gradient is dissipated by a back flow of protons, which is linked to the phosphorylation of ADP to ATP (figure 21), energy is stored (Henderson, 1977; Lanyi, 1978; Stoeckenius et al., 1979). Using isolated purple membrane lipid vesicles it is possible to demonstrate the establishment of a proton gradient by measuring the pH change of the suspending solution when the vesicles are illuminated (Renthal & Lanyi, 1976). In the presence of ADP, phosphate, and an ATPase it is also possible to demonstrate photophosphorylation, which is affected by uncouplers of phosphorylation (Stoeckenius et al., 1979). This membrane is, therefore,attributed the status of a primitive photosynthetic membrane (Stoeckenius, 1978a).

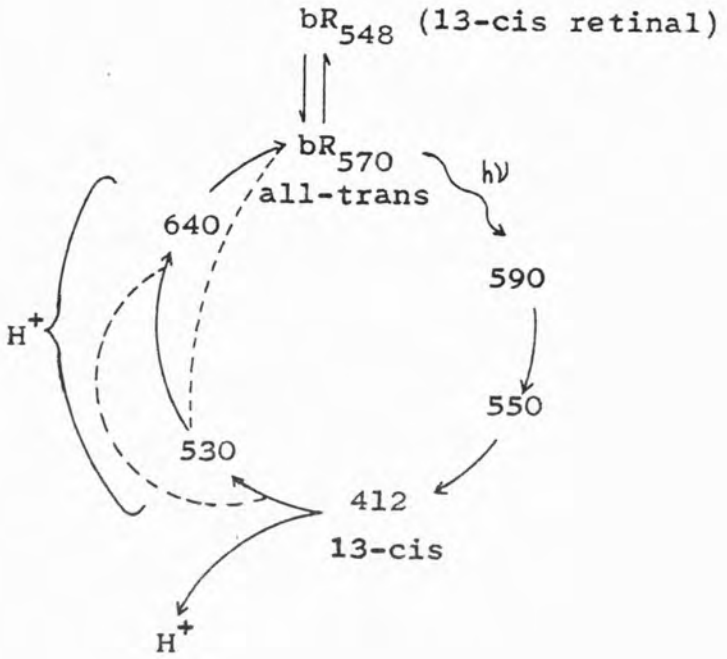
The purple membrane is a semi-crystalline unit, which owes its physical peculiarities to its chemical constituents. Some 75% of the membrane is the single opsin-like protein, bacterio-opsin, the remaining 25% being lipid (Oesterhelt & Stoeckenius, 1971). In an investigation of the lipid content of the purple and red membranes, Kushwaha et al. (1975a; 1976) found that glycerol diether lipids phosphatidyl glycerosulphate and glycolipid sulphate (figure 16) were almost exclusively contained in the purple membrane. It has been suggested that the glycolipid sulphate is involved in the formation of lipid bilayers, since without it the polar lipids do not form into bilayers in the presence of salt (Chen et al., 1974). Involvement

Figure 19. Steroisomeric and spectral intermediates of bacteriorhodopsin in the light and dark.

Dark adapted bacteriorhodopsin (bR) is a mixture of 13-cis (bR_{548}) and all-trans retinal (bR_{570}), and has an absorption maximum at 560nm. The various spectral intermediates in the cyclic isomerisation of bacteriorhodopsin are denoted by their absorption maxima (Stoeckenius et al., 1979), and are normally very unstable. Proton translocation is associated with this light sensitive cycle.

Figure 20. Reversible stereoisomerisation of retinal.

Retinal, which is bound to the lysine residue of the opsin-like protein via a Schiff base, undergoes a stereoisomeric change in the presence of light, from 13-cis to all-trans retinal, accompanied by proton translocation and changes in the spectral characteristics of bacteriorhodopsin.


bR548 (13-cis retinal)
bR570
all-trans
hν
590
550
412
13-cis
530
640
H+
H+

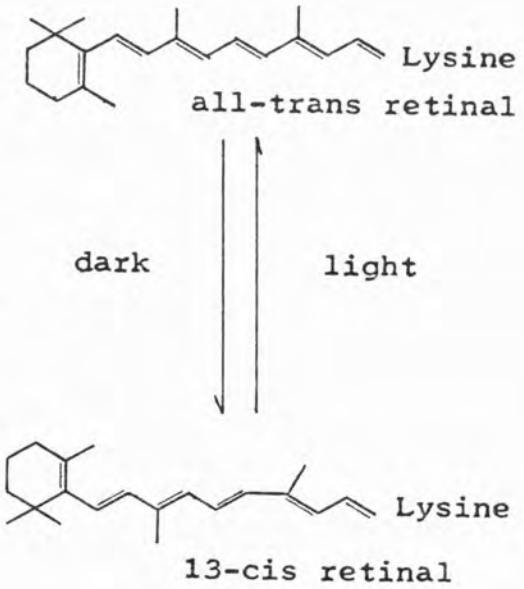
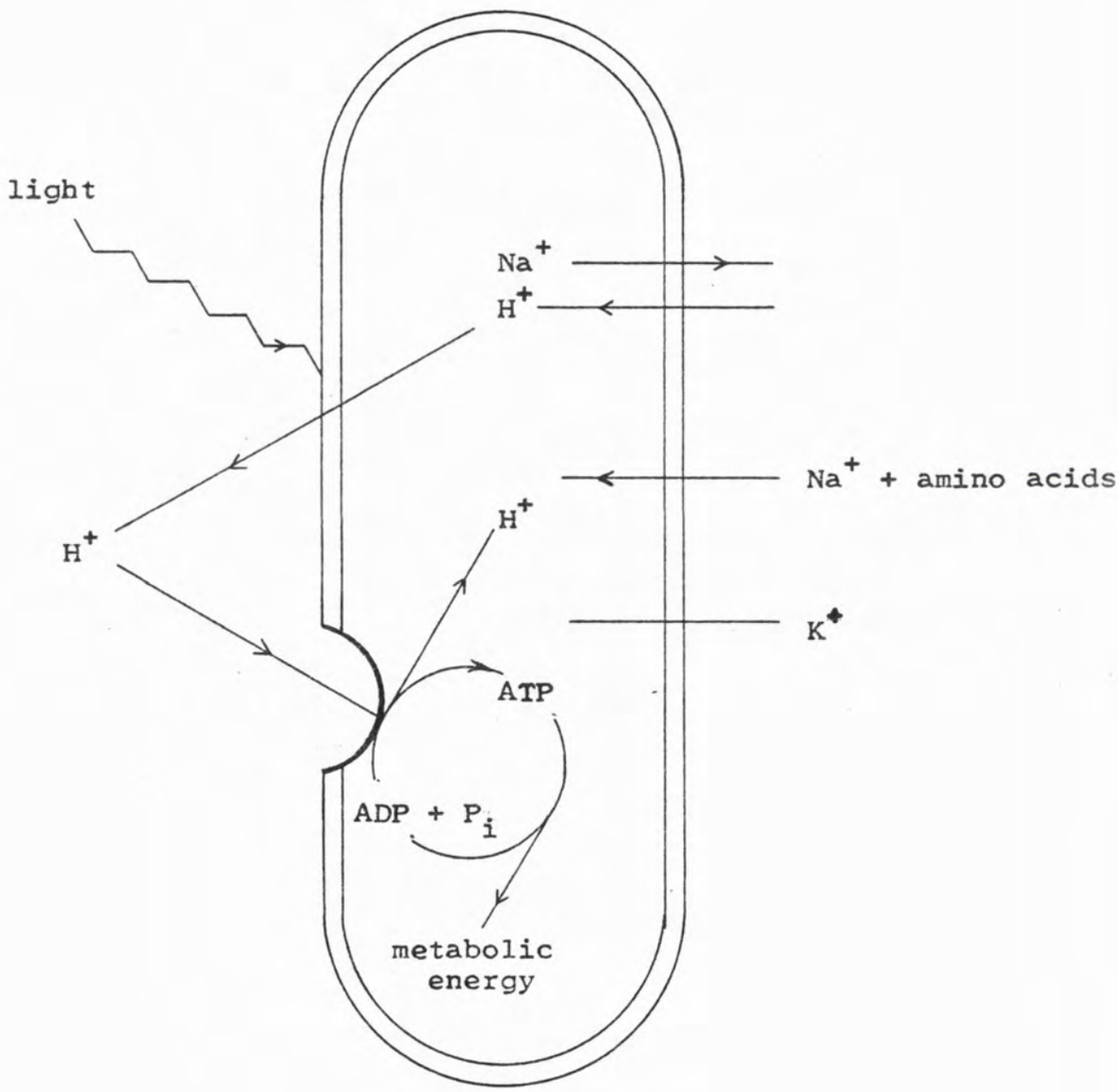

Lysine
all-trans retinal
dark
light
Lysine
13-cis retinal

Figure 21. Ion transport across the cell membrane of halobacteria

The mechanisms of ion transport across the cell membrane of the halobacteria are unusual among the porkaryotes in the co-transportation of Na^+ and amino acids, and the stimulation of H^+ translocation in the presence of light. The translocation of H^+ in the presence of light involves a specialised part of the cell membrane, the purple membrane, which also results in the synthesis of energy, in the form of ATP. Proton translocation across the purple membrane involves the reversible stereoisomerisation of retinal in the light sensitive protein, bacteriorhodopsin. ATP synthesised during this translocation of protons is used in the organisms' metabolism, although it is not sufficient to maintain the organism by this mechanism alone.


light
Na^+
H^+
Na^+ + amino acids
H^+
H^+
K^+
ATP
ADP + P_i
metabolic
energy

in facilitating proton transport is also postulated (Kates & Kushwaha, 1978). Proteins of the halobacteria and halococci contain a large excess of charged amino acids. There is a much greater excess of acidic amino acids over basic amino acids than in non-halophiles (Brown, 1963; Mullakhanbhai & Larsen, 1967; Steensland & Larsen, 1969). It has been suggested that this is a property of the halophilic mode of life (Bayley & Morton, 1978; Larsen, 1967; Kushner, 1978b), since an increase in the glutamic acid content in the cell wall of a facultative halophile with increasing salinity was reported (Novitsky & Kushner, 1976). Despite the lack of comparative data the excess acidity of the proteins of the Halobacteriaceae is regarded as characteristic of the family (Gibbons, 1974). The non-halophiles studied for their amino acid content are relatively few, and there is also little data on the amino acid composition of other archaebacteria, perhaps the closest relatives of the Halobacteriaceae. The amino acid analysis of Sulfolobus acidocaldarius gives an acidic to basic ratio similar to that for non-halophilic eubacteria (Weiss, 1974), but in the cell envelope of Methanospirillum hungatii, the ratio is very similar to that for the bulk protein of members of the Halobacteriaceae (Kandler & Konig, 1978). This property needs to be examined more closely to determine whether this is a property found in many of the archaebacteria, or in more bacteria than was previously thought.

The DNA composition of a number of strains of halobacteria and halococci shows a GC base content of 66-68 mole%, with a satellite band of 57-60 mole%, making up 10-30% of the total DNA (Joshi et al., 1963; Moore & McCarthy, 1969a; 1969b). The investigations of the DNA content are, however, almost entirely restricted to H.cutirubrum, H.halobium, H.salinarium, and Hc.morrhuae. The GC base content of H.volcanii was reported as 63.4 mole%, but there was no information on the presence of a satellite band (Mullakhanbhai & Larsen, 1975).

Apart from plasmids, satellite bands are unknown in the prokaryotes, including the archaebacteria (Balch et al., 1979; Buchanan & Gibbons, 1974; De Rosa et al., 1974b). The satellite band of most of the halobacteria do not behave like a multiple copy plasmid (Moore & McCarthy, 1969b), and the cells cannot be cured of it by acridine orange or acriflavin treatment (Joshi et al., 1963). However, the satellite band of Halobacterium strain 5 has been resolved into three plasmids of different molecular weight (Simon, 1978) while, in Halobacterium halobium, only a single plasmid could be attributed to tha satellite band (Wiedinger et al., 1979). The plasmids were not affected by teatment of cells with curing agents. One of the plasmids in Halobacterium strain 5, and the single plasmid in H.halobium appears to carry information coding for the formation of gas vacuoles (Simon, 1978; Wiedinger et al., 1979). Wiedinger et al. (1979) have also suggested that their single plasmid also codes for the formationn of the purple membrane and bacterioruberins. There are, however, no reports of satellite bands from gas vacuolate members of the methanogenic bacteria or Rhodospirillales (Mandel et al., 1971; Zhilinia & Zavarzin, 1979).

An interesting feature of the satellite band is its apparent constant relative amount, which is possibly characteristic of the individual species (Moore & McCarthy, 1969a). Few strains of each species have been examined in this respect, so no definite conclusions can be drawn.

4.3. Ecological aspects.

Our current knowledge of the Halobacteriaceae is based on a relatively restricted physiological-ecological group. Most of the isolates come from sea water salterns or solar salt, the chemical composition of which is relatively constant. Highly saline environments also occur where the major salts are not only NaCl, and

pH acidic, neutral, or alkaline. Apart from the studies on the Dead Sea (Elazari-Volcani, 1940; Kaplan & Baedecker, 1970; Kaplan & Friedman, 1970; Nissenbaum, 1975) and the Great Salt Lake (Post, 1977) few of the naturally occuring saline lakes have been systematically examined for the presence of halobacteria and halococci. Isolates from the Dead Sea (Mullakhanbhai & Larsen, 1975) and the Wadi Natrun, Egypt (Imhoff et al., 1978b) indicate that the environment may be an important factor in modifying the physiological requirements of these obligately extremely halophilic bacteria.

In particular, in their investigation of the Wadi Natrun, Imhoff et al. (1978b) described the mass accumulations of halobacteria in the water of some of the more saline lakes. An organism was isolated, which resembles members of the genus Halobacterium. It was different in some respects, namely its ability to grow at high pH (above pH 8.5) and in media almost devoid of Mg^{2+}. The chemical analysis of the lake waters in the Wadi Natrun (Imhoff et al., 1978b) showed that the level of Mg^{2+} was below the level of detection. The organism, therefore, appeared to differ from recognised species, which are unable to grow well at concentrations of Mg^{2+} below 10mM (Brown & Gibbons, 1955) and at pHs above 9.0 (Colwell et al., 1979).

MATERIALS AND METHODS

1. ECOLOGICAL OBSERVATIONS.

Observations of the ecology of the sampling areas were made during August 1976 (Dr.A.A.Mills, Dept of Geology) and September 1978;a number of samples were also collected during August 1977 (Dr.A.Khan, Dept of Geology). During September 1978 specimens were examined microscopically as soon as possible after collection, using an Olympus microscope fitted with phase optics (courtesy of Dr.D.Widdowson, Botany Dept University of Nairobi). A written record of the areas was kept, and a photographic record was also made using a Yashica TL electro camera fitted with either a Yashinon-DS f1.9 50mm automatic lens, or a Vivitar series I f3.8 85-205mm automatic/manual zoom lens. Skylight 1A filters (Hoya) were fitted to reduce the effects of UV haze over distance, while a polarising filter (Vivitar) was used to reduce reflection from the surface of water. Kodak Vericolour professional II film (ASA 100) was used for all photography, and was processed by Colab (Coventry). A number of people at the Magadi Soda Company Ltd., Ministry of Water Development, Kenya, and the Botany Department, University of Nairobi were able to provide additional information on the climate, ecology, and water chemistry of the lakes.

2. CHEMICAL ANALYSIS OF THE LAKE WATER.

Water samples were collected and stored in clean polythene bottles. In some cases a 40% formaldehyde solution was added to give a final concentration of 0.1% (v/v) in samples to aid preservation. With the exception of samples used for the determination of pH, CO_3^{2-}, HCO_3^-, and conductivity, the water was passed through a 0.45µm membrane filter (Millipore or Sartorius) prior to analysis (Golterman & Clymo, 1969; Golterman et al., 1978). Samples were analysed as soon as possible after collection.

2.1. pH.

Initially, in 1976, pH was determined on site using narrow range indicator papers (Merck). In 1978, pH was measured on site with both indicator papers and a portable pH meter (EIL). Subsequently, the pH of the samples was determined in the laboratory using an Ingold combination electrode, which was corrected for the sodium ion effect (Pye-Unicam). For all methods the magnitude of the deviation of the pH with varying salinity was estimated by measuring the pH of a series of buffers, containing varying concentrations of NaCl.

2.2. CO_3^{2-}, HCO_3^-, total alkalinity.

These measurements were made by titration, using 0.1N or 1.0N HNO_3, or HCl. Samples were titrated in triplicate to the end point pH 8.3, for CO_3^{2-}, and pH 4.2, for total alkalinity (Golterman & Clymo, 1969; Golterman et al., 1978). In these waters the major ions causing the high alkalinity are HCO_3^- and CO_3^{2-} and, therefore, the sum of these values are approximately equal to the total alkalinity (Talling & Talling, 1965). End points were determined by pH electrode, or by the indicators phenolphthalein (pH 8.3) and bromophenol blue (pH 4.2). Samples were stirred with a magnetic stirrer to aid the release of free CO_2.

2.3 Na^+, K^+, Mg^{2+}, Ca^{2+}.

An atomic absorption flame spectrophotometer (Varian Tectron) was used to measure the concentrations of the ions Na^+ and K^+ in an acetylene-air flame, and Mg^{2+} and Ca^{2+} in an acetylene-nitrous oxide flame (Golterman & Clymo, 1969; Golterman et al., 1978; Ratcliffe, personal communication). For the determination of Na^+ and K^+ the samples were diluted until the concentration of the ions were within the range 1.0 to 0.2mM. Samples for the determination of Mg^{2+} and Ca^{2+} were not diluted, due to the low levels of these ions in the

samples.

Standards for the determination of Na^+ were prepared within the range 1.0 to 0.2mM NaCl in the presence of $(NH_4)_2CO_3$, which was added to the standards to give CO_3^{2-} concentrations comparable with those in the samples. Standards for the determination of K^+ were prepared within the range 1.0 to 0.2mM KCl, in the presence of Na_2CO_3 added to the standards to give concentrations comparable with those in the samples. For the determination of Ca^{2+} and Mg^{2+}, standards of either 0.2 and 0.05mM Ca^{2+}, or 0.2 and 0.05mM Mg^{2+} were made up in the presence of either 1.0% (w/v) Na_2CO_3 and 3.0% NaCl, or 15% Na_2CO_3 and 15% NaCl, to reflect the appropriate ionic composition of the samples. The concentrations of the ions were measured at the following wavelengths: Na^+, 5890.0µm; K^+, 7664.9µm; Ca^{2+}, 4226.7µm; Mg^{2+}, 2852.1µm.

2.4. Cl^-.

The concentrations of Cl^- was measured by selective ion electrode (Orion Cl^- electrode), in conjunction with a pH and selective ion meter (EIL 7050). Standards of 100mM, 10mM, and 1mM NaCl were prepared to which Na_2CO_3 was added to give values of CO_3^{2-} concentration similar to those in the samples. Portions of the collected water samples were diluted with deionised water to give Cl^- concentration in the samples within the range of the standards.

2.5. NH_4^+.

The concentration of NH_4^+ was measured by a spectrophotometric method (Havilah et al., 1977), using the colour reaction of salicylate and cyanurate reagents with NH_4^+. Salicylate reagent was prepared from 34g sodium salicylate and 0.24g sodium nitroprusside in 1 litre of deionised water. Cyanurate reagent was prepared from 0.25g sodium dichloroisocyanurate, dissolved in 200ml of deionised water, to which was added 15ml of sodium hydroxide (400g/l), the volume

being made up to 1 litre. The determination of the concentration of NH_4^+ was made using sample volumes of 100µl, in clean universal bottles, to which was added 5.0ml salicylate reagent and 5.0ml cyanurate reagent (Ratcliffe, unpublished). The tops were screwed down tightly and the bottles inverted to ensure thorough mixing of the sample and reagents. The colour was allowed to develop for 30min before the absorbance of the samples were measured at a wavelength of 667nm. Standards of 0.2mM and 1.0mM NH_4^+, and a deionised water blank were prepared in a similar manner.

2.6. Total reducible nitrogen.

The total reducible nitrogen (NO_2^- and NO_3^-) was measured by reducing NO_3^- and NO_2^- to NH_4^+, in the presence of Devarda's alloy and, then determining the total NH_4^+ as described above (2.5). Samples of 100µl were reduced in the presence of a small quantity of Devarda's alloy and 1.0ml of NaOH (Ratcliffe, unpublished). The reduction was allowed to proceed overnight, and 5.0ml each of salycilate and cyanurate reagent were added, and the absorbance determined as described above (2.5). Standards of 0.2mM and 1.0mM, and a deionised water blank were treated similarly.

2.7. PO_4^{3-}.

The concentration of PO_4^{3-} was determined spectrophotometrically using vanadate reagent (Kaila, 1955), which was prepared by mixing 300ml of nitric acid diluted 1:1 (v/v) with water, 300ml 5% (w/v) ammonium molybdate solution, 300ml 0.25% (w/v) ammonium vanadate solution, making up the volume of the final solution to 1 litre with deionised water. 5.0ml of water sample were dispensed into clean universal bottles and 10ml of the vanadate reagent was added. The samples were allowed to stand for 1hr after mixing before the absorbances were measured at a wavelength of 250nm. Standards of 0.01mM and 0.025mM PO_{43}^{3-}, and a deionised water blank were treated

similarly.

2.8. Conductivity.

The electrical conductivity of the samples were measured directly without dilution, using a conductivity meter (Electronic Switchgear type MC1) at a temperature of 20°C.

3. CHARACTERISATION OF PHOTOTROPHIC AND EXTREMELY HALOPHILIC BACTERIA.

3.1a. Reference strains of Rhodospirillaceae.

Chromatium gracile	DSM 203
Chr.minus	DSM 178
	DSM 179
	BN 1211 (from H.G.Trüper)
Chr.vinosum	DSM 180
	strain D (from H.G.Trüper)
Chr.violascens	DSM 198
Chr.minutissimum	strain Moskau (from H.G.Trüper)
Ectothiorhodospira mobilis	DSM 239
E.shaposnikovii	DSM 243
E.halophila	DSM 244
E.halochloris	DSM 1059
Ectothiorhodospira sp.	strain G_1 (from W.D.Grant)
Lamprocystis roseopercina	DSM 229
Thiodictyon bacillosum	DSM 234
Amoebobacter pendens	DSM 236
Rhodopseudomonas sulfidophila	W_4 (from H.G.Trüper)
Rhodospirillum rubrum	NCIB 8255
Rhodomicrobium vannielii	NCIB 10020
Chromatium purpuratum	BN 5500 (from H.G.Trüper)

3.1b. Reference strains of halobacteria

Halobacterium halobium	NCMB 736
	strain R_1 (from Ms.E.Dibb)
H.cutirubrum	NCMB 763
H.salinarium	NCMB 764
H.trapanicum	NCMB 767
H.volcanii	NCMB 2012

DSM = Deutsche Sammlung von Mikroorganismen

NCIB = National Collection of Industrial Bacteria

NCMB = National Collection of Marine Bacteria

3.2. Culture media for the maintainance of reference strains.

a. Phototrophic bacteria.

Ectothiorhodospira mobilis and E.shaposnikovii were maintained on a modification of the medium of van Niel (1931; 1944) used by Grant et al. (1979), of the following composition (%w/v): NH_4Cl, 0.1; $MgSO_4.7H_2O$, 0.04; $CaCl_2$, 0.05; KH_2PO_4, 0.1; sodium acetate, 0.1; sodium sulphide, 0.01; $Na_2CO_3.10H_2O$, 3.0; NaCl, 0.04 (3.0 for E.mobilis); 1.0ml trace element solution of the following composition: $ZnSO_4$, 2.2; $CaCl_2.2H_2O$, 0.55; $MnCl_2.4H_2O$, 0.55; $FeSO_4.7H_2O$, 0.5; Na_2MoO_4, 0.1; $CuSO_4.5H_2O$, 0.15; $CoCl_2$, 0.15. The pH was adjusted to 9.0 to 9.5 with concentrated HCl.

E.halophila and E.halochloris were maintained on the medium of Imhoff and Trüper (1977), of the following composition (%w/v): $CaCl_2.2H_2O$, 0.005; KH_2PO_4, 0.05; NH_4Cl, 0.08; $MgCl_2$, 0.01; NaCl, 18.0; Na_2CO_3, 2.0; Na_2SO_4, 2.0; $Na_2S.9H_2O$, 0.1; sodium succinate, 0.1; yeast extract, 0.05; 1.0ml trace element solution ; 1.0ml vitamin solution.
Trace element solution; $FeCl_2.4H_2O$, 0.18; $CoCl_2.6H_2O$, 0.025; $NiCl_2.6H_2O$, 0.001; $CuCl_2.2H_2O$, 0.001; $MnCl_2.4H_2O$, 0.007; $ZnCl_2$, 0.01; H_3BO_3, 0.05; $NaMoO_4.2H_2O$, 0.003; $Na_2SeO_3.5H_2O$, 0.001.
Vitamin solution; biotin, 0.001; nictotinamide, 0.0035; thiamine dichloride, 0.003; p-aminobenzoic acid, 0.002; pyridoxal chloride, 0.001; Ca-pantothenate, 0.001; vitamin B_{12}, 0.0005.

Rhodospirillum rubrum and Rhodomicrobium vannielii were maintained on a modification of the medium of van Niel (1931; 1944), of the following composition (%w/v): NH_4Cl, 0.1; $MgSO_4.7H_2O$, 0.04; $CaCl_2$, 0.05; NaCl, 0.04; KH_2PO_4, 0.1; sodium acetate, 0.1; 1.0ml of the trace element solution used in the medium for the culture of E.mobilis and E.shaposnikovii. The pH was adjusted to 6.5 to 7.0

with 1.0% (w/v) K_2HPO_4.

All other reference strains were maintained on the medium of Pfennig (1965a; 1965b), as described in the DSM catalogue:

solution 1: 1.5g $CaCl_2.2H_2O$ in 2.7l distilled water.

solution 2: 3.0g $Na_2S.9H_2O$ in 400ml distilled water.

solution 3: 9.0g $NaHCO_3$ in 2.8l distilled water.

solution 4: 2.0g NH_4Cl; 3.0g $MgSO_4.7H_2O$; 2.0g KCl; 2.0g KH_2PO_4; 12.0ml vitamin B_{12} solution (1.0mg/100ml); 60ml trace element solution; 140ml distilled water.

solution 5: 0.125g $CaCl_2.2H_2O$ in 500ml distilled water.

Trace element solution (% w/v): EDTA di-sodium salt, 0.15; $FeSO_4.7H_2O$, 0.02; $ZnSO_4.7H_2O$, 0.01; $MnCl_2.4H_2O$, 0.002; 6.0ml Hoagland trace elements.

Hoagland trace element solution (% w/v): $AlCl_3$, 0.1; KI, 0.05; KBr, 0.05 LiBr, 0.05; $MnCl_2.4H_2O$, 0.7; H_3BO_3, 1.1; $ZnCl_2$, 0.1; $NiCl_2$, 0.1; $CoCl_2$, 0.5; $SnCl_2.2H_2O$, 0.05; Na_2MoO_4, 0.05; $NaVO_3.H_2O$, 0.01; selenium salt, 0.05

Solutions 1, 2, and 5 were autoclaved separately, solution 1 was usually autoclaved as 46ml portions in 100ml glass bottles. CO_2 was bubbles through solution 3 for about 30min, the pH having fallen to about 6.2, this solution was added to solution 4, and the resulting solution (3 + 4) was filter-sterilised by passing through 0.22um membrane filters (Sartorius or Millipore), using CO_2 over pressure. 50ml of sterile solution 3 + 4 was added to the sterilised solution 1. To this solution was added 6ml of sterile solution 2, and the pH was adjusted to 7.2 to 7.5 with sterile 0.2M Na_2CO_3. After inoculation with the culture, the bottles were filled, leaving a small air space the size of a pea, with sterile solution 5. For the growth of large batches of organisms, sodium acetate (0.1% w/v) was added, reduced to 0.025% (w/v) for Lamprocystis roseopercina and Thiodictyon bacillosum (Pfennig et al., 1968). The following

additions were made to the above medium for the culturing of the following strains: Chr.gracile and Chr.minutissimum, 1.0% (w/v) NaCl; Chr.purpuratum and Rhps.sulfidophila, 3.0% (w/v) NaCl; Amoebobacter pendens, 0.05% (w/v) yeast extract.

In all media described semi-solid agar tubes and agar plates were prepared by the addition of 1.0% and 1.5% (w/v) agar respectively.

b. Extremely halophilic bacteria.

The reference strains of halobacteria were maintained on the medium of Payne et al. (1960), of the following composition (%w/v): yeast extract, 1.0; casamino acids (Difco), 0.75; KCl, 0.2; $MgSO_4.7H_2O$, 2.0; trisodium citrate, 0.3; Fe^{2+}, 10ppm; Mn^{2+}, 0.1ppm. 2.0M NaCl was added to the medium for the culture of H.volcanii, the pH was adjusted with sterile NaOH. 4.3M NaCl was added to the medium for the culture of all other strains, and the pH was adjusted to 7.4 with sterile NaOH. A stock culture of H.volcanii was also maintained on the medium of Mullakhanbhai and Larsen (1975), of the following composition (%w/v): NaCl, 12.5; $MgCl_2.6H_2O$, 5.0; K_2SO_4, 0.5; $CaCl_2.6H_2O$, 0.2; tryptone (Oxoid), 0.5; yeast extract, 0.5; pH 6.8.

3.3. Culture media for the maintainance of isolated strains.

a. Phototrophic bacteria.

The medium used for the maintainance of E.mobilis and E.shaposnikovii (3.2a), containing either 1.0% or 3.0% (w/v) NaCl was used to maintain the strains isolated from the Kenyan soda lakes.

b. Extremely halophilic bacteria.

The extremely halophilic bacteria were initially isolated using a modification of the medium of Brown (Brown, 1963; Grant & Tindall, 1980), of the following composition (%w/v): bacto-peptone (Oxoid L37), 1.0; trisodium citrate, 0.3; KCl, 0.2; $MgSO_4.7H_2O$, 2.0; NaCl, 25.0;

$Na_2CO_3.10H_2O$, 5.0; pH 9.0. Subsequently the isolates were found to give higher growth yields on the medium of Tindall et al. (1980), which was a modification of the medium of Payne et al. (1960), to which 5.0% (w/v) $Na_2CO_3.10H_2O$ was added and the $MgSO_4.7H_2O$ content was lowered to 0.1% (w/v); the pH was 9.5 (see 3.2b)

3.4. Physiological and morphological tests.

a. Phototrophic bacteria.

The optimal salinity for growth of the organisms was determined in the basic medium free of Na^+ at pH 9.5, 1.5% (w/v) K_2CO_3 being added to the medium instead of $Na_2CO_3.10H_2O$. NaCl was then added to the medium to give a range of NaCl concentrations from 0.0 to 10.0% (w/v). The medium was filter sterilised by passing through 0.22µm membrane filters (Millipore or Sartorius), and dispensed in 2.5ml portions into sterile repli dishes (Sterilin Products). The medium was inoculated in quintuplet with the organisms under test and incubated in an anaerobic atmosphere at 26 °C, at a light intensity of 750 lux for 7 days. The optical density of the cultures were determined at 650nm using a Beckman model 35 spectrophotometer.

The optimal pH for growth was determined in completely filled 3.0ml specimen tubes (Sterilin Products). The basic medium was prepared without the addition of $Na_2CO_3.10H_2O$, and the pH was then adjusted with 0.2M Na_2CO_3 and 0.2M $NaHCO_3$ so that the salinity was constant within the pH range 7.5 to 11.0. NaOH and HCl were used to adjust the pH above 10.5 and below 8.5 respectively. The medium was prepared with either 0.1% (w/v) $Na_2S.9H_2O$, 0.1% (w/v) sodium acetate, or both. The medium was sterilised by passing through 0.22µm membrane filters (Millipore or Sartorius), dispensed as 3.0ml portions, inoculate in triplicate, and incubated under the conditions described above. The optical density of the cultures was determined at 650nm after 7 days.

Growth was tested in the light and dark in soft agar tubes (1.0% w/v). The medium was prepared with 0.1% (w/v) sodium acetate, without sulphide or any other reducing agent. The warm, liquified agar was dispensed into sterile test tubes and maintained at 45°C in a water bath. The tubes were inoculated with the organisms under test and the suspension mixed as described by Pfennig (1965b). The agar was solidified in cold water, and the tubes incubated at 26°C, either in the dark, or in the light, at 750 lux until growth appeared (up to 1 month).

To test the utilisation of various carbon sources and sulphur compounds the basic medium was prepared without the addition of carbon sources or sulphide. The salinity and pH was adjusted to the optimal values for the strains under test. To test for the utilisation of carbon compounds 0.1% (w/v) sulphide was added to the medium together with the appropriate concentration of carbon compound as described by Thiele (1968), the control containing only sulphide and lacking any carbon compounds. The utilisation of sulphur compounds was tested in media devoid of carbon sources, the individual sulphur compounds being added at the concentrations described by Thiele (1968) and Trüper (1968). The media were sterilised by filtration through 0.22µm membrane filters (Millipore or Sartorius). The sterile medium was dispensed in 2.5ml portions into sterile repli dishes (Sterilin Products) and placed in an anaerobic chamber, incubated in the light at 750 lux, at 26°C for 7 days. The optical density of the cultures was determined at 650nm.

The in vivo absorption spectrum of intact cells was determined by saturating a cell suspension with sucrose (Eimhjellan et al., 1967) and measuring the absorption spectrum between 350 and 950nm, using a Ziess PM QII or a Beckman model 35 spectrophotometer.

The morphology of the cells was examined using a Leitz Orthoplan

microscope fitted with phase optics. The microscope was fitted with a camera attachment (Leitz) for photomicroscopy. Cellular inclusions were investigated by staining or by their physical properties. Gas vacuoles and sulphur globules were distinguished by their appearance and the collapse of the former when the culture was subjected to an increase in pressure (Walsby, 1974). Polyhydroxybutyrate was stained with Sudan Black, by the method of Burdon (1946). Polysaccharides were dectected by the method of Hotchkiss (1948). The cells were Gram-stained according to the method of Hucker and Cohn (1923). Catalase activity was determined by the production of gas from hydrogen peroxide (Trüper, 1968).

The purity of all cultures was determined by microscopy and by inoculation into Difco AC medium, Difco AC medium adjusted to pH 9.5 with the addition of 3.0% (w/v) $Na_2CO_3.10H_2O$, Starskey's Desulphovibrio medium (Abd-el-Malek & Rizk, 1958), and Starskey's Desulphovibrio medium adjusted to pH 9.5 with the addition of 3.0% (w/v) $Na_2CO_3.10H_2O$.

b. Extremely halophilic bacteria.

To determine the pH range and optimal Mg^{2+} concentration required by the organisms, the medium of Payne et al. (1960) was prepared without the addition of $MgSO_4.7H_2O$. The medium was divided into three portions, and the pH was adjusted to pH 7.5, with 0.2M NaOH or HCl, pH 8.5, with 3.0% (w/v) $NaHCO_3$, and pH 9.5 with 3.0% (w/v) Na_2CO_3. Media at each of the three pHs was divided into three aliquots, and the final concentration of $MgSO_4.7H_2O$ was adjusted to 0.0%, 0.1%, and 2.0% (w/v) at each pH. Media were sterilised by passing through 0.22µm membrane filters (Millipore or Sartorius), and dispensed in 2.5ml aliquots into sterile repli dishes (Sterilin Products). The media were inoculated with the organisms under test and incubated at 37°C for 14 days, after which

growth was determined qualitatively (Tindall et al., 1980).

Mg^{2+} optima were determined more accurately by preparing the medium of Tindall et al. (1980), in which the $MgSO_4.7H_2O$ concentration (%w/v) was adjusted to 0.0, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0. The medium was sterilised by passing through 0.22µm filters (Millipore or Sartorius) and dispensed in 100ml aliquots into sterile 250ml flasks. The inoculated flasks were incubated at 37°C at a shaking rate of 100rpm for 10 days.

The optimal salinity for growth was determined in the medium of Tindall et al.(1980), in which the NaCl concentration was adjusted to 0.0M, 0.5M, 1.0M, 1.5M, 2.0M, 2.5M, 3.0M, 3.5M, 4.0M, 4.5M, and 5.0M. Media were also prepared in which $Na_2CO_3,10H_2O$ was replaced by 1.5% (w/v) K_2CO_3 over the same range of NaCl concentration. Media were filter sterilised by passing through 0.22µm membrane filters (Millipore or Sartorius), dispensed as 100ml aliquots into 250ml flasks, and incubated in triplicate at a shaking rate of 100rpm at 26°C and 37°C for 14 days. The NaCl optimum was further established by three passages of the organisms in media of the same salinity, under the conditions described above. The optical density of the cultures was determined at a wavelength of 650nm.

Gelatinase activity was determined by the method of Gonzalez et al. (1978), by adding 1.0% (w/v) gelatin to the medium of Payne et al. (1960) or Tindall et al. (1980), solidified with 2.0% (w/v) agar, and examining the plates for zones of clearing (Clarke, 1953) after flooding the plates with Frazier's reagent (Frazier, 1926). Gelatinase activity was also determined by the inclusion of gelatin-charcoal discs (Oxoid) in liquid cultures, and examining the cultures for the liberation of charcoal particles.

Starch hydrolysis was determined in solid media to which 0.2% (w/v) starch was added (Gibbons, 1957; Gonzalez et al., 1978).

Starch hydrolysis was detected by flooding the plates with Lugol's iodine.

Reduction of nitrate or nitrite was tested in liquid media to which 1.0% (w/v) or 0.1% potassium nitrate or potassium nitrite was added, gas production being detected by the inclusion of Durham tubes in the cultures (Gonzalez et al., 1978). The presence of nitrite was determined by the addition of Greiss' reagent to the medium (Greiss, 1971). Nitrate was detected by the addition of zinc dust to the culture followed, after 30min, by the addition of Greiss' reagent for the detection of nitrate.

The production of sulphide from thiosulphate was tested in liquid media to which 0.1% (w/v) sodium thiosulphate was added. H_2S production was detected by lead acetate impregnated filter papers (Colwell et al., 1979; Gonzalez et al., 1978; Kocur & Hodgkiss, 1973).

Production of indole from peptone was determined in the medium of Gibbons (1957), of the following composition (%w/v): casamino acids, 0.5; yeast extract, 0.5; bacto-tryptone, 0.05; glutamic acid, 0.25; trisodium citrate, 0.03; $MgSO_4.7H_2O$, 2.5 (pH 7.2) or 0.1 (pH 9.5); KCl, 0.2; NaCl, 25.0. The pH was adjusted to 7.2 with HCl, or 9.5 with 5.0% (w/v) $Na_2CO_3.10H_2O$. The presence of indole was detected with Ehrilich's reagent, p-dimethylaminobenzaldehyde (Gonzalez et al., 1978).

The ability to grow under anaerobic conditions was tested by incubation of plates in an anaerobic atmosphere (Gaspak Systems) or by observing growth in agar stabs.

The utilisation of carbon compounds was tested in liquid and solid media (Gochnauer & Kushner, 1969; Gonzalez et al., 1978; Tomlinson & Hochstein, 1976). The agar medium of Tindall et al. (1980) was prepared, and in some experiments the casamino acids and yeast extract contents were lowered to 0.75% (w/v) and 0.1%

respectively. Wells were cut in the agar with a sterile cork borer and were filled with 10% (w/v) solutions of the carbon compounds under test. Plates were dried at 37°C for 15min, spread with the organism under test and incubated at 37°C for 7 days, after which the plates were examined for zones of increased growth.

In liquid cultures, 10% (w/v) solutions of the carbon sources under test were added to the medium of Tindall et al. (1980) to give a final concentration of 1.0% (w/v). The cultures were incubated at 37°C for 10 days in an illuminated incubator (Gallenkamp), at a shaking rate of 100rpm. The optical density of the cultures was recorded daily at a wavelength of 650nm, and at 570nm and 500nm at the end of the incubation period.

The method of Colwell et al. (1979) was used to test the sensitivity of the organisms to antibiotics. The media of Payne et al. (1960) and Tindall et al. (1980) were solidified with 2.0% (w/v) agar and then spread with the organisms under test. Antibiotics were applied to the surface of the agar either in crystalline form (0/129) or in filter paper dics containing known amounts of the antibiotic. The plates were incubated for up to 3 weeks at 37°C, and were examined at 7 day intervals for inhibition of growth. The following antibiotics were used: penicillin G, 10 units; tetracycline, 30µg; dihydrostreptomycin, 10µg; $HgCl_2$, 20µg; novobiocin, 30µg; ampicillin, 30µg; polymixin B, 300 units; 0/129, crystalline. All antibiotics, with the exception of $HgCl_2$ (BDH) and 0/129 (BDH) were obtained from Sigma.

The absorption spectrum of whole cells was determined using a Beckman model 35 spectrophotometer.

Cells were Gram-stained according to the method of Dussalt (1955). Catalase activity was determined by the production of gas from hydrogen peroxide and the production of a green colour with 1.0%

benzidene solution and 0.15% (w/v) hydrogen peroxide (Gonzalez et al., 1978). Oxidase activity was tested by the production of a pink colouration with 1.0% (w/v) dimethyl-p-phenylenediamine (Gonzalez et al., 1978).

3.5. Lipid analyses.

a. Extraction and identification of polar lipids from phototrophic bacteria.

The polar lipids of the phototrophic bacteria were extracted by the method of Takacs and Holt (1971). Cells were harvested at 20,000g for 15min at 4°C and washed twice with the salt solution of the growth medium. 100mg of freeze dried cells were extracted with 10ml chloroform:methanol (2:1) overnight (extract I). The cells were harvested at 12,000g for 15 min at 4°C and extracted overnight with chloroform:methanol (1:1) (extract II). The cells were harvested again and extracted with methanol overnight (extract III). All extractions were carried out at room temperature under an oxygen-free nitrogen atmosphere. The polar lipids from the combined extracts (I, II, & III) were transfered to chloroform by the addition of 5.0ml of water followed by 5.0ml of chloroform. The chloroform phase was collected, evaporated to dryness under a stream of nitrogen, and the residue dissolved in a small volume of chloroform. The polar lipid mixtures were separated by thin layer chromatography on silica gel in the solvent systems chloroform:methanol:water (65:25:4) (Wagner et al., 1961), and chloroform:methanol:acetic acid: water (80:12:15:4) (Collins, 1978).

Phosphatidic acid, phosphatidyl glycerol, phosphatidyl choline, phosphatidyl inositol, phosphatidyl serine, and diphosphatidyl glycerol (cardiolipin) were obtained from Sigma as standards.

b. Extraction and identification of lipids from extremely halophilic bacteria.

The lipids of the extremely halophilic bacteria were extracted by the modified method of Bligh and Dyer (1959) used by Kates et al. (1966). 0.5g of wet packed cells were suspended in 5.0ml of water to which was added 7.5ml chloroform:methanol (2:1). The suspension was extracted overnight under oxygen-free nitrogen with stirring, and then passed through a G3 sintered glass filter to remove cell debris. The filtrate was collected in a separating funnel and diluted with 2.5ml of water followed by 2.5ml of chloroform. The chloroform phase was collected, diluted with an equal volume of benzene and brought to dryness under reduced pressure at 35°C. The residue was redissolved in 5.0ml chloroform and stored under nitrogen at 4°C. The lipid composition was determined by separating the lipids on silica gel thin layer plates.

Acid methanolysis of freeze dried cells was preformed by the method of Minnikin et al. (1975) in 10ml methanol:toluene (1:1) acidified with 0.2ml concentrated sulphuric acid. The non-polar components were extracted into hexane after heating to 50°C overnight. The extracts were separated by thin layer chromatography on silica gel plates in the solvent petroleum ether:ether (85:15). The non-fatty acid components were isolated by preparative thin layer chromatography and examined by infra-red spectroscopy using a Perkin-Elmer 580 spectrophotometer.

Squalenes were characterised by co-chromatography with authentic squalene (Sigma) in hexane:ether (99.75:0.25) on silica gel thin layer plates (Kushwaha et al., 1972). Menaquinones were identified by reverse phase chromatography using Merck HPTLC RP-18F_{254} reverse phase plates in the solvent system acetone:water (99:1) as described by Collins et al. (1980). Standard menaquinones were obtained from organisms as described by Collins et al. (1980). The identification was confirmed by Dr.M.D.Collins. Polar lipids were

identified by their chromatographic behaviour in the solvent systems chloroform:methanol:concentrated ammonia (65:35:5) (Kates, 1978), chloroform:methanol:water (65:25:4) (Wagner et al., 1961), chloroform: methanol:90% acetic acid (30:4:20) (Kates, 1978), and chloroform: methanol:acetic acid:water (80:12:15:4) (Collins, 1978), and by specific spray reagents.

c Non-specific spray reagents.

Iodine vapour was used to detect lipids by placing a few crystals in a warm chromatography tank with the developed chromatgram the lipids appearing as brown spots (Krebs et al., 1969).

30-40% (v/v) aqueous sulphuric acid was sprayed onto the chromatogram which was then heated for 20min at 180-190°C in a force draught oven, lipids appearing as black spots (Collins, 1978).

5% (w/v) ethanolic molybdophosphoric acid was sprayed onto the chromatograms which were heated for 20min at 120°C, the lipids appearing as blue to black spots (Krebs et al., 1969).

0.001% (w/v) rhodamine 6G in acetone was sprayed onto the plates, which were examined under long wavelength UV light, lipids appearing as different coloured spots according to their chemical properties (Kates, 1978; Kates et al., 1966; Krebs et al., 1969; Witter et al., 1957).

d Specific spray reagents

Phospholipids were detected by the modified Zindzadze reagent of Vaskovsky and Kostetsky (1968), phospholipids appearing as blue spots after spraying.

Free amino groups were detected by spraying the plates with a 0.2% (w/v) solution of ninhydrin in butanol (Collins, 1978; Skipiski et al., 1961) saturated with water. Plates were heated for 5min at 100-105°C, lipids containing free amino groups appearing as red-violet spots.

Lipids containing vicinal diol groups were detected by the Schiff-periodate reagent (Baddiley et al., 1956; Shaw, 1968). Glycolipids appeared as blue spots, phosphatidyl glycerol appearing almost immediately as a blue spot, while other lipids may take several hours to appear. Phosphatidyl inositol appears as a brown spot.

Glycolipids were also detected by the α-naphthol spray of Jacin & Mischkin (1965). The plates were heated at 120°C for 10min, glycolipids appearing as purple-violet spots.

Dragendorff's reagent (Wagner et al., 1961) was used for the detection of choline containing lipids, which appeared as orange spots after gentle warming.

3.6 Analyses of carotenoids.

a Phototrophic bacteria.

The carotenoids of the phototrophic bacteria were extracted by a modified method of Schmidt et al. (1965) and Schmidt and Trüper (1971). 100mg of feeze dried cells were suspended in 1.0ml of water and extracted overnight, with stirring, at 4°C, under an oxygen-free nitrogen atmosphere, with 20ml acetone:methanol (7:3). The cell residue was separated from the solution by passing through a G3 sintered glass filter, and washing with acetone and finally chloroform, until no more pigment was extracted. The combined extract was brought to dryness under reduced pressure at 30°C and dissolved in 2.5ml methanol:ether (2:1), and saponified according to the method of Schmidt et al. (1965). The carotenoid compositiom of the cells was determined by thin layer chromatography on silica gel, using standard carotenoids for co-chromatography. The standard carotenoids were obtained by the method of Schmidt et al. (1965), and identified by chromatography and absorption spectrum (Schmidt et al., 1965; Pfennig et al., 1968; Schmidt & Trüper, 1971). Standard carotenoids

were obtained from E.mobilis, L.roseopercina, Rhm.vannielii, Rhsp.rubrum, Chr.minus, and Chr.violascens.

b. Extremely halophilic bacteria.

The carotenoids of the extremely halophilic bacteria were extracted by the method of Kates et al. (1966) described in 3.5b, except that extraction was carried out in the dark at 4°C under an oxygen-free atmosphere. The carotenoids of H.salinarium were used as standards for comparison in the solvent system chloroform:ether (99:1), benzene:acetone (65:35), and chloroform:methanol (93:7) on silica gel thin layers (Kushwaha et al., 1975b).

3.7. Electron microscopy of the phototrophic bacteria.

a. Negative staining.

Cells were negatively stained with 1.0% (w/v) or 2.0% phosphotungstic acid (Imhoff & Trüper, 1977; Oyewole & Holt, 1976) on carbon coated formvar covered copper grids, and examined at an accelerating voltage of 80Kv in a Joel 100S electron microscope.

b. Thin sections.

Cells were harvested, washed, and prefixed with 0.1% (w/v) osmium tetroxide, using the modified method of Kellenberger et al. (1958) described by Murray and Watson (1965). Blocks were transfered to 0.5% (w/v) uranyl acetate solution, followed by dehydration in a graded acetone series (Murray & Watson, 1965). The blocks were permeated overnight with propylene oxide, followed by transfer to propylene oxide:araldite (1:1) overnight. Blocks were then transfered to araldite for 5h followed by polymerisation in fresh araldite resin for 48h at 60°C. Blocks were sectioned on a ultramicrotome (Cambridge Huxley-Pattern mk2) with a glass or diamond knife. Sections were cut on a diamond knife by Ms.S.Bulman. Thin sections were taken up on copper grids and stained with uranyl acetate or lead citrate (Murray & Watson, 1965; Remsen et al., 1968), and examined

at an accelerating voltage of 80Kv in an AEI 802 or Joel 100S electron microscope.

3.8. Determination of GC base content.

The DNA from phototrophic and extremely halophilic bacteria was isolated by the method of Marmur(1961). Cells were harvested and washed in the basal salts solution of the growth medium, suspended in saline-EDTA solution followed by treatment with sodium dodecyl sulphate and sodium perchlorate (Marmur, 1961). The suspension was deproteinised with 4% (v/v) iso-amyl alcohol in chloroform followed by treatment with ribonuclease (BDH). The DNA was again deproteinised, selectively precipitated with iso-propyl alcohol and stored in saline-citrate over a small volume of chloroform at $4^{o}C$. The purity of the DNA sample was determined by the absorption spectrum in the UV range, using a Beckman model 35 spectrophotometer. DNA from Esherichia coli strain K_{12} was extracted by the method of Marmur (1961) and used as a standard, "Micrococcus lysodiekticus" DNA was obtained from Sigma and used as a standard.

The GC base content was determined by melting point by the method of Marmur and Doty (1963) in either 1/10th or normal strength standard saline citrate, using a Pye-Unicam SP800 fitted with an SP876 automatic temperature programmer. The GC base content was calculated from the relationships (Owen & Lapage, 1976):

i) for normal saline citrate

$$\%GC = 2.44T_m - 169.3$$

ii) for 1/10th saline citrate

$$\%GC = 2.08T_m - 106.4$$

where T_m is the metlting point of the DNA.

Buoyant demsity determinations were carried out using an MSE centriscan ultracentrifuge at a speed of either 38,000 or 40,000rpm at room temperature. The centrifuge cells were loaded according to

the method of Mandel et al. (1968). The density of the final solution was adjusted to approximately 1.71g/ml using an Abe refractometer (Mandel et al., 1968). The position of the DNA bands was determined when the cells had reached equlibrium after 20-24h. An expanded scale trace was produced directly from the scan of the cell. The buoyant density and, hemce, the GC base content was determined from the relationship (Mandel et al., 1968):

$$p = p_o + 4.2w^2 (r^2 - r_o^2) \times 10^{-10} \text{ g/ml}$$

where p_o is the density of the reference DNA

p is the density of the DNA to be determined

r_o is the distance of the reference DNA from the centre of rotation

r is the distance of the sample DNA from the centre of rotation

w is the speed of rotation in radians/second

$$\%GC = \frac{p - 1.660}{0.098} \text{ g/ml}$$

3.9. Analysis of the cellular components of extremely halophilic bacteria.

a. Detection of muramic acid and sugar amines.

Muramic acid and sugar amines were detected in the hydrolysed cell walls by the method of Rondle and Morgan (1955) as described by Mullakhanbhai and Larsen (1975). Cell envelopes were isolated by the method of Holmes et al. (1965), dialysed against distilled water overnight at 4°C, and hydrolysed at 105°C for 4h in evacuated glass tubes in 4N HCl. After passing through glass wool and evaporating to dryness under reduced pressure at 30°C, the residue was dissolved in the minimum quantity of distilled water and purified by elution from a Dowex-50 W (H^+) X8 (200-400 mesh) (Sigma) column with 20ml 0.5N HCl. The eluate was evaporated to dryness under

reduced pressure, dissolved in 2ml distilled water, and muramic acid and sugar amine determined by the colorimetric method of Rondle and Morgan (1955) using glucosamine (Sigma), galactosamine (Sigma), and muramic acid (Sigma) as standards (Mullakhanbhai & Larsen, 1975). The initial concentration of protein in the samples was determined by the method of Lowry et al. (1951), using bovine serum albumin (Sigma) as a standard.

b. Amino acid composition of bulk protein.

The cell pellet was lysed by the method of Holmes et al. (1965). The cell envelopes were collected after dilution with distilled water, and harvested at 20,000g for 1h at 4°C. The supernatant was decanted, mixed with trichloroacetic acid (TCA) and heated at 90°C to precipitate protein (Reistad, 1970). After washing twice with TCA the collected protein was resuspended in 50mM NH_4HCO_3 and hydrolysed in 6M HCl for 48h at 105°C. The suspension was evaporated to dryness under reduced pressure and redissolved in 0.2M HCl. The amino acid composition was determined using an automatic amino acid analyser (Locarte mk IV floor model) by Dr.J.W.Keyte.

c. SDS polyacrylamide gel electrophoresis.

SDS polyacrylamide gel electrophoresis of cell envelopes or membrane fractions was carried out by the method of Mescher et al. (1974). SDS polyacrylamide gels were prepared by the method of Laemnli (1970) modified according to Hancock et al. (1976), either as tube or slab gels. Samples were dissolved in the buffer of Mescher et al. (1974) and heated for 10min in a boiling water bath before loading onto the gel. Standards of Xenopus vitellogen, phosphorylase A, transferin, bovine serum albumin, ovalbumin, lactate dehydrogenase, chymotrypsinogen, and lysozyme were obtained from Sigma. Gels were run according to the method of Weber and Osborn (1969), and stained for protein with Coomassie Brilliant Blue, and

carbohydrate with periodate-Schiff as described by Fairbanks et al. (1971). Slab gels were run and stained by Ms.V.Darby.

d. Isolation and characterisation of the purple membrane.

The purple membrane from H.halobium strain R_1 was isolated by sucrose density gradient centrifugation (Becher & Cassim, 1975; Kushwaha et al., 1975a; Oesterhelt & Stoeckenius, 1974). Cells were grown according to the method Kushwaha et al. (1975a), harvested at 10,000g for 20min, and washed twice with the basal salt solution of the growth medium (Kushwaha et al., 1975a). The cell pellet was frozen at -70°C, thawed and dialysed against distilled water overnight at 4°C (Kushwaha et al., 1975a; Oesterhelt & Stoeckenius, 1974). 5.0mg DNAase (Sigma) was added to reduce the viscosity caused by the release of DNA from the lysed cells. The dialysed cell membranes were then purified by centrifugation to remove traces of cell wall at 10,000g for 20min at 4°C. The supernatant was then centrifuged at 50,000g for 1.5h at 4°C to collect the cell membranes (Kushwaha et al., 1975a). The membrane pellet was resuspended with the aid of a tissue grinder, and then subjected to repeated centrifugation at 10,000g followed by centrifugation at 50,000g until the membranes were free of cell wall debris, and the supernatant obtained after the cell membranes were collected was free of carotenoids (Kushwaha et al., 1975a; Oesterhelt & Stoeckenius, 1974). The membrane pellet was then resuspended in water and layered on to a 1.3M/1.5M sucrose step gradient and centrifuged at 200,000g for 20h at 4°C in a Sorval T-865 fixed angle rotor. The purple membrane fraction, which collects at the interface of the 1.3M and 1.5M sucrose layers, was collected from below and layered onto a second 1.3M/1.5M sucrose gradient and centrifuged as before (Kushwaha et al., 1975a; Oesterhelt & Stoeckenius, 1974). The purity of the membrane was determined by measuring the absorption spectrum using a Beckman

model 35 spectrophotometer, and by SDS polyacrylamide gel electrophoresis (Becher & Cassim, 1975; Oesterhelt & Stoeckenius, 1974). Sucrose was removed from the purple membrane by dialysis against distilled water.

Percoll, polyvinyl pyrrolidone coated silica particles (Pharmacia) was also used for the isolation of membrane fractions. The density of the purple membrane in Percoll was determined by layering purple membrane, isolated from sucrose density gradients, on to Percoll solutions and determining the density at which the membrane banded after centrifugation at 20,000g for 20min at 4°C in a fixed angle rotor. The density in Percoll was measured using an Abe refractometer. Purple and red membrane fraction could be isolated by loading Percoll gradients with either uncentrifuged dialysed membrane preparations or membranes partially purified by centrifugation. Purity of the purple membrane fractions obtained after repeated centrifugation in Percoll gradients at 20,000g for 20 min was determined by measuring the absorption spectrum before and after the removal of Percoll from the membrane suspension, and by SDS polyacrylamide gel electrophoresis of the Percoll free membranes. Percoll was removed from the membrane suspension by centrifugation at 100,000g for 2h at 4°C in a Sorval AH-650 swinging bucket rotor, and twice washed with water, followed by centrifugation at 100,000g for 2h.

e. Isolation of retinal.

Retinal was isolated from purple membranes or from whole cells by the methods of Oesterhelt et al. (1973) and Kushwaha et al. (1975a). Purple membranes were extracted with 0.01M cetyltriammonium bromide (CTAB, Sigma):acetic acid (9:1) in the dark and transfered to petroleum ether after the addition of ethanol (Oesterhelt et al., 1973). Whole cells were extracted with chloroform:methanol (2:1) and the retinal separated into the chloroform phase as described by Kushwaha

et al. (1975a). Retinal was identified by co-chomatography with authentic all-trans and 13-cis retinal (Sigma) in the solvent system hexane:ether (85:15) on silica gel thin layers (Oesterhelt et al., 1973). Retinal was detected by the fluorescence of the retinol in long wavelength UV light, after spraying with 1.0% $NaBH_4$ solution, and by the Carr-Price reaction (Oesterhelt et al., 1973).

RESULTS

1. ECOLOGY, CLIMATE, AND GEOGRAPHY OF THE LAKES.

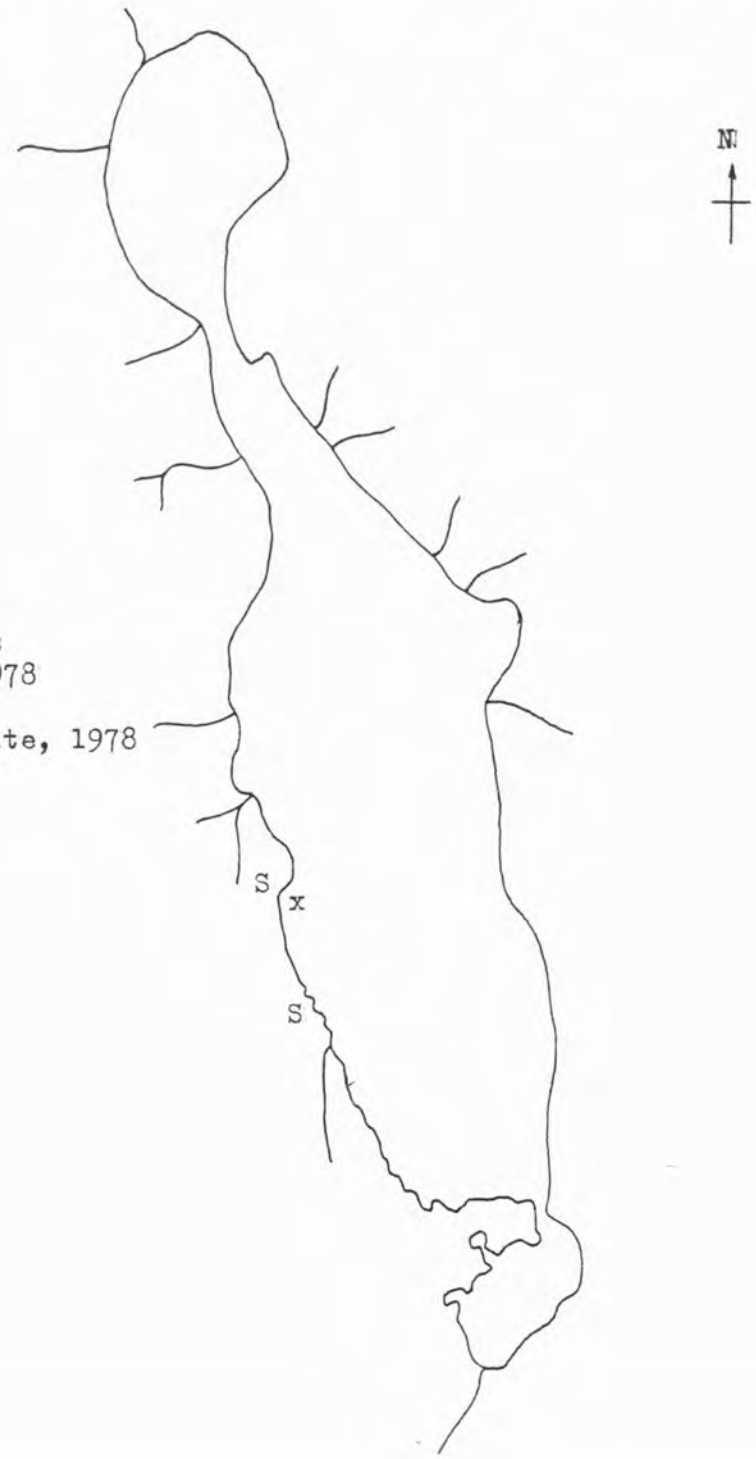
1.1. Lake Bogoria (formerly Lake Hannington).

Lake Bogoria lies about 30 miles north of the township of Nakuru (figure 5), below the Siracho Escarpment, which rises sharply from the eastern shore. The lake lies in a trough created by tilt faulting, which is characteristic of the area (McCall, 1967). Along the shore are numerous hot springs and geysers (figure 22), which may be submerged by the fluctuating water level. North of the lake is the swampy area of the Loboi Plain (figure 22), which indicates a possible subterranean outflow, and a prehistoric connection with the freshwater Lake Baringo, about 15 miles north of Lake Bogoria (figure 5). Small seasonal streams flow into the lake.

The lake water was pea green in colour in 1978, and vast numbers of Lesser Flamingos as well as Greater Flamingos were feeding along the shore (figure 23). The predominant alga present was a Spirulina sp., which formed a dense pellicle on the surface along the shore, and also coloured the lake water (figure 24). The level was higher than in 1976, when the predominant alga observed in samples returned from the site was a uni-cellular blue-green. No flamingos were observed at the lake in 1976. During the heavy rainfall in 1978, the lake level had probably risen to cover all the hot springs and geysers examined in 1976, although several of them were exposed at the time of sampling in 1978. A group of geysers on a rocky outcrop (figure 22), which were probably not covered by the high water level, were also examined in 1978. Most of the hot spring outflows were colonised by dense algal growths (figures 25, 26, 27). Close examination of the growth in some of the springs showed that it was stratified similar to the Chloroflexus-algal associations of the hot springs on other continents (figures 25, 26) (Bauld & Brock, 1973; Doemel & Brock, 1974; 1976; Florenzano et al., 1978;

Figure 22. Map of Lake Bogoria.

Position	36°.06'E 0.15'N
Altitude	3,200ft
Area	13sq miles
Average temperatures:	
Minimum	10-14°C
Maximum	30-34°C
Average rainfall	20-30in per year



S hot springs sampled, 1978

x sampling site, 1978

Figure 23. View east over Lake Bogoria.

Lake Bogoria, looking east towards the Siracho Escarpment, which rises sharply from the eastern shore (top). The green coloured water is speckled with thousands of Lesser Flamingos, which form a dense pink band along the shore line.

Figure 24. Algal pellicle at Lake Bogoria.

The algae in Lake Bogoria, in 1978, formed a dense brown and green pellicle along the edge of the lake, and were also responsible for the pea-green colour of the lake water. These algae were found to consist primarily of members of the genus Spirulina, on which the flamingos feed.

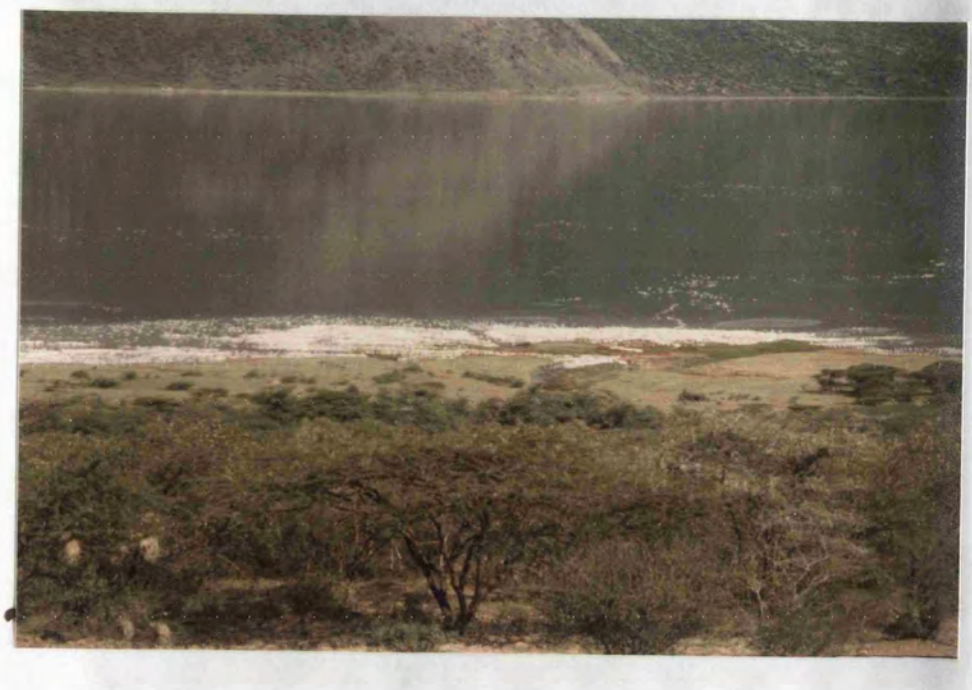


Figure 25. Hot springs, Lake Bogoria.

The hot springs and geysers, which line the western shore of the lake contained gelatinous/spongy masses, which formed thick, stratified mats, covering large areas of the rocks. The prolific growths seemed to be little affected by submersion in the alkaline lake water.

Figure 26. Chloroflexus/algal mat.

The mats which form the gelatinous/spongy masses in the hot springs and geysers contained algae and a filamentous organism resembling Chloroflexus aurantiacus. The mass development of these mats is typical of the Chloroflexus/algal mats found in other hot springs, in its layered structure of green algae and blue-green algae overlying the white or orange lower layer of the Chloroflexus-like organism.

Figure 27. Typical V-shaped growth in the hot springs outflow.

In some of the hot springs the Chloroflexus/algal mat may be seen to form a typical V-shaped pattern. This pattern is due to the different rates of cooling at the centre and edge of the outflow, and the inability of the organisms to grow above a certain temperature.

Madigan et al., 1974). In some springs the typical V-shaped pattern of growth was evident (figure 27). To examine the associations for the presence of organisms similar to Chloroflexus, enrichments were made on site in medium D of Castenholz (1969), and incubated in the light at 50°C. After 3-4 weeks, growth appeared on the side of the bottles, and was examined microscopically. Although uni-cellular and filamentous algae were present in the enrichments and the growths from the springs, the predominant organisms were fine filamentous forms resembling Chloroflexus. It was not isolated for further identification.

1.2. Lake Elmentieta.

Lake Elmentieta lies west of the Aberdare Range (figure 5), although this region of fairly high rainfall appears to contribute little to the drainage into the lake (McCall, 1967). The diatomite deposits of Kariandus, above the present lake level (figure 28), are indicative of the prehistoric Gamblian Lakes, which covered the present day lakes Elmentieta, Nakuru, and Naivasha (McCall, 1967; Thompson & Dodson, 1963). The topography of the area now effectively isolates the lake from the other lake basins (figure 5).

At the time of sampling in 1976 the lake level was low, and the shore line was marked by the exposed mud of the lake bed, which was overlain by a deposit of drying trona. In 1978 the lake level was much higher, having flooded surrounding pasture land (figure 29). In both years the lake water was coloured by algae (figure 30), which were predominantly uni-cellular in 1978, and filamentous in samples brought back from site in 1976. There was no evidence, in 1978, of the trona deposits visible in 1976 (figures 29, 30).

1.3. Lake Magadi.

The Magadi trough lies at the lowest point of the Rift Valley in southern Kenya (figure 5), and contains extensive saline deposits,

Figure 28. Map of Lake Elmentieta.

Position	36°.16'E 0.27'S
Altitude	5827ft
Area	7sq miles
Average temperatures:	
Minimum	10-14°C
Maximum	26-30°C
Average rainfall	30-40in per year

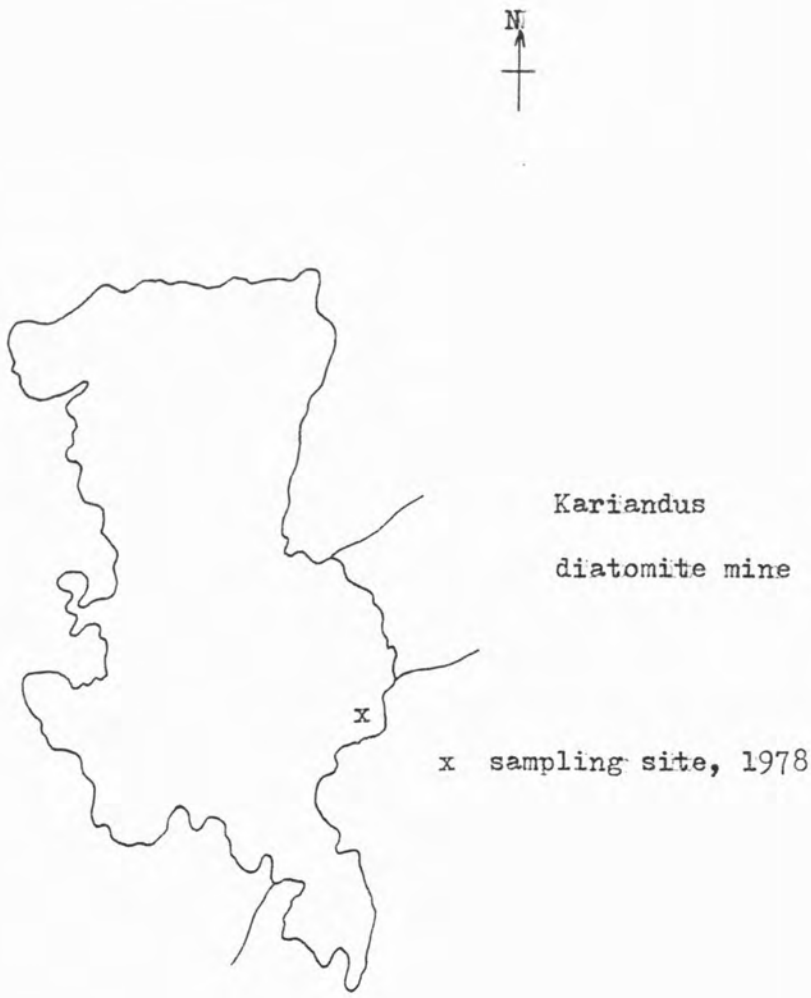


Figure 29. View north-west over Lake Elmentieta.

The view north-west over Lake Elmentieta, showing the location of the lake in a natural drainage basin. The high water level in 1978 is visible in the areas of partly submerged pasture land along the western shore.

Figure 30. Lake water at Lake Elmentieta.

Close inspection of the lake water shows that algae colour the water green. The algae present in 1978 were not, however, Spirulina sp., and no flamingos were observed at the lake.

Figure 31. Map of Lake Magadi.

Position	36°.15'E 1°.53'S
Altitude	1,900ft
Area	40sq miles
Average temperatures:	
Minimum	20-25°C
Maximum	35-40°C
Average rainfall	10-20in per year

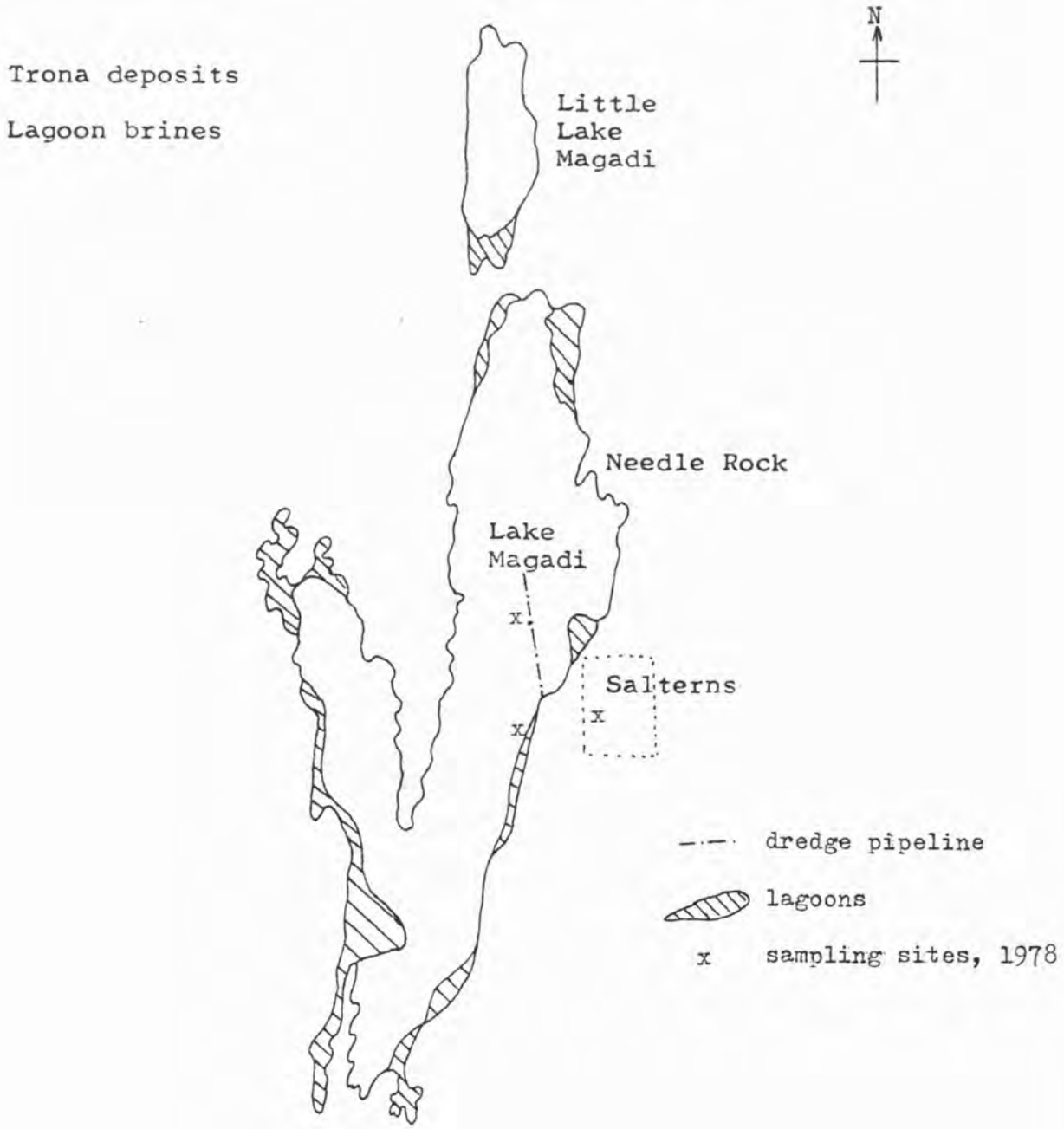
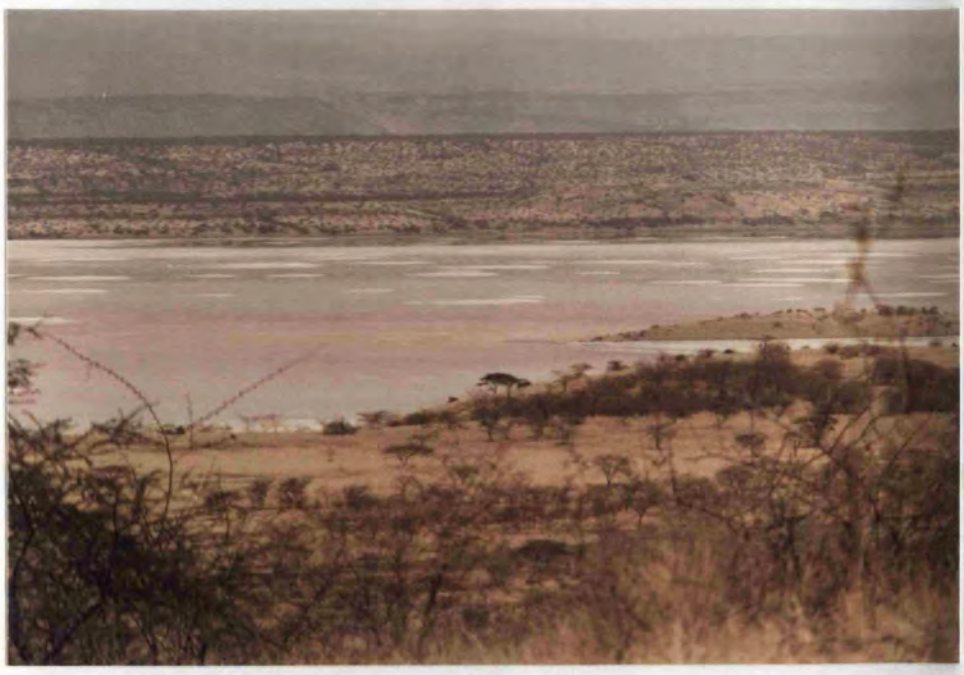


Figure 32. Trona crust, Lake Magadi.

The trona crust along the dredge pipe-line is off white in colour, with patches of pink, due to the presence of microorganisms containing carotenoids. The upper crust is brittle and crystalline, but the lower layers are strong enough to support the weight of a man.

Figure 33. View of Needle Rock, Lake Magadi.

The view west across Lake Magadi, with Needle Rock projecting into the lake from right to left. At the tip of Needle Rock a pool of water overlies the trona, and the surrounding area is coloured a variety of hues, from orange, through red, to purple. Along the western shore three distinct escarpments may be seen, merging with the skyline in the distance.



which are predominantly trona, making up the body of Lake Magadi (Baker, 1958). Older lake beds are visible in rocky outcrops at levels higher than that of the present lake, indicating that the lake was once much more extensive, and also more dilute (Baker, 1958). Although the main body of the lake is solid trona, lagoons lie along the edge of the deposits, and are fed by peripheral springs and seasonal streams (figure 31). Freshwater is pumped by pipeline to the works and township of Magadi, and the resulting sewage and effluent enters the lake directly at several places near the town. Water is never far below the surface of the trona and, although the lake does not usually support a free standing body of water above the trona, this has been known in years of exceptional rainfall, as in 1978. At the time of sampling in 1978 the water had receded below the trona.

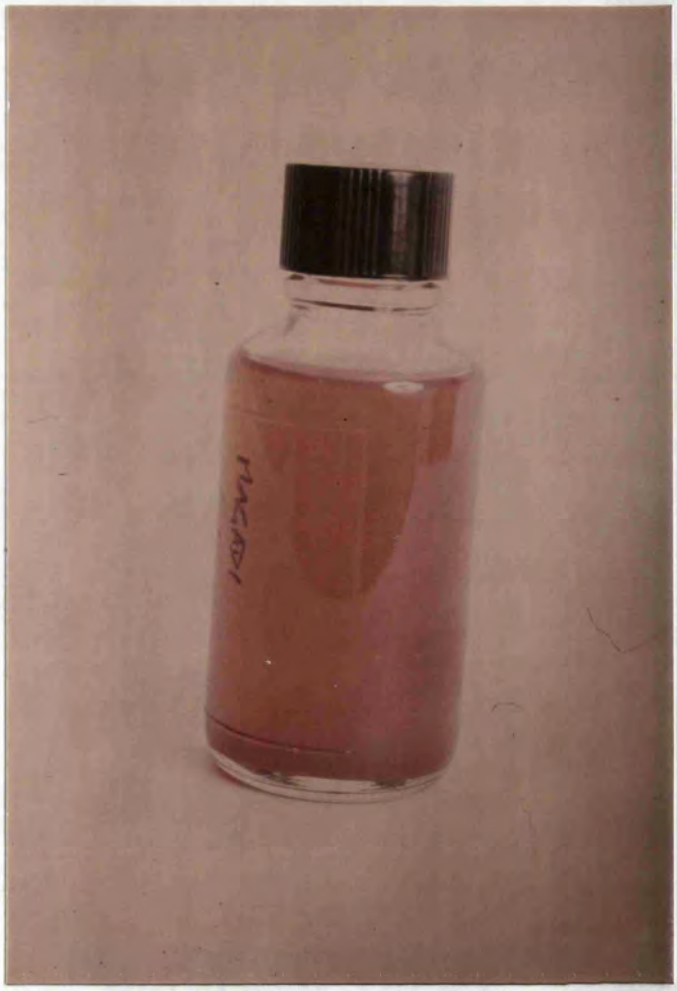
The surface trona, which is mined by a floating dredger, is variously coloured from off-white to orange, or even purple, according to the location and the time of year (figures 32, 33). This localised colour difference was best illustrated at Needle Rock (figure 33), where there was a small body of water. Around this area was a zone of coloured trona, which changed colour towards the main body of the trona deposits from orange to pinkish purple (figure 33). Closer examination of the trona along the dredge pipe (figure 31) showed that the upper most layer was a dry, cracked crust, with patchy colouration (figure 34). The crust was probably bleached by the sun, since to a depth of about 2cm the pink colouration intensified (figure 34). Directly below this was a pale green layer about 0.5cm thick, consisting almost exclusively of algae, which overlay thin bands of purple, dark green, and beige (figure 34). It was not possible to easily determine the predominant organisms in the thinner layers due to mixing of the zones in

Figure 34. Section of the trona crust, Lake Magadi.

The layers of trona immediately below the surface of the lake are strongly coloured in a series of sharply defined bands. The uppermost layer is pink, due to the presence of extremely halophilic bacteria, and the layer immediately below that is green, due to the presence of algae. The purple and dark green layers may be due to the presence of purple and green phototrophic bacteria, but only purple bacteria were isolated from the lake. The water below the surface is black, and has a characteristic foul smell, which was also noticed in cultures of the extremely halophilic bacteria isolated from the lake.

Figure 35. Lake Magadi water after storage.

The lake water collected and stored rapidly turned red-pink, due to the presence of purple sulphur bacteria, which included both gas vacuolate and non-gas vacuolate organisms.


MAGADI

transit to Nairobi for microscopic examination. However, the purple layer seemed to be composed of motile spirilla, probably phototrophic purple bacteria. No bacteria could be identified in the upper most layer by microscopy. Water collected from the dilute lagoon lying below the town rapidly turned reddish purple upon storage (figure 35). The colour was found to be due to motile rods and spirilla, the identity of which as purple phototrophic bacteria was tentatively confirmed by measuring the absorption spectrum using a Beckman model 35 spectrophotometer (figure 36).

At Lake Magadi, common salt is also harvested from the lake brines which percolate through the lower trona deposits. Pits are dug in the trona, which fill with the more concentrated lower brines, and typically contain 10-12% (w/v) NaCl and 12-16% (w/v) Na_2CO_3 (Magadi Soda Co. Ltd., analysis). The alkaline,highly saline water is then pumped into large, shallow salterns, where the liquor evaporates, concentrating the solutes present. Consequently, as the liquor concentrates the NaCl content inceases, and the liquor begins to turn pink to orange (figures 38, 39). Eventually Na_2CO_3 precipitates as the sesquicarbonate, and leads to selective enrichment for NaCl in the brine. The brine reaches saturation with respect to NaCl, and it is precipitated, harvested, and washed. All the saltern lagoons are highly alkaline due to the presence of Na_2CO_3, which is never completely lost from the brine. As the brine begins to concentrate and NaCl begins to precipitate, initially forming a surface crust, the colour of the brine varies from orange to claret, pink, or even purple (figures 38, 39). The intensity of the colour is a good indication of the final yield of NaCl (General Manager, Magadi Soda Co. Ltd., personal communication). There is a strong similarity between the colour of the saltern brines and that of the pink to orange areas of the lake trona (figures 33, 38,

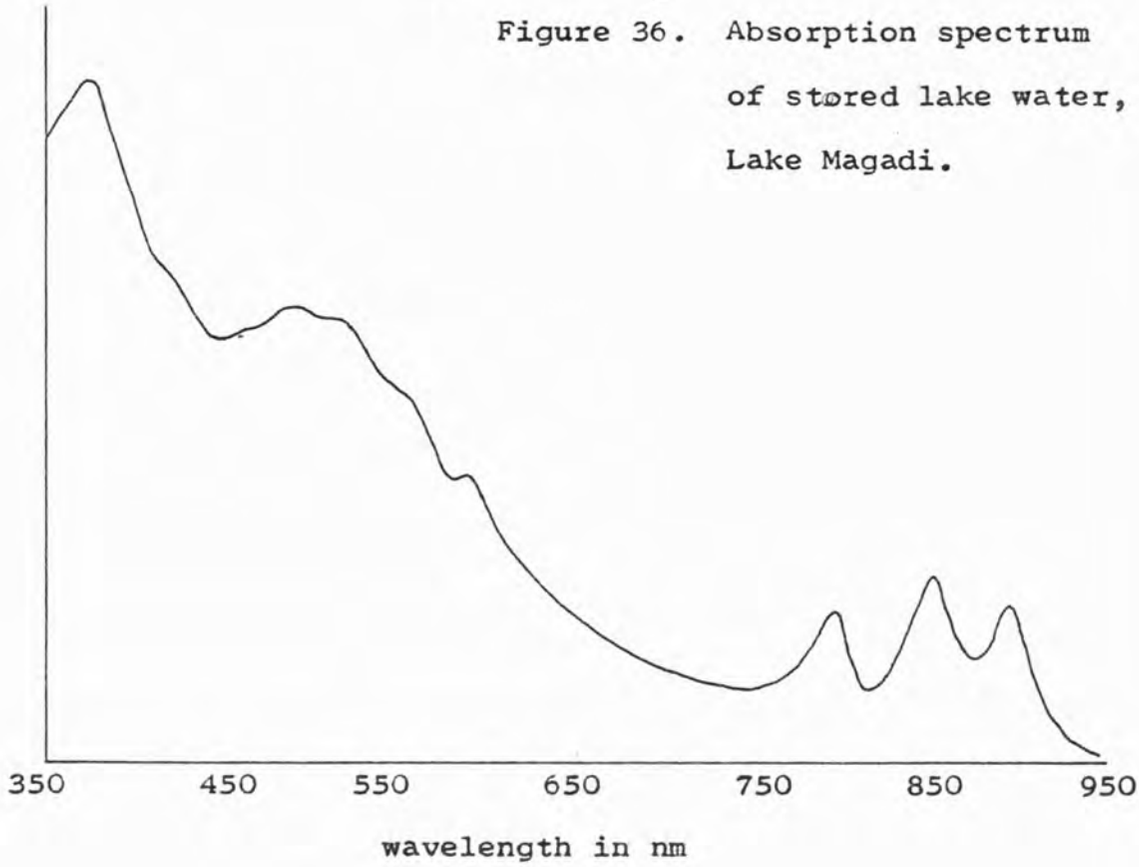


Figure 36. Absorption spectrum of stored lake water, Lake Magadi.

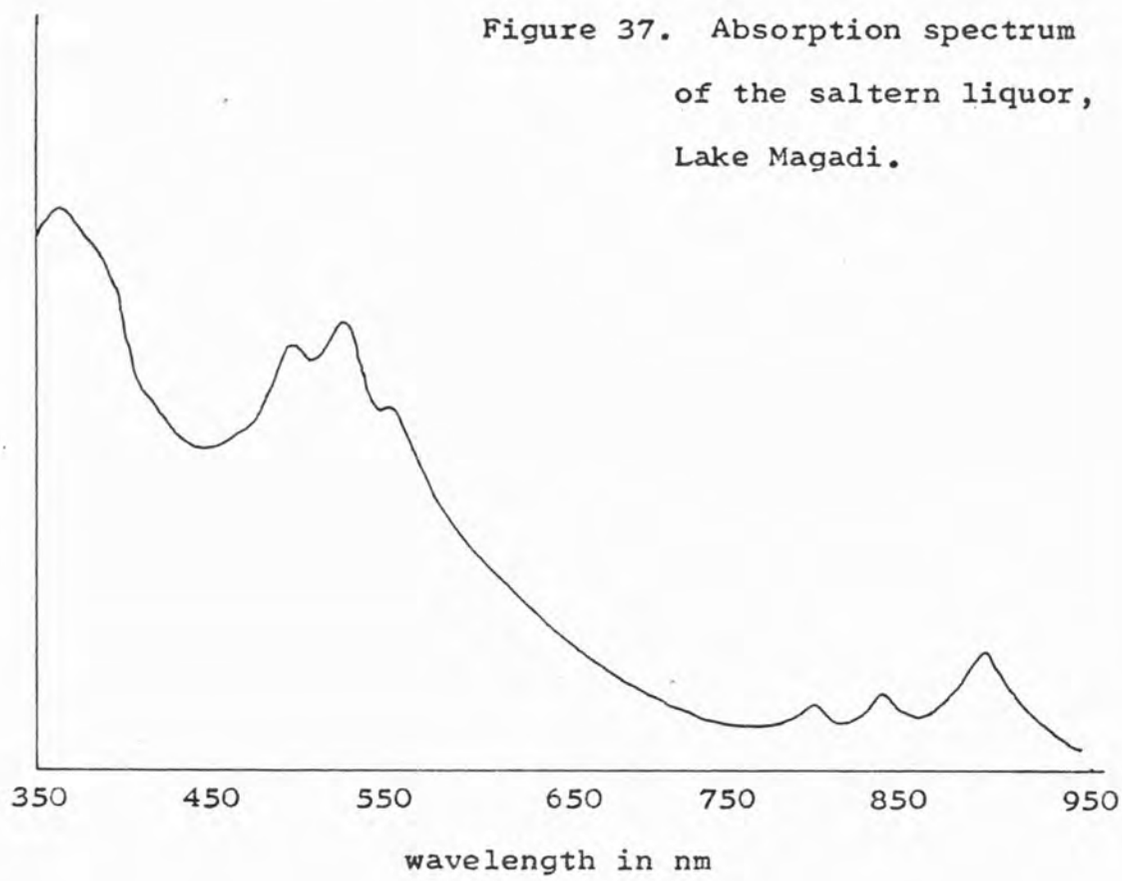


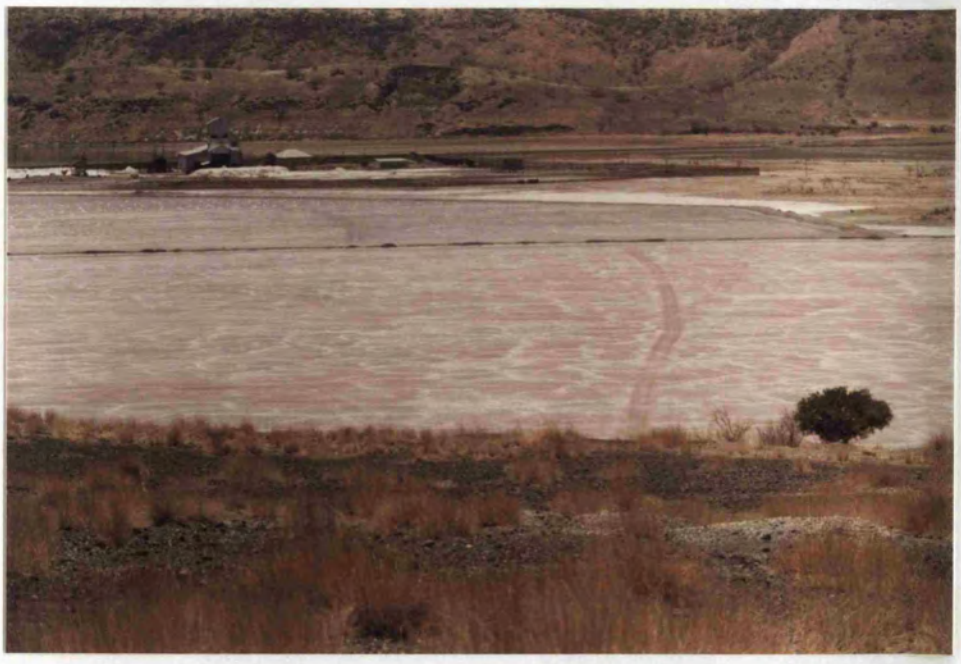
Figure 37. Absorption spectrum of the saltern liquor, Lake Magadi.

Figure 38. Saltern liquor, Lake Magadi.

Close examination of the saltern liquor, during the deposition of salt, shows that the liquor is covered by a thin crust of either sodium carbonate (during the day), or salt (during the night). The salt crust precipitates, while the sodium carbonate redissolves during the cool evening.

Figure 39. Salterns, Lake Magadi.

The shallow salterns at Lake Magadi are filled with water from pits dug in the lake trona. The water is initially black, due to the presence of mud, which precipitates in the first salterns. As the liquor concentrates it is transfered to further salterns, where it begins to turn red-orange in colour, due to the growth of pigmented halophilic microorganisms. From some angles the saltern liquor may appear to have a purple hue.



39). Microscopic examination of the saltern brines revealed the presence of numerous bacilli, cocci, and long fusiform organisms. Measurement of the absorption maxima showed that the pigmentation was due to the presence of carotenoids and bacteriochlorophylls present in some of these organisms (figure 37).

1.4. Lake Nakuru.

Lake Nakuru lies about 10 miles north-west of Lake Elmentieta, and drains the Mau Escarpment to the west (figure 5). Famous for its vast flocks of flamingos, the ecology of the lake is undergoing change due to the introduction of the alkali-tolerant Tilapia grahamii from Lake Magadi, and the concomitant increase in the numbers of fish eating birds. The lake has been known to dry up almost completely, with the exception of the north-east corner, where freshwater springs enter the lake (figure 40). The lake is also fed by seasonal and aseasonal streams and rivers, and by treated sewage effluent from the town of Nakuru.

During 1976 flamingos were present on the lake, and the predominant alga observed in samples brought back was a Spirulina sp. In 1978, when the lake level was higher, and some of the surrounding area was flooded, the predominant alga was a uni-cellular organism, no flamingos were present. Several of the tracks at the southern end of the lake were flooded by the high water level in 1978. At one point, where the road was raised above the surrounding grassland, a large area of water was separated from the lake (figure 41). Since the water level of the lake had previously been higher, and the pH of the isolated pools was the same as the pH of the lake water, it was assumed that the pools had been separated from the lake as the high water level receded. The water of the lake was brownish-green in colour, due to the presence of algae (figure 41), but the isolated pools were red in colour (figure 42,

Figure 40. Map of Lake Nakuru.

Position	36°.06'E 0.23'S
Altitude	5765ft
Area	11.5sq miles
Average temperatures:	
Minimum	10-14°C
Maximum	26-30°C
Average rainfall	30-40in per year

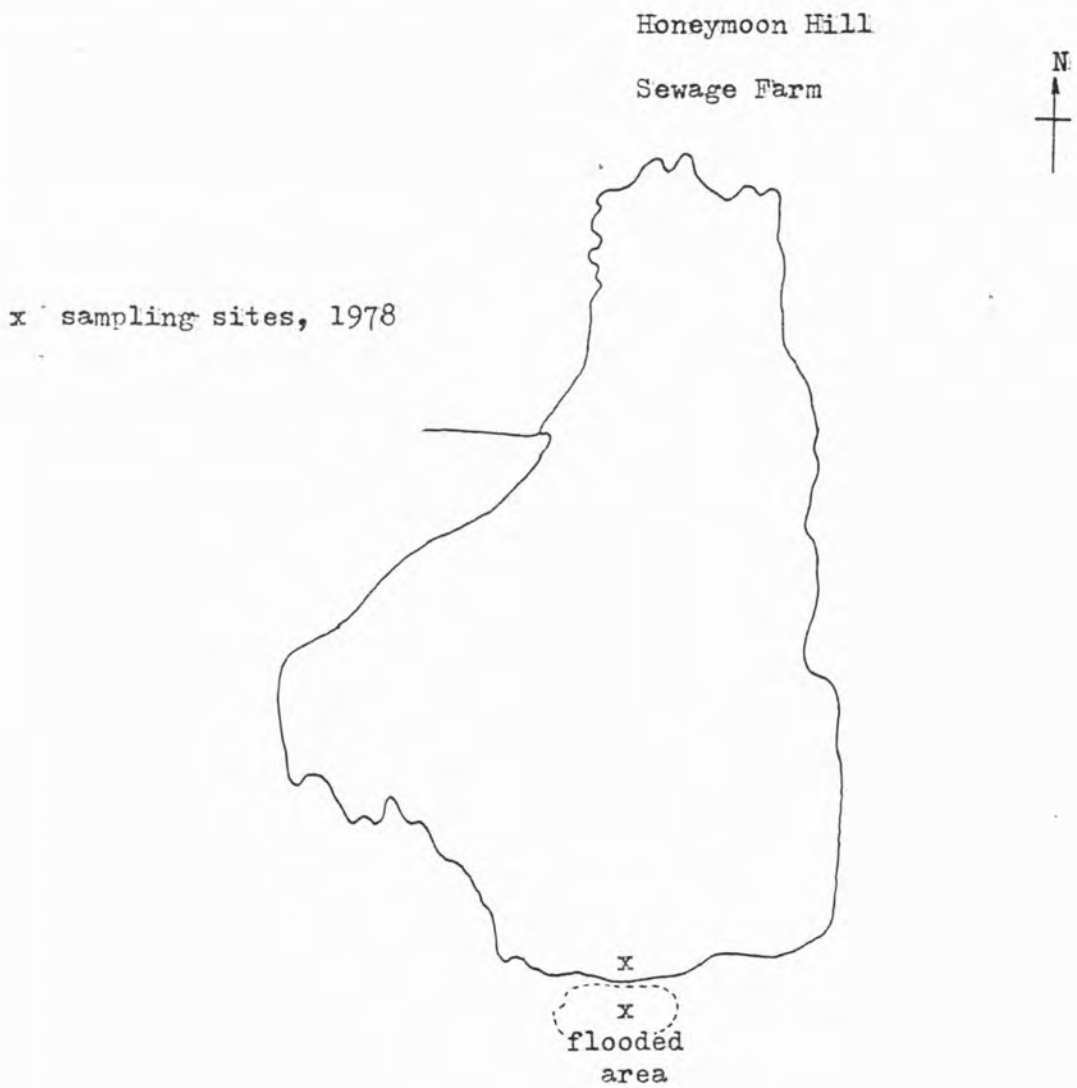


Figure 41. Lake Nakuru water.

The lake water at Lake Nakuru was coloured green-brown, in 1978, due to the presence of algae. The higher lake level, in 1978, had caused flooding of the surrounding area. At one point, along the southern part of the lake, a raised road had divided the flood water from the main body of the lake.

Figure 42. Isolated pools, Lake Nakuru.

The isolated pools of water, lying south of the raised road, were coloured brown-red, and contrasted strongly with the colour of the lake water. The colour of the isolated pools was caused by the presence of large numbers of purple phototrophic bacteria, predominantly gas vacuolate strians.

Figure 43. Lake water from Lake Nakuru.

The water from the isolated pools at Lake Nakuru was stored and examined at a later date. The water remained red-brown in colour, and the organisms observed in the water were gas vacuolate and non-motile, but became motile during storage, and lost the gas vacuoles.

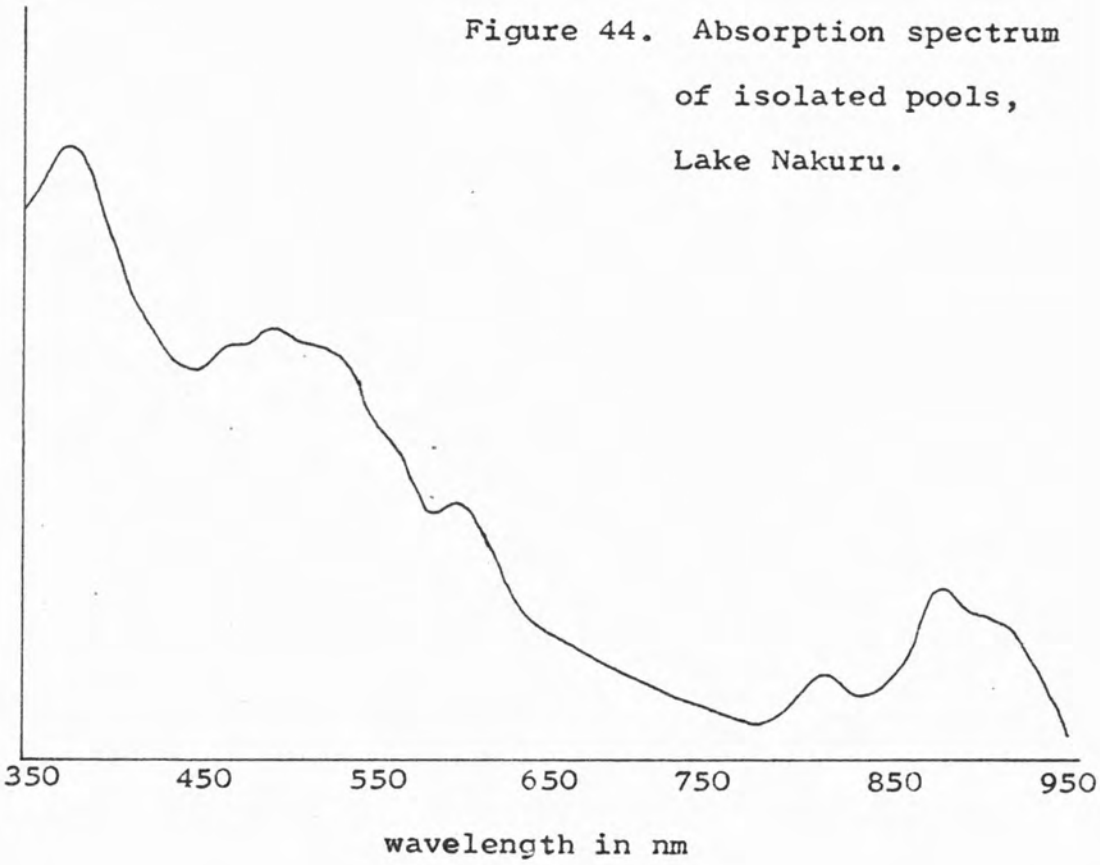


Figure 44. Absorption spectrum of isolated pools, Lake Nakuru.

43), and the pool water was examined in more detail. Microscopic examination of the water revealed the presence of large numbers of motile rods and spirilla, some of which contained refractile inclusions. The colouration was shown to be due to the presence of carotenoids and bacteriochlorophyll a in the cells, by measuring the absorption maxima, using a Beckman model 35 spectrophotometer (figure 44). Flowing into the northern end of the lake is the effluent from the Honeymoon Hill sewage farm. In 1978 the oxidation ponds at the sewage farm were pink-red in colour due to the presence of phototrophic bacteria, which were apparently enriched by the high levels of sulphide reaching the oxidation ponds from the anaerobic digestion tank (Tindall, unpublished). Enrichments made at pH 9.5 failed to yield growth, although the organisms grew at lower pH. The organisms isolated (Grant & Schofield, unpublished) did not resemble any of the organisms isolated from the lake, and the sewage farm was, therefore, discounted as a possible source of the phototrophic bacteria in the lake.

1.5. Naivasha Crater Lake.

Naivasha Crater Lake is a small lake lying in the crater of an extinct volcano. The lake lies about 3½ miles west of Hippo Point, and almost due north of Little Lake Naivasha (figure 5, 45). It is sometimes confused with the lake which forms in the crater at the north-east edge of Lake Naivasha. The rim of this crater is usually partly submerged, and forms Crescent Island. During dry years the whole of the crater rim may be exposed, cutting off the water within the crater. Naivasha Crater Lake is never connected to Lake Naivasha, as are other lakes in the complex, and its water level is about 50ft higher than that of the other lakes. The lake water of Naivasha Crater Lake is always alkaline by comparison with Lake Naivasha, which has a pH of 7.5, and Little Lake Naivasha, in

Figure 45. Map of the Naivasha lakes.

Position	36°.16'E 0.47'S
Altitude	6180ft
Area of Crater Lake	1sq mile
Average temperatures:	
Minimum	10-14°C
Maximum	26-30°C
Average rainfall	10-20in per year

Figure 46. View north over Naivasha Crater Lake.

As it's name implies this lake lies in the crater of an extinct volcano, west of Lake Naivasha. The lake is effectively isolated from the drainage into Little Lake Naivasha and Lake Naivasha by the sides of the volcano. The water level in the crater lake is, apparently, 50ft higher than that of the other two lakes, and there appears to be no subterranean inflow. The lake water was characteristically pea-green in colour, in 1978, as was the lake water of the less alkaline Little Lake Naivasha.

which the pH fluctuates between pH 8.5 and 9.5 (Gaudet, personal communication). Although the lake was only sampled in 1976, due to difficulties of access in 1978, the lake level was higher in 1978 than in 1976. The lake appears to be fed mainly by rainfall, and not by subterranean springs, as also indicated by the fluctuation in the lake level with the annual rainfall. In both years the water was characteristically pea green in colour (figure 46), and in 1976 the most numerous organism observed in samples brought back was a filamentous blue-green alga. The colour of the lake water was similar to that observed in Little Lake Naivasha, although this lake was not investigated.

2. WATER CHEMISTRY.

The chemical analysis of the water samples brought back from the lakes was carried out as soon as possible after collection. Where possible the pH and $CO_2/HCO_3^-/CO_3^{2-}$ concentration was measured on site, and again in Leicester. The results of the chemical analysis are presented in table 6. The pH, as determined by a sodium ion corrected Ingold combination electrode (Pye-Unicam), has been compared with the pH calculated from the relationship:

$$pH = pK + \log \frac{[CO_3^{2-}]}{[HCO_3^-]}$$

where $pK = -\log K$

$K = 5.61 \times 10^{-11}$, and is the equilibrium constant of the reaction (after Golterman et al., 1978):

$$HCO_3^- \rightleftharpoons H^+ + CO_3^{2-}$$

the values of $[CO_3^{2-}]$ and $[HCO_3^-]$ are those measured by chemical analysis.

The values obtained for the calculated pH and those obtained by direct measurement are in close aggrement, illustrating that, allowing for deviations from the calculated pH caused by the method of measurement, the actual pH of the lake waters would not be significantly different. The pH of the water is directly related to both the relative concentration of the anions HCO_3^- and CO_3^{2-}, and to their absolute concentration, and hence, the salinity of the water. These results are not significantly different from those obtained by other workers, who have examined the same lakes (Baker, 1958; Eugster, 1970; Jones B et al., 1977; McCall, 1967; Talling & Talling, 1965; Thompson & Dodson, 1963), or other soda lakes (Eugster & Hardie, 1978; Imhoff et al., 1978b).

Eugster (1970) has shown that the effect of evaporative concentration may be followed in East African waters by comparing

Table 6. Chemical analyses of the lake waters.

a. Analyses made in 1976

	Lake Bogoria	Lake Elmentieta	Lake Magadi	Lake Nakuru	Naivasha Crater Lake	Salterns
Na^+(mM)	494	1,725	4,600	410	154	-
K^+ (mM)	4.64	31.8	42.0	7.8	5.8	-
Mg^{2+}(mM)	0.002	0.001	0.001	0.005	0.038	-
Ca^{2+}(mM)	0.061	0.006	0.006	0.018	0.039	-
Cl^- (mM)	37.5	200	640	32.5	11.0	-
CO_3^{2-}(mM)	75	445	1,950	60	28	-
total alkalinity (mM)	382	1,075	2,380	291	137	-
pH (measured)	10.1	10.4	10.5	10.15	10.0	-
pH (calculated)	9.76	10.22	11.03	9.79	9.78	-

b. Analyses made in 1978

	Lake Bogoria	Lake Elmentieta	Lake Magadi	Lake Nakuru	Naivasha Crater Lake	Salterns
Na^+(mM)	652	300	2,000	400	-	10,200
K^+(mM)	5.0	4.5	16.0	3.1	-	215
Mg^{2+}(mM)	0.002	0.001	b.l.d.	0.001	-	b.l.d.
Ca^{2+}(mM)	0.045	0.165	0.0175	0.040	-	b.l.d.
Cl^-(mM)	85	67	400	33	-	4,500
CO_3^{2-}(mM)	220	54	582	57	-	1,654
total alkalinity (mM)	546	133	1,304	123	-	2,233
pH (measured)	10.4	10.5	10.35	10.45	-	10.8
pH (calculated)	10.2	10.21	10.28	10.31	-	10.8
PO_4^{3-}(mM)	0.004	0.05	0.008	0.06	-	0.10
NH_4^+(mM)	0.23	0.042	0.42	0.042	-	0.25
total nitrogen (mM)	0.25	0.25	2.2	0.20	-	0.80
conductivity x 10^4 mho	24.0	1.2	52.0	1.2	-	90.0

(b.l.d.; ion concentrations below the level of detection)

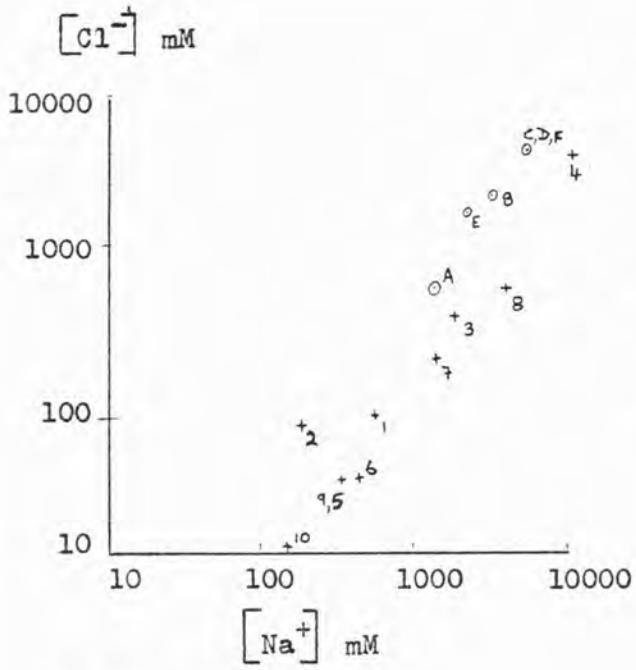
the concentrations of cations and anions with the concentration of Cl^-, which is one of the most soluble anions in these waters. If the water analyses are subject to analysis in this manner, and are compared with the results obtained from previous studies, it may be seen that there is no significant deviation among the results, with the exception of the Magadi saltern liquor, which is affected by the precipitation of NaCl (figure 47). The linear relationship between the different lake waters shows that the major controlling effects in the water chemistry are the nature of the surrounding rock and evaporative concentration, rather than solute loss in the lake. Comparison with results obtained from other soda lakes outside the Kenyan Rift Valley shows that they are influenced by the geology of the area, due to the different solute concentrations in the leaching waters (figure 47). Thus, even the more dilute lakes in the Rift Valley, Kenya would form soda lakes like those at Nakuru, Elmentieta, and Bogoria on further evaporative concentration. Concentration of the less concentrated soda lakes would also lead eventually to the deposition of trona, as has been seen at lakes Nakuru and Elmentieta in the past, when trona was also mined, as at Lake Magadi. Similarly, under the same geological conditions dilution of Lake Magadi would result in the formation of more dilute water, tending towards the comparatively freshwater, found in lakes Naivasha, Turkana, and Baringo. The geological evidence shows that the present lakes have been subject to variations in lake level, which were accompanied by changes in the concentration of solutes in the water (Baker, 1958; Butzer et al., 1972; McCall, 1967; Richardson & Richardson, 1972; Thompson & Dodson, 1963). The changes in the lake level in the geological past were due to climatic and topographical changes. Climatic changes have been observed at a number of the lakes as a cyclic series of wet and dry periods over

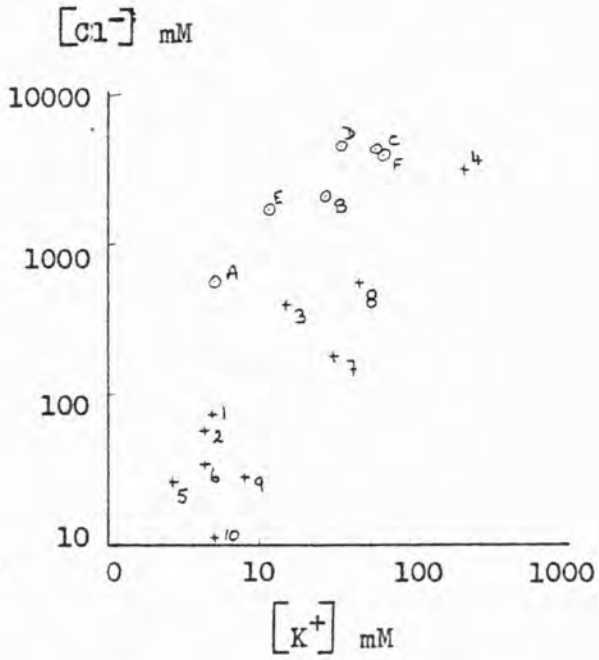
Figure 47. Concentration of the major ions in the soda lakes.

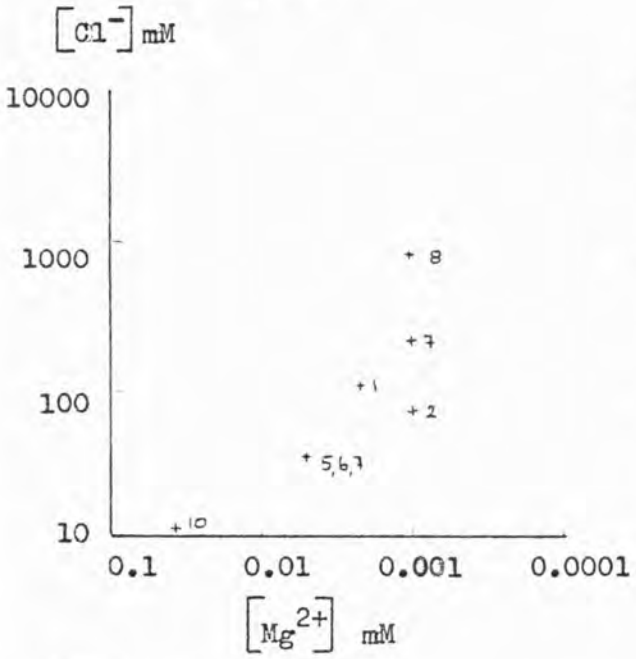
The concentration of the major ions in the lake water is compared to the concentration of the chloride ion, which is not precipitated until the later stages of evaporative concentration. The values obtained from the Kenyan soda lakes are compared to the values from the lakes of the Wadi Natrun (Imhoff et al., 1978b).

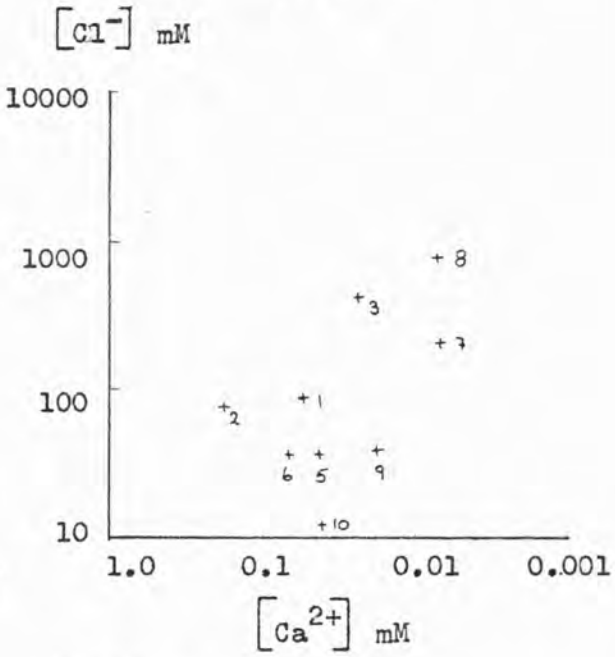
Kenyan lakes: 1, Lake Bogoria, 1978; 2, Lake Elmentieta, 1978; 3, Lake Magadi, 1978; 4, Lake Magadi salterns, 1978; 5, Lake Nakuru, 1978; 6, Lake Bogoria, 1976; 7, Lake Elmentieta, 1976; 8, Lake Magadi, 1976; 9, Lake Nakuru, 1976; 10, Naivasha Crater Lake, 1976

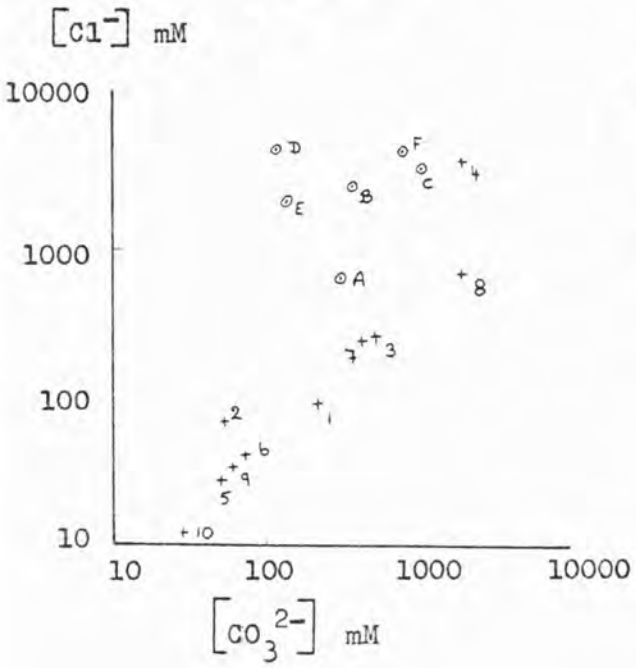
Wadi Natrun lakes: A, Lake Gabara; B, Lake Hamara; C, Lake Zugum; D, Lake Gaar; E, Lake Muluk; F, Lake Rizunia.

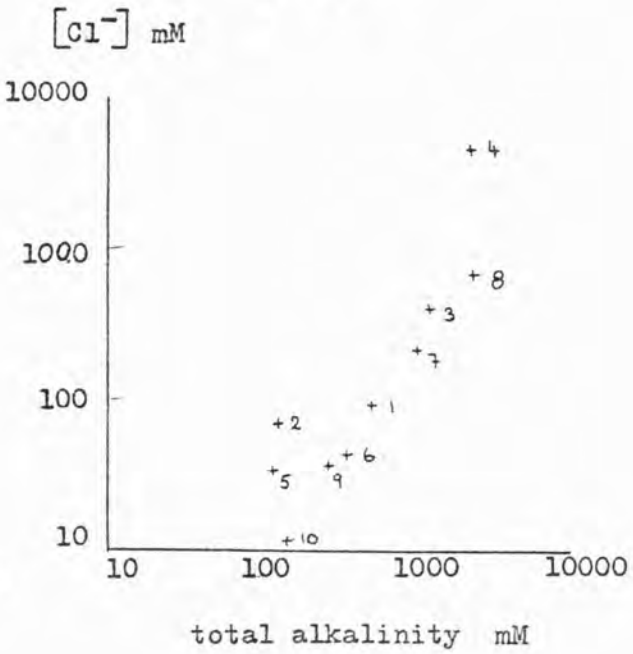

[Cl⁻] mM
10000
1000
100
10
10
100
1000
10000
[Na⁺] mM


[Cl⁻] mM
10000
1000
100
10
0
10
100
1000
[K⁺] mM


[Cl⁻] mM
10000
1000
100
10
0.1
0.01
0.001
0.0001
[Mg²⁺] mM


[Cl⁻] mM
10000
1000
100
10
1.0
0.1
0.01
0.001
[Ca²⁺] mM


[Cl⁻] mM
10000
1000
100
10
10
100
1000
10000
[CO₃²⁻] mM


[Cl⁻] mM
10000
1000
100
10
10
100
1000
10000
total alkalinity mM

a number of years, and is reflected in the changes in the water chemistry, as has been observed in other closed-basin lakes (Eugster & Hardie, 1978). The present minor climatic changes may be observed in the loss of trona production in the 1950's at Lake Nakuru, and the recent flooding of the lake observed intermittently over the last twenty years. These fluctuations in the water chemistry of the soda lakes of the Kenyan Rift Valley, whether over a period of tens of years, or thousands of years would have some influence on the biota of the lakes.

3. ISOLATION AND CHARACTERISATION OF PHOTOTROPHIC BACTERIA.

Phototrophic bacteria were enriched from the lakes by inoculation of liquid and soft agar media with mud samples, either on site, in 1978, or from samples brought back in 1976 and 1977, and by Winogradsky and Molisch type columns, after Pfennig (1965a). Similar enrichments were made using mud samples from a local Leicestershire stream at the natural pH of the stream, and at pH 9.5.

3.1. Enrichments.

Four different types of Winogradsky and Molisch column were prepared, using mud and water from the same source. The pH of the local stream water was adjusted to pH 9.5 by the addition of 3.0% (w/v) $Na_2CO_3.10H_2O$. One group of enrichments were prepared by filling boiling tubes to a depth of 15-18cm with mud, which was then covered with 2.0-3.0cm of water from the same source. The tubes were incubated either by a window, or in front of fluorescent strip lights (Philips daylight), at a light intensity of 1,000 lux, until growth was observed on the side of the tube. A second group of tubes were prepared by mixing mud, water, and plant remains from the same source with a small quantity of calcium sulphate (Pfennig, 1965a). The third group of tubes were prepared by mixing a small quantity of mud with eggwhite to give a layer about 2-3cm deep, on top of which was placed 5-7cm of mud from the same source, containing a small quantity of potassium sulphide and calcium sulphate (Pfennig, 1965a). A 7-10cm layer of water was introduced above the mud. These two groups of tubes were incubated in a window away from direct sunlight. The final group of tubes were prepared by mixing mud rich in organic matter with calcium sulphate in the ratio 2.0:0.5, to a depth of 5cm, and was then covered with lake water to a depth of 12-15cm. This group of tubes were incubated in the dark for 14 days before they were placed alongside the others, in a window away from

direct sunlight (Pfennig, 1965a). The temperature in the window varied from 15 to 26°C depending upon the outside temperature, while the temperature in front of the strip lights was relatively constant at 25-27°C. The tubes were examined periodically for pigmentation.

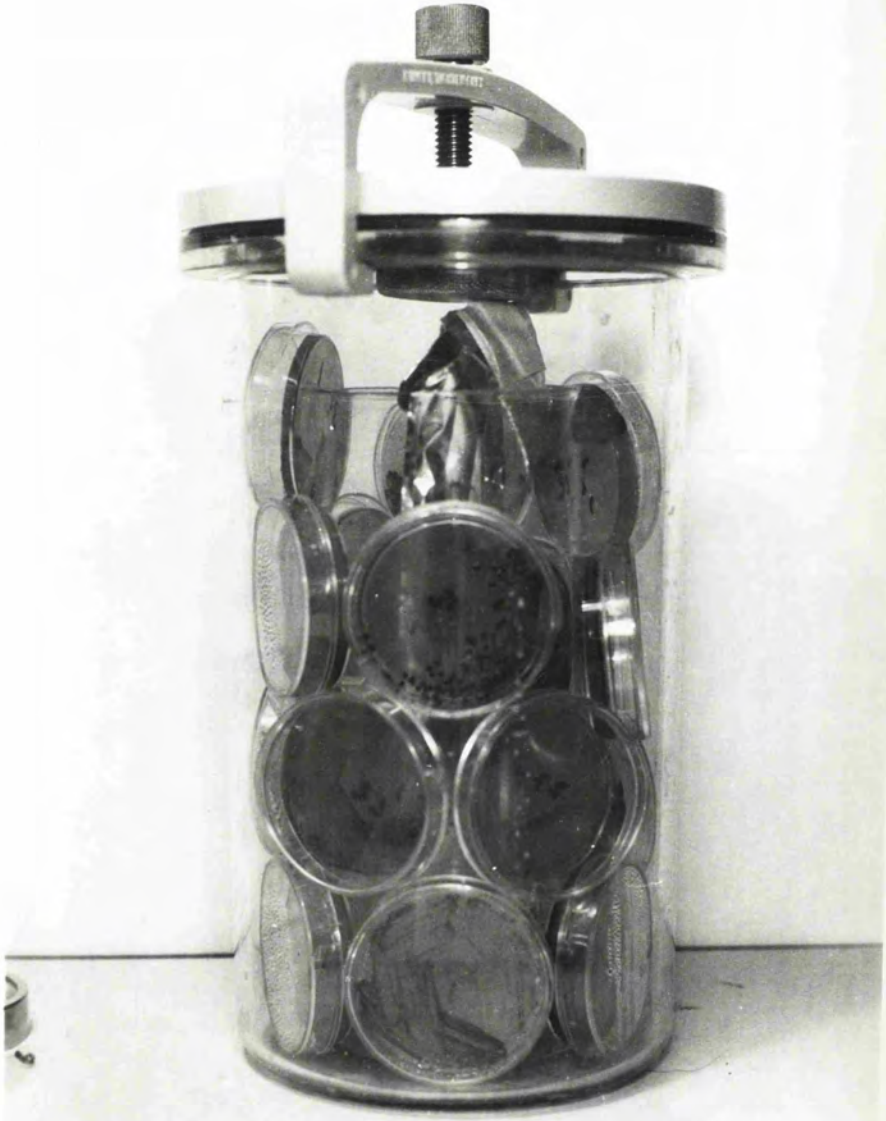
Liquid enrichments were prepared by the addition of mud samples to 30ml of sterile culture media, in filled universal bottles. The media used were those of Pfennig (1965a), or the modified medium of van Niel (1931, 1944), adjusted to either pH 7.5 or 9.5 as described earlier (Materials & Methods, 3.2a, 3.3a). Enrichments made in 1976, on samples collected, contained the carbon sources malate, succinate, or ethanol at concentrations of 0.1% (w/v), with or without the addition of 0.1% (w/v) $Na_2S.9H_2O$. In 1977 and 1978 all enrichments were made using 0.1% (w/v) acetate, without the addition of sulphide. All cultures were incubated at 1,000 lux in front of fluorescent strip lights, either Philips daylight, or Philips red, for the selective enrichment of purple or green phototrophic bacteria (Pfennig, 1965a; Whittenbury, 1971).

3.2. Isolation of pure cultures.

Pure cultures were obtained by the repeated inoculation of dilutions of enrichments, or mud directly into soft agar tubes, or on to agar plates. Agar plates were incubated in a 5% CO_2, 10% H_2, and 85% N_2 (v/v) atmosphere. Anaerobiosis was achieved by the use of palladium catalyst (Englehart D). Small, clear, polycarbonate jars (GasPak Systems) were used with an inner glass sleeve, which supported the small petri dishes (50mm dia Sterilin Products) in an upright position (figure 48). The jars were placed between parallel strips of lights during incubation of the plates. Single colonies were streaked on to plates containing the same medium as the enrichment, and were incubated anaerobically in the light in the polycarbonate jars. Purity was determined after three successive

Figure 48. Small anaerobic chamber.

Phototrophic bacteria were grown on 50mm dia plates under anaerobic conditions in a Gas-Pak polcarbonate jar, in which an upright glass sleeve was placed to allow the plates to stand vertically. The gas generating pack and anhydrous calcium carbonate, to absorb water, were placed inside the glass sleeve.



passages of single colonies on media of the same composition. The cultures were examined microscopically for the presence of different morphological forms, and for the presence of sulphate reducing and anaerobic bacteria by inoculation into the Desulphovibrio medium of Starkey (Abd-el-Malek & Rizk, 1959), at pH 7.5 and 9.5, and Difco AC medium, at pH 7.5 and 9.5 (Trüper, 1968; Imhoff & Trüper, 1977). Pure cultures were maintained on agar plates containing 1.5% (w/v) NaCl, which supported the growth of all the strains irrespective of their Na^+ requirement in liquid media. Growth was found to be enhanced when agar plates were prepared by pouring the agar growth medium over a 1.0ml layer of 1.5% (w/v) agar containing 0.3% (w/v) $Na_2S.9H_2O$, which had been allowed to solidify in the base of the plate. Agar plates were dried at 37°C for 30min, and used the same day. The effect of sulphide appeared to be lowering of the redox potential of the plates, enabling growth to occur more rapidly after inoculation of the plates. Reference strains were also maintained on agar plates prepared by the solidification of the appropriate medium with 1.5% (w/v) agar. Ectothiorhodospira halophila and E.halochloris could not easily be maintained on agar plates as growth was sparse, and often failed. Both species grew in agar deeps containing the medium of Imhoff and Trüper (1977), solidified with 1.0% (w/v) agar, and these strains were, therefore, maintained in liquid culture and in soft agar tubes.

3.3. Organisms which were not isolated or fully characterised.

A liquid enrichment at pH 9.5 from a mud sample brought back in 1977 from Lake Nakuru, containing 1.0% (w/v) NaCl, malate, and sulphide, gave rise to a green coloured organism, which morphologically resembled members of the genus Chlorobium. The organism, which was isolated, but grew poorly and was lost before it could be studied, grew as chains of cells, 1.0µm wide by 1.0-2.0µm long. The organism

was not motile. Attempts to isolate similar organisms from the lakes by the use of restricted wavelength light were not successful, and this organism was not observed in any other enrichments. The presence of members of the Chlorobiineae in the soda lakes would not be unusual, since Baas-Becking and co-workers (Baas-Becking & Wood, 1955; Baas-Becking et al., 1960) found green phototrophic bacteria grew up to pH 9.0-10.0, and some members of the Chloroflexaceae have pH optima of 8.5 (Dubinina & Gorlenko, 1975; Gorlenko & Pivovarova, 1977; Pivovarova & Gorlenko, 1977). An organism similar to Chloroflexus aurantiacus was present in the hot springs at Lake Bogoria and Lake Magadi. The Chloroflexus/algal mats in some of the springs may occasionally be covered by the highly alkaline lake water, without apparent affect on the association, suggesting that the organisms are tolerant of these conditions. It has recently been suggested that some members of the genera Oscillatoria, Spirulina, Phormidium, and Lyngbya, some species of which are present in the Rift Valley lakes, may belong to the Chloroflexaceae (Dubinina & Gorlenko, 1975; Gorlenko & Pivovarova, 1977; Jenkin, 1932; 1936; Rich, 1932; 1933), and the green phototrophic bacteria may be present in the lakes as these filamentous organisms.

A liquid enrichment at pH 9.5, from mud samples brought back from Lake Nakuru, in 1976 and 1977, produced a large spirillum, 2-3µm wide by 10-15µm long. Also present in the culture obtained in 1976 were a number of large rods, 2-4µm wide by 5-10µm, which contained centrally located gas vacuoles. The individual cells of the spirillum were coloured pink, and grew prolifically in the initial enrichments, but in sub-cultures smaller rods and spirilla outgrew them. All attempts to isolate the organisms failed, and their identity as phototrophic bacteria was not confirmed. The

similarity of the large spirillum to members of the genus Thiospirillum was suggested by morphology. Members of the genus Thiospirillum have been reported to occur in other soda lakes (Butlin & Postgate, 1954; Isachenko, 1934; 1951), but have not been isolated from these environments.

A small pleomorphic rod was observed in most of the lakes in enrichments made at pH 9.5, in 1978. The organisms formed small lens shaped colonies in soft agar, and a single isolate was obtained from Lake Bogoria in enrichments made in 1976. The organism was purified on agar plates, and was shown to contain carotenoids and bacteriochlorophyll a (figure 49). The isolate, which grew on plates in the presence of 0.03% (w/v) $Na_2S.9H_2O$, without the apparent deposition of sulphur, grew poorly and erratically in liquid culture despite the use of a number of different media. The organism, which resembled members of the genus Rhodopseudomonas morphologically, and was brown in colour, was not studied further, but was maintained in the culture collection (isolate B4). The organism is unusual in that members of the purple non-sulphur bacteria have not been isolated from alkaline, saline lakes, although they have been isolated from the sea (Imhoff & Trüper, 1976; Imhoff et al., 1978a; 1978b; Trüper, 1970; 1976).

3.4. Characterisation of isolates of phototrophic bacteria.

a. Morphology.

The majority of liquid enrichments, soft agar tubes, and mud columns contained small rods or spirilla as the major morphological groups of phototrophic bacteria. The organisms, which were purified and studied could be divided into five types on the basis of colony colour and cellular morphology, represented by the type strains B3, E7, M2, M4, and NCL5 (table 7). Cells of strain B3 were Gram-negative rods, 1.5µm wide by 2.0-4.0µm long. Colonies and liquid

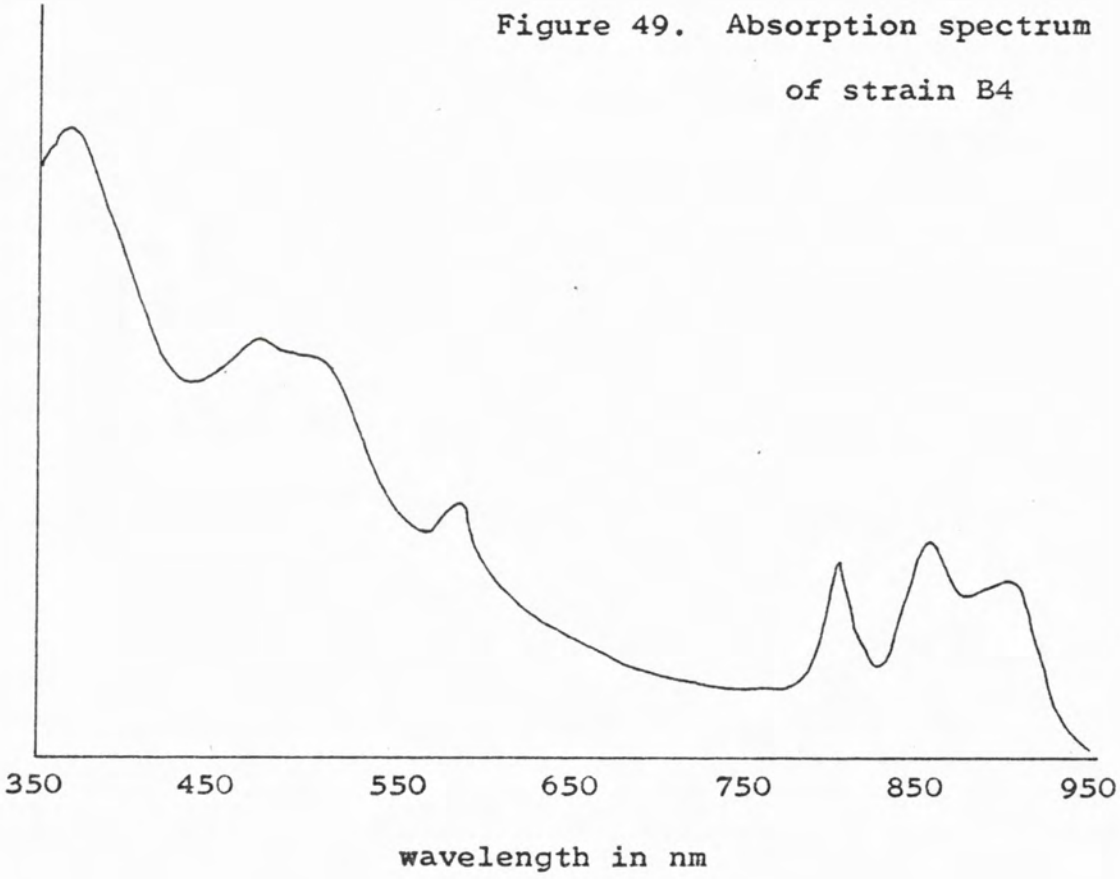


Figure 49. Absorption spectrum of strain B4

Table 7 List of strains of phototrophic bacteria isolated from the Kenyan soda lakes.

Strain	Isolated from Lake	Year of isolation	Colony colour	NaCl content (% w/v) of enrichment	Gas vacuoles present
B2	Lake Bogoria	1976	pink	1.0	+
B3*	Lake Bogoria	1976	pink	1.0	+
B6	Lake Bogoria	1978	pink	2.0	+
B7	Lake Bogoria	1978	pink	2.0	+
E2	Lake Elmentieta	1976	pink	1.0	+
E3	Lake Elmentieta	1976	pink	1.0	+
E5	Lake Elmentieta	1976	pink	1.0	+
E10	Lake Elmentieta	1978	pink	2.0	+
E11	Lake Elmentieta	1978	pink	2.0	+
E13	Lake Elmetieta	1978	pink	2.0	+
E14	Lake Elmentieta	1976	pink	1.0	+
N1	Lake Nakuru	1976	pink	1.0	+
N4	Lake Nakuru	1978	pink	2.0	+
N5	Lake Nakuru	1978	pink	2.0	+
N8	flooded ponds Nakuru	1978	pink	2.0	+
N10	flooded ponds Nakuru	1978	pink	2.0	+
N11	flooded ponds Nakuru	1978	pink	2.0	+
M1	Lake Magadi lagoons	1976	pink	1.0	+
NCL1	Naivasha Crater Lake	1976	pink	1.0	+
NCL2	Naivasha Crater Lake	1976	pink	1.0	+
NCL4	Naivasha Crater Lake	1976	pink	1.0	+
NCL6	Naivasha Crater Lake	1976	pink	1.0	+
E7*	Lake Elmentieta	1978	green-red	10.0	-
N3	Lake Nakuru	1976	green-red	10.0	-
M5	Lake Magadi lagoons	1976	green-red	10.0	-

Table 7. (cont.)

Strain	Isolated from Lake	Year of isolation	Colony colour	NaCl content (% w/v) of enrichment	Gas vacuoles present
M12	Lake Magadi trona	1978	red	10.0	-
M17	Lake Magadi trona	1978	red	10.0	-
M2*	Lake Magadi lagoons	1976	violet	10.0	+
M3	Lake Magadi lagoons	1976	violet	10.0	+
B5	Lake Bogoria	1976	violet	2.0	+
M4*	Lake Magadi trona	1978	red-violet	10.0	+
M6	Lake Magadi trona	1978	red-violet	10.0	+
M7	Lake Magadi trona	1978	red-violet	10.0	+
M8	Lake Magadi trona	1978	red-violet	10.0	+
M9	Lake Magadi trona	1978	red-violet	10.0	+
M10	Lake Magadi trona	1978	red-violet	10.0	+
M11	Lake Magadi trona	1978	red-violet	10.0	+
M13	Lake Magadi trona	1978	red-violet	10.0	+
M14	Lake Magadi trona	1978	red-violet	10.0	+
M15	Lake Magadi trona	1978	red-violet	10.0	+
M16	Lake Magadi trona	1978	pink	10.0	+
M18	Lake Magadi trona	1978	pink	10.0	+
M19	Lake Magadi trona	1978	red-violet	10.0	+
M20	Lake Magadi trona	1978	red-violet	10.0	+
M21	Lake Magadi trona	1978	red-violet	10.0	+
B1	Lake Bogoria	1976	red	1.0	-
B7	Lake Bogoria	1978	red	2.0	-
B8	Lake Bogoria	1978	red	2.0	-
B9	Lake Bogoria	1978	red	2.0	-
B10	Lake Bogoria	1978	red	2.0	-
E1	Lake Elmentieta	1976	red	1.0	-
E4	Lake Elmentieta	1976	red	1.0	-

Table 7 (cont.)

Strain	Isolated from Lake	Year of isolation	Colony colour	NaCl content (% w/v) of enrichment	Gas vacuoles present
B6	Lake Elmentieta	1978	red	2.0	-
B8	Lake Elmentieta	1978	red	2.0	-
B9	Lake Elmentieta	1978	red	2.0	-
N2	Lake Nakuru	1976	red	1.0	-
N6	Lake Nakuru	1978	red	2.0	-
N7	Lake Nakuru	1978	red	2.0	-
N9	flooded ponds Nakuru	1978	red	2.0	-
NCL3	Naivasha Crater Lake	1976	red	1.0	-
NCL5*	Naivasha Crater Lake	1976	red	1.0	-
B4*	Lake Bogoria	1976	brown	1.0	-

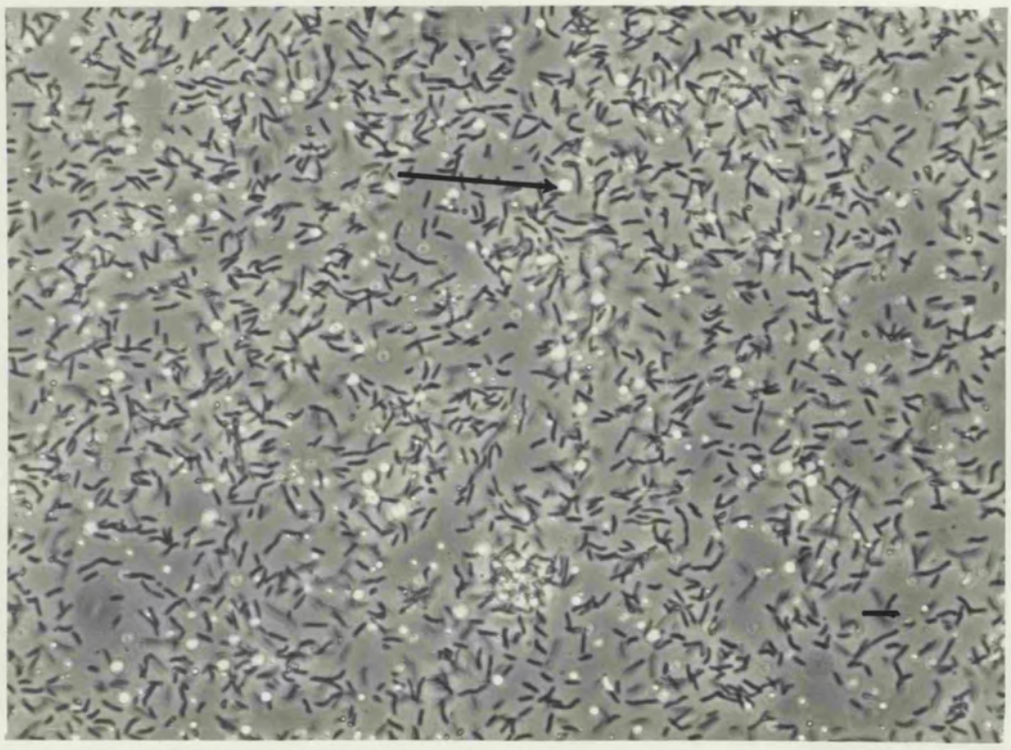
All strains were Gram-negative and motile. Cells of most strains could store polyhydroxybutyrate and all strains were catalase positive. With the exception of strain B4 sulphide was oxidised to sulphur, which was deposited outside the cell, and was further oxidised.

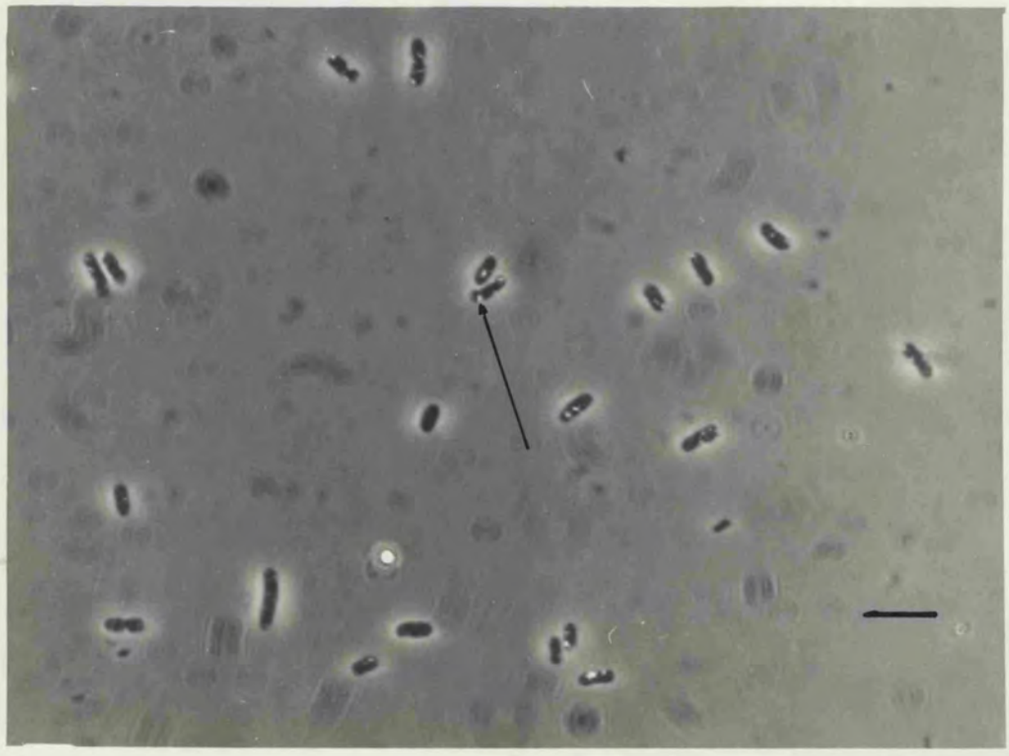
(strains marked * are the type strains of the six groups of phototrophic bacteria observed in the soda lakes, and subsequently isolated).

cultures of strain B3 and related organisms were red or pink in colour. Cells of strain M2 were Gram-negative cocco-bacilli 1.5µm wide by 1.5-2.5µm long, and cells of strain M4 were Gram-negative short rods, 1.5µm wide by 2.0-3.0µm long. Colonies of strains M2 and M4 were violet-red in colour, liquid cultures of strain M4 being similar in colour to that of Chromatium minus, which contains the carotenoid okenone (table 5). Cells of strain M2 and similar organisms often formed long chains, with individual cells 10-20µm in length under sub-optimal conditions, and the cells were usually bound together by a viscous extracellular slime. In strains B3, M2, M4, and similar organisms the cells contained refractile areas located at the periphery of the cell (figures 51, 52). These inclusions did not stain for any storage material, and did not resemble sulphur globules, which have a birefringent edge (Trüper, 1978). The refractile areas disappeared when the cells were subjected to an increase in pressure, indicating that they were gas vacuoles (Walsby, 1972; 1974; 1978). In cells of strain B3 and similar organisms the gas vacuoles were more commonly present in the cells at an early stage of growth of the culture, when the cells were also non-motile. Once the cultures had reached the stationary phase of growth, the cells had become motile, and did not contain gas vacuoles. Initially it was thought that the cultures were impure, and were a mixture of non-motile gas vacuolate rods, and motile non-gas vacuolate vibrios. However, observations of the cultures throughout the incubation period showed that the cultures were not mixed. In the early phase of growth of the cultures most cells were non-motile and gas vacuolate, and as the cells approached the stationary phase they became poorly motile. At this stage of growth the cells still contained gas vacuoles, and moved slowly, but as the cultures aged further, the gas vacuoles were lost and the cells became vigorously

Figure 50. Cells of strain E7, showing sulphur deposition (arrowed) x400, bar indicates 10μm.

Figure 51. Cells of strain B3, showing gas vacuoles (arrowed) x1000, bar indicates 10μm.





motile, morphologically resembling cells in which the gas vacuoles had been artificially collapsed. In strain B3 and similar organisms the loss of gas vacuoles, either during the course of growth, or by collapse, was accompanied by a change in colour of liquid cultures or colonies from pink to red (figure 53). There was also an accompanying change in the refractility of the cultures, which is also associated with the collapse of gas vacuoles (Walsby, 1972; 1974; 1978).

In strain M4 and similar organisms the transitions were less marked, whereas in strain M2 and similar organisms few, if any of the cells were motile once the gas vacuoles were lost, the cells forming gelatinous aggregates in the culture medium. There was no marked change in colour of cultures of strains M2 and M4 when the gas vacuoles were lost, although there was a change in the refractility of colonies, becoming translucent.

Strains E7, NCL5, and similar organisms were never observed to contain gas vacuoles (figure 50). Cells of strain NCL5 were usually short vibrios, 1.0µm wide by 2.0-3.0µm long, whereas cells of strain E7 were spirilla of 1-2 turns, 1.0µm wide by 4.0-5.0µm long. Both strains were Gram-negative. Liquid cultures of strain NCL5 and E7 were red, but when grown on plates, colonies of strain E7 and similar organisms were first green in colour, turning brown-red after 5-7 days growth. Cells of strain NCL5, E7, and similar organisms were motile, but in the presence of 0.1% (w/v) $Na_2S.9H_2O$ cells were initially non-motile. All the strains isolated from the soda lakes were capable of growing in the presence of 0.1% (w/v) $Na_2S.9H_2O$, when sulphur globules were deposited outside the cells (figures 50, 52), suggesting similarities to member of the genus Ectothiorhodospira, certain Rhodopseudomonas spp, or members of the Chlorobiineae. All the strains contained bacteriochlorophyll a as

Figure 52. Cells of strain B3, showing gas vacuoles and extracellular sulphur deposition (arrowed), x1000, bar indicates 10μm.

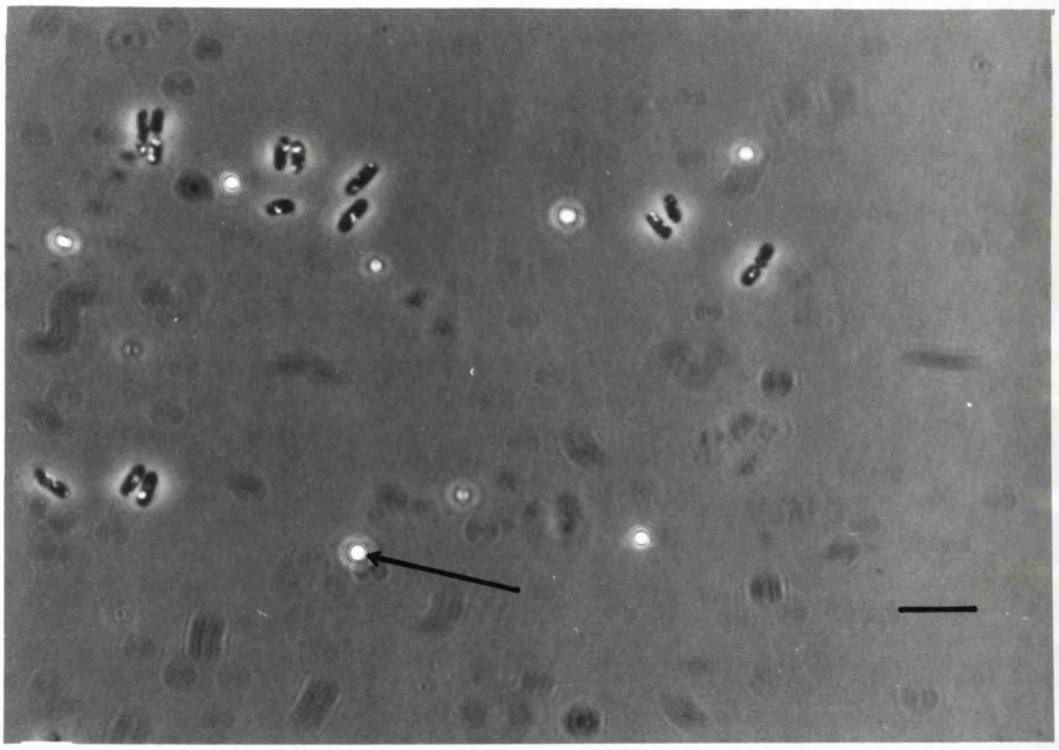
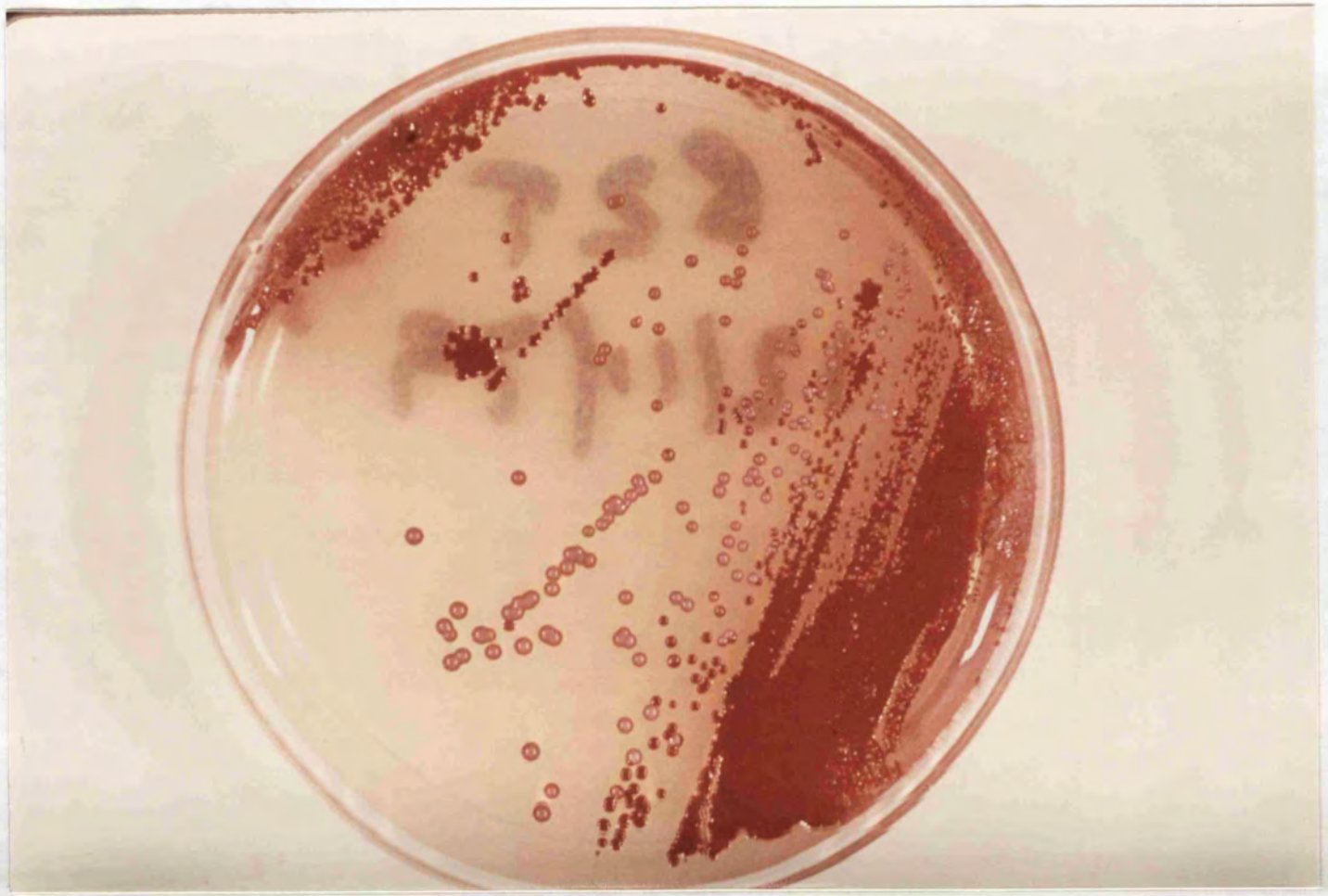


Figure 53. Colour change in gas vacuolate strain.

Colonies of strain B3, showing the colour change from pink to red, associated with the loss of gas vacuoles from the cells.


PSA
Pilis

the major chlorophyllous pigment, illustrated by the characteristic in vivo absorption maxima at 375, 580-590, 800-900nm (Biebl & Drews, 1969; Schlegel & Pfennig, 1961; Thornber et al., 1978), identifying them as members of the Rhodospirillineae (figures 63-68) (Pfennig & Trüper, 1974; Trüper & Pfennig, 1978).

A number of species in the genus Rhodopseudomonas, and all members of the genus Ectothiorhodospira have been shown to be capable of depositing sulphur globules outside the cell, however, none of these organisms have been observed to contain gas vacuoles (Cherni et al., 1969; Hansen & van Gemerden, 1973; Imhoff & Trüper, 1977; Imhoff et al., 1978a; 1978b; Keppen & Gorlenko, 1975; Kondrat'eva & Krasil'nikova, 1979; Raymond & Sistrom, 1967; 1969; Trüper, 1968). These results suggest that the organisms isolated from the Kenyan soda lakes may be divided into two distinct groups, on the basis of the possession of gas vacuoles. According to present taxonomic criteria used to distinguish members of the Rhodospirillales, these two groups may be representative of at least two different taxa (Pfennig & Trüper, 1974; Trüper & Pfennig, 1978).

Organisms isolated from the local Leicestershire stream, at the natural pH 7.3, included members of the genera Rhodospirillum, Rhodopseudomonas, Rhodomicrobium, Chromatium, Thiocystis, and Thiopedia, but no phototrophic green and purple bacteria were observed in enrichments prepared at pH 9.5 from Leicestershire streams. None of the organisms isolated at pH 7.3 were capable of depositing sulphur globules outside the cell, or shown to contain peripherally located gas vacuoles.

Observations of the cells of all strains isolated from the Kenyan soda lakes showed that the cells contained dark inclusions, which did not stain for storage material (figure 51). Similar inclusions have been reported in members of the genus

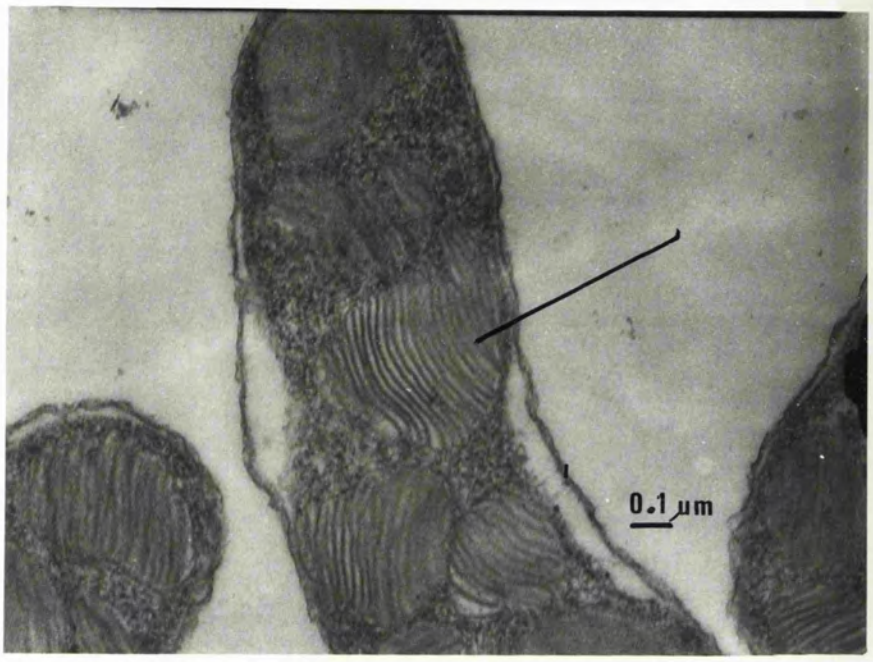
Ectothiorhodospira when viewed under the light microscope (Imhoff & Trüper, 1977; Remsen et al., 1968; Trüper, 1968), and could be seen in cells of E.shaposnikovii and E.mobilis. These areas may be attributed to the presence of a lamellar intracytoplasmic membrane system. The nature of the dark areas was further investigated by negative staining, with phosphotungstic acid, and by thin section, using an electron microscope. In negatively stained cells, the stain penetrates the space within the intracytoplasmic membrane, which is continuous with the outside of the cell. The intracytoplasmic membranes, therefore, take in the stain, and appear as an electron dense area within the cell, which is characteristic of members of the genus Ectothiorhodospira (Imhoff & Trüper, 1977; Holt et al., 1968; Oyewole & Holt, 1976; Remsen et al., 1968; Trüper, 1968). Electron dense areas were observed in all of the strains examined, including E.mobilis and E.shaposnikovii, showing that the membrane system within the cells were similar to that found in members of the genus Ectothiorhodospira. However, it is possible that any intracytoplasmic membrane system, which is continuous with the outside of the cell, would allow the stain to enter, and produce electron dense areas within the cell. To confirm the structure of the intracytoplasmic membranes, thin sections of cells were examined. All of the strains examined showed that the cells had a similar sub-cellular organisation. In the gas vacuolate strains B3, M2, and M4, and in the non-gas vacuolate strains E7 and NCL5, the intracytoplasmic membranes were located in groups of parallel double membranes (figures 54, 55, 56). A similar pattern was observed in E.mobilis and E.shaposnikovii, which was not significantly different from that observed previously in members of the genus Ectothiorhodospira (Holt et al., 1968; Cherni et al., 1969; Oyewole & Holt, 1976; Remsen et al., 1968; Trüper, 1968). Lamellar membrane

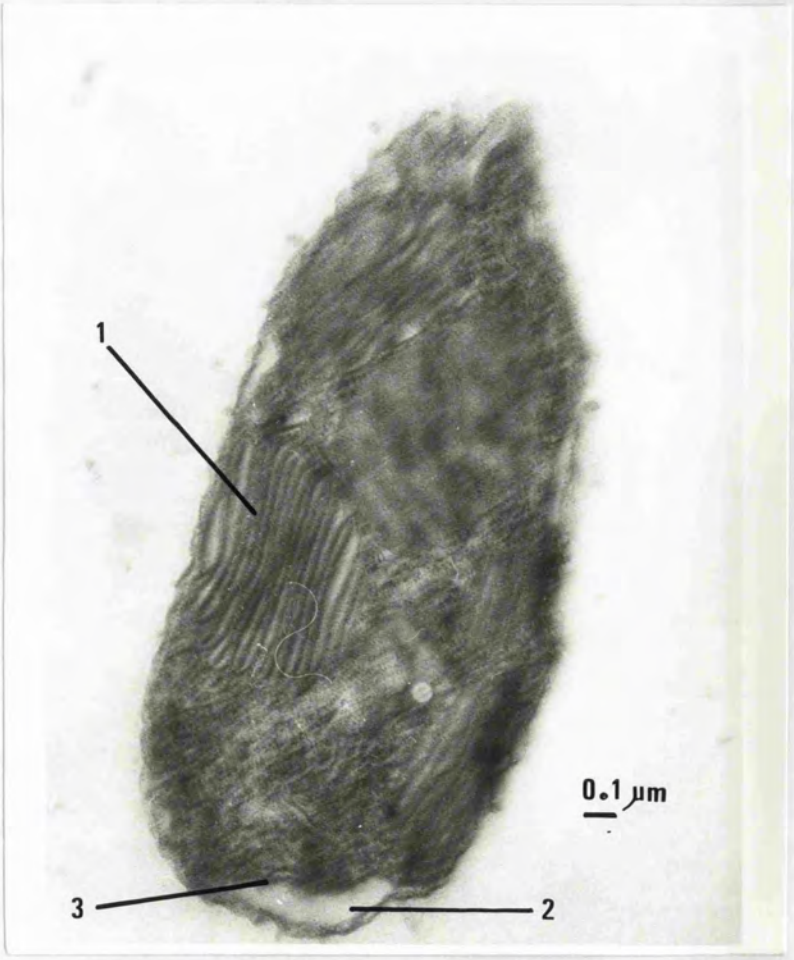
Figure 54. Thin section of non-gas vacuolate strain.

Electron micrograph of a thin section of strain NCL5, showing the lamellar type of intracytoplasmic membranes (arrowed).

Figure 55. Thin section of gas vacuolate strain.

Electron micrograph of a thin section of strain M2, showing the presence of intracytoplasmic membranes of the lamellar type (arrowed 1). The possible location of partially collapsed gas vacuoles is suggested by the electron transparent regions underlying the cell envelope (arrowed 2), and appear to be bounded by a membrane on the cytoplasmic side, as well as the cell envelope side (arrowed 3).


0.1 µm

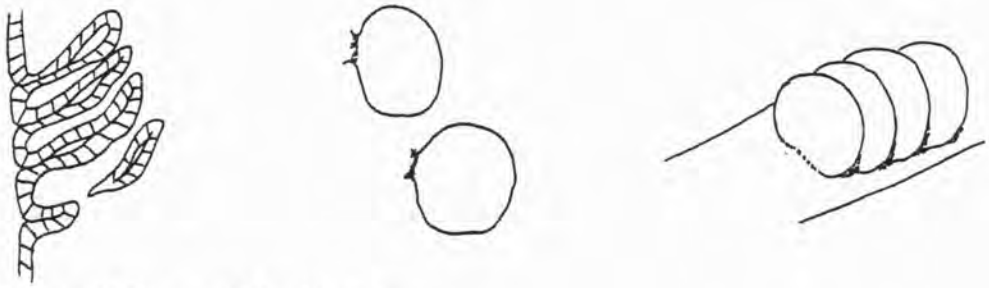

1
0.1 µm
3
2

stacks are also present in members of the genera Rhodospirillum (Gibbs et al., 1965; Hickman & Frenkel, 1965; Pfennig, 1967), Rhodopseudomonas (Giesbrecht & Drews, 1966; Oelze & Drews, 1972; Pfennig, 1967; Remsen, 1978), and Rhodomicrobium (Oelze & Drews, 1972; Remsen, 1978). However, the membranes may be divided into several distinctive morphological types, which are restricted to certain taxa. In the brown species of the genus Rhodospirillum the membrane stack is composed of several separate invaginations, which become appressed together, and lie at an acute angle to the cell membrane (figures 11.2, 12.2, 56). In the budding species of the genera Rhodopseudomonas and Rhodomicrobium the intracytoplasmic membrane is typically composed of single invaginations, which lie parallel to the cell membrane, and overlie one another (figures 11.3, 12.3) (Oelze & Drews, 1972; Remsen, 1978). In some species the membranes may branch. The membranes usually form an extensive sheet-like double membrane, which may encircle the interior of the cell, with the exception of the poles of the cell. In Rhps.viridis a lamellar stack has been described, which is similar to the grana found in chloroplasts (Giesbrecht & Drews, 1966). The membrane stack is made up of several slightly curved vesicles, which are appressed together, forming a group of flat membranes. It is not clear whether each constituent plate of the stack arises as a separate invagination, or if they are formed by pinching off from a single invagination (figure 56). However, in all these membranes the stack integrity is disrupted when the cell is lysed, and the flattened vesicles are evident as separate entities. The intracytoplasmic membrane seen in the strains examined, and in members of the genus Ectothiorhodospira, the stack is formed from a single invagination, which develops secondary folds (figures 13.1, 14.1, 56). In lysed cells the lamellar stacks retain their integrity

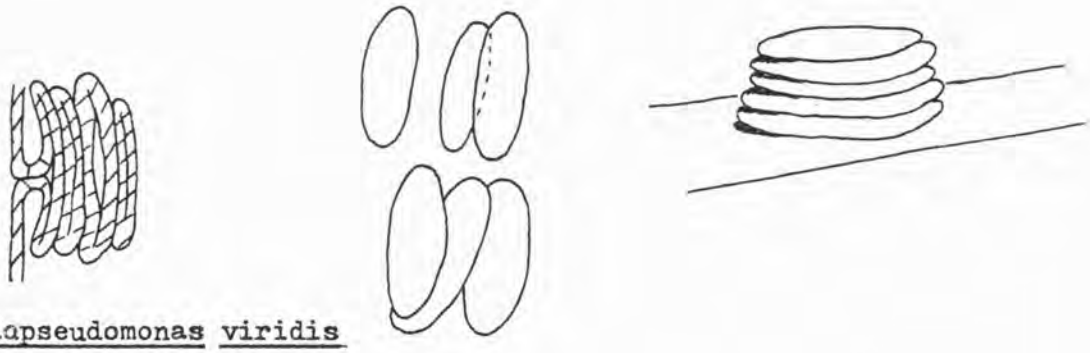
Figure 56. Interpretation of the structure of the intracytoplasmic membranes from the lamellar stack containing species of the genera Rhodospirillum, Rhodopseudomonas, and Ectothiorhodospira.

The form of the various membrane systems from the genera Rhodospirillum, Rhodopseudomonas, and Ectothiorhodospira are illustrated as they would appear in thin sections and negatively stained lysed cell preparations (after Geisbrecht & Drews, 1966; Hickman & Frenkel, 1966; Holt et al., 1968; Remsen, 1978; Remsen et al., 1968). The three dimensional interpretation of the structures are also visualised, and are consistent with the available data.

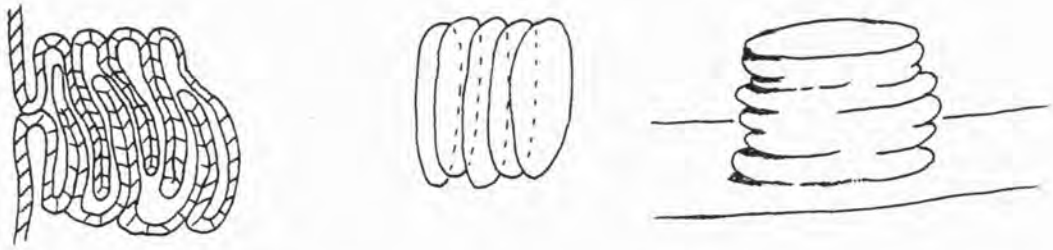
Thin section: Negatively stained lysed cells Three dimensional interpretation.



Rhodospirillum molischianum



Rhodopseudomonas viridis



Ectothiorhodospira sp.

as though the stack were bounded by a membrane (Holt et al., 1968; Oyewole & Holt, 1976; Remsen et al., 1968). In thin section the membranes are of a typical double membrane structure folded back on itself (figures 54, 55) (Holt et al., 1968; Cherni et al., 1969; Oyewole & Holt, 1976; Remsen et al., 1968; Trüper, 1968). The extracellular space contained within the lamellar stack is continuous further illustrating that the intracytoplasmic membranes of the members of the genus Ectothiorhodospira consists of a continuous membrane system. The presence of this type of lamellar intracytoplasmic membrane system is characteristic of members of the genus Ectothiorhodospira, and suggests that the strains NCL5, E7, and similar organisms are members of this genus, which they also morphologically resemble. The gas vacuolate strains B3, M2, M4, and similar organisms were also observed to contain an intracytoplasmic membrane system of the same type as that found in members of the genus Ectothiorhodospira, suggesting that by present taxonomic criteria they are similar to members of this genus, but may be taxonomically distinct from this genus.

The ability to deposit sulphur extracellularly, and further metabolise it to sulphate seems to be restricted to those members of the Rhodospirillineae which have and intracytoplasmic membrane system resembling that of the genus Ectothiorhodospira. This may be related to a restriction of space within the cell due to the presence of thses membranes, or to differences in the metabolism of this genus. The presence of the photosynthetically active intracytoplasmic membranes within cells seems to influence the position of the gas vacuoles in those strains which produce them. Observations of cells containing gas vacuoles showed that they were always peripherally located (figures 51, 52), at a position between two adjacent intracytoplasmic membrane stacks (figure 55). In all

species of the Rhodospirillales examined the gas vacuoles are centrally located (Cohen-Bazire et al., 1969; Pfennig & Trüper, 1974; Remsen, 1978; Trüper & Pfennig, 1978). In thin sections of the strains B3, M2, and M4 the regular honey-comb structure typical of gas vesicles in other prokaryotes was not evident (figure 55) (Cohen-Bazire et al., 1969; Remsen, 1978; Walsby, 1972; 1974, 1978). However, in most of the cells of strains B3, M2, and M4 examined, hollow spaces were observed lying immediately below the cell envelope. In all organisms examined by thin section, including E.mobilis and E.shaposnikovii, there was some degree of detachment of the cell envelope from the cytoplasm (figures 54, 55), forming an electron transparent region at the periphery of the cell. In the non-gas vacuolate strains the whole of the cell envelope appeared to be detached from the cytoplasm, whereas in the gas vacuolate strains there was a densely staining band bounding the cytoplasm, which was suggestive of a membrane (figure 55). The points of detachment in the gas vacuolate strains were always between two adjacent intracytoplasmic membrane stacks, the position in which the gas vacuoles were observed under the light microscope. In the non-gas vacuolate strains the points of detachment were random, and often occured directly above the lamellar stack (figure 54). These results suggest that the gas vacuoles in the strains B3, M2, and M4 may be different, and may arise as a single chamber underlying the cell envelope, and immediately adjacent to it. Freeze fracture of the cells should provide further information on the structure and nature of the gas vacuoles in these organisms, which otherwise resemble members of the genus Ectothiorhodospira in their fine structure.

Negatively stained cells showed that all of the strains examined were polarly flagellate when motile, the flagella occuring as a tuft, and never as single flagella (figures 57, 58). None of the

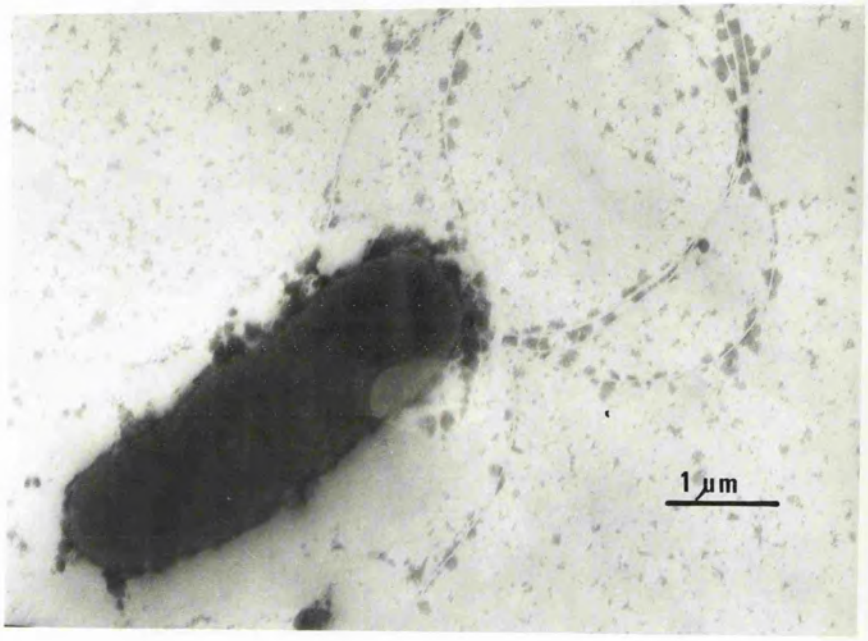
organisms examined were bipolarly flagellate, as are the extremely halophilic species E.halophila (Raymond & Sistrom, 1969), E.halochloris (Imhoff & Trüper, 1977), and E.abd-el-malekii (Imhoff & Trüper, personal communication). In negatively stained preparations from cultures grown in the presence of sulphide there were regular groups of extracellular material. The extracellular material was only present in cultures which contained sulphur globules. From the size and shape of the material, and the absence of any other particulate matter in the cultures, it was concluded that the extracellular material was sulphur globules. Close examinatiom of the surface of the globules revealed there was no regular pattern (figure 59), indicative of a bounding membrane (Remsen, 1978). All the extracellular material examined was composed of numerous small globules stuck together, forming an irregular shape (figure 59). The small globules had an irregular shape, which resembles that of ortho-rhombic sulphur, the stable enantiotropic form of sulphur at room temperature and pressure (Durrant, 1964). The shape of the globules is also slightly spherical, suggesting that the sulphur was not fully crystallised. This is consistent with the finding that sulphur deposited in cells of members of the Chromatiaceae is in the non-crystalline, plastic form (Trüper, 1978). When plastic sulphur dries it changes to the crystalline ortho-rhombic form (Trüper & Hathaway, 1970). It is possible that the small globules were, therefore, deposited in the plastic form, and the semi-crystalline appearance is due to the transition to a more stable allotrope of sulphur. Remsen (1978) has postulated that the sulphur globule within the cells of members of the Chromatiaceae is formed by accretion of small membrane bound globules. The membranes are thought to arise from the photosynthetically active intracytoplasmic membranes which degrade to form the largely proteinaceous membrane that has

Figure 57. Negative stain of non-gas vacuolate strain.

Negatively stained electron micrograph of strain E7, showing the location of the polar tuft of flagella.

Figure 58. Negative stain of gas vacuolate strain.

Negatively stained electron micrograph of strain B3, showing the location of the polar tuft of flagella.



1

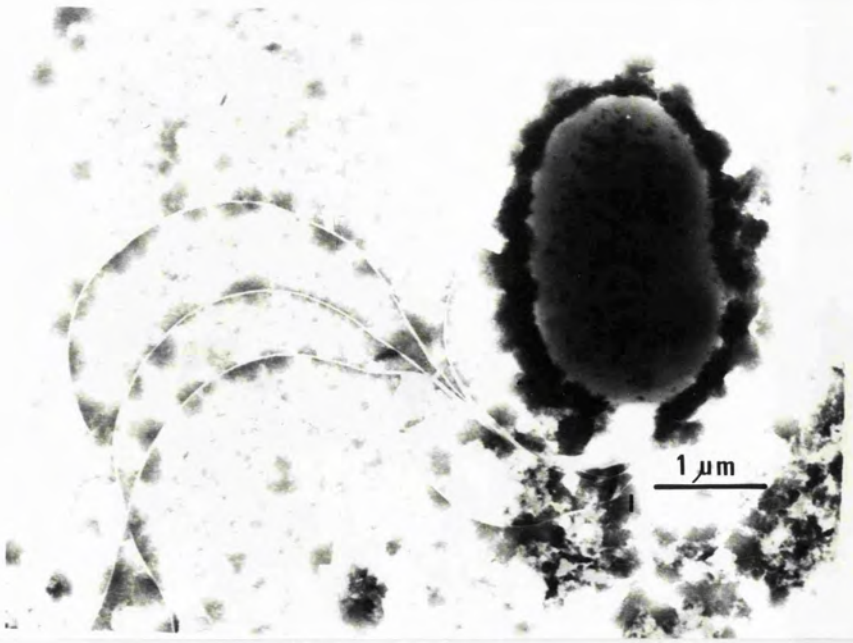
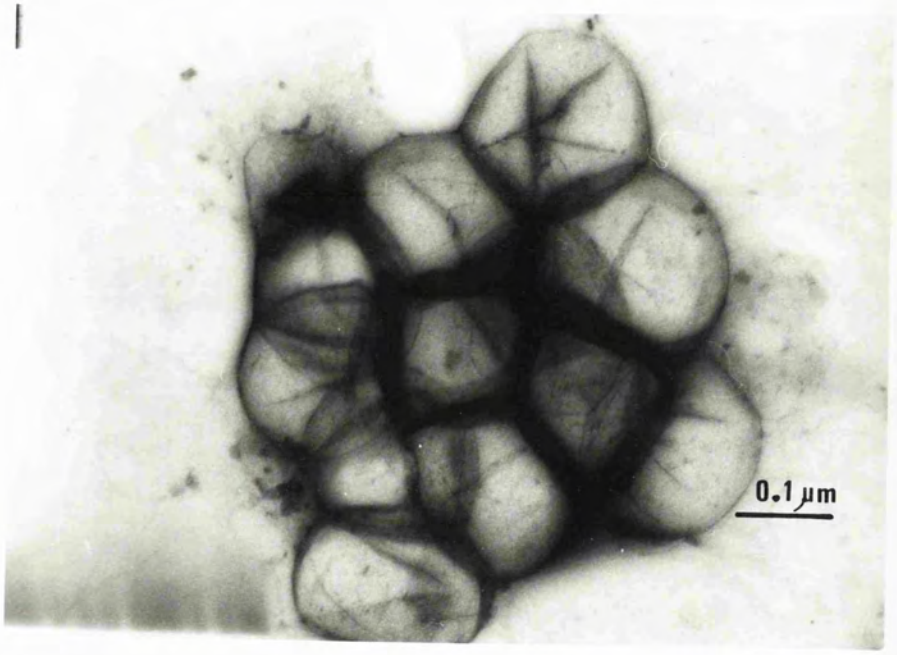


Figure 59. Negatively stained sulphur globule.

In negatively stained preparations from cultures containing sulphur globules, regular semi-crystaline, extracellular material was observed, which may have been sulphur globules. The structures did not appear to be membrane bound, and consisted of a number of smaller globules appressed together.


0.1 μm

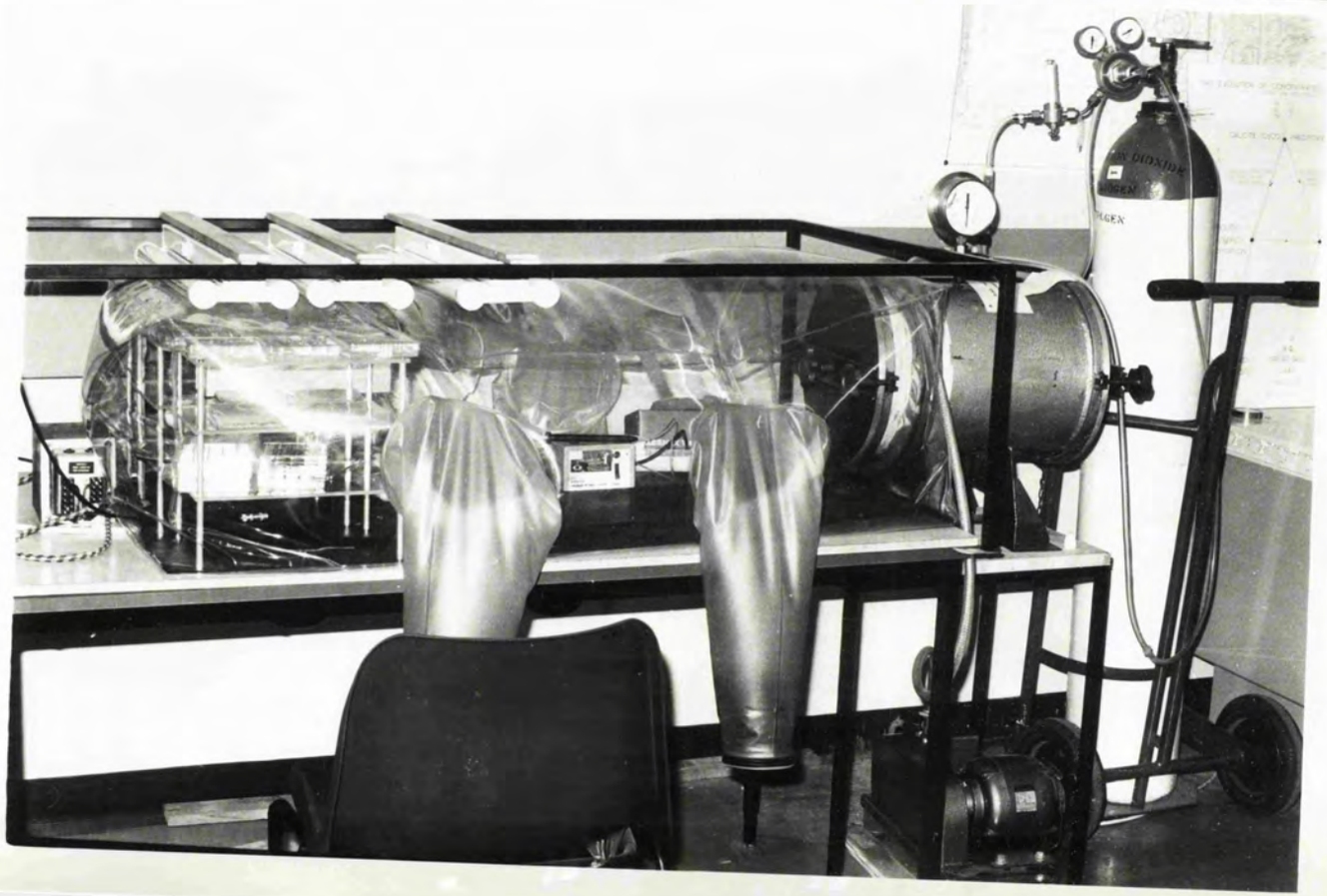
been found in members of the Chromatiaceae (Goyne et al., in Remsen, 1978; Nicholson & Schmidt, 1971; Schmidt et al., 1971). In cultures of Ectothiorhodospira sp. sulphur globules were occassionally observed attached to the cells, indicating a close association between the cell and the sulphur globule during deposition. It is possible that small sulphur globules are formed at the cell surface, and become detached from the cell when of sufficient size. The formation of the aggregates may occur either at the cell surface, or after detachment from the cell. Whether the sulphur globule is excreted as a membrane bound body, in which the membrane degrades completely, or as a membrane free body remains to be determined.

b. Physiology.

The optimal conditions for growth, and the ability to grow phototrophically in the presence of various carbon and sulphur compounds was determined in liquid culture in repli dishes, or in filled bottles. Repli dishes were incubated in an anaerobic environment, containing 5% CO_2, 10% H_2, 85% N_2 (v/v), kept oxygen free by the presence of palladium catalyst (Englehart D). Two types of chamber were used to maintain an anaerobic atmosphere, and were made to specification by Plysu Ltd, from 20thou thick clear polythene. The large chamber was similar to the chamber described by Aranki et al. (1969), entry being gained through a large metal airlock (kindly given by Dr.Jayne-Williams, N.I.R.D., Reading), which could be flushed with the anaerobic gas mixture, or evacuated with an oil pump (Edwards High Vacuum Ltd.). Sleeves and gloves in the side of the chamber allowed manipulation of cultures inside the chamber (figure 60). Clear perspex tables were provided inside the chamber to raise the cultures to a constant height below the fluorescent strip lights (Philips daylight), which were suspended above the chamber. The light intensity at the upper surface of the table was

Figure 60. Large anaerobic chamber (after Aranki et al., 1969).

The chamber was constructed from clear polythene, and filled with a 5% CO_2, 10% H_2, and 85% N_2 atmosphere, kept anaerobic by the presence of palladium catalyst. Entry to the chamber was achieved by evacuating the metal air lock several times, followed by flushing with the gas mixture.


DIOXIDE

750 lux. Small, clear perspex chambers were also used in growth experiments. The chambers were provided with two valves, to allow the evacuation, or flushing of the chamber, which had its free end sealed with a large clamp. Anaerobiosis was achieved by placing the cultures under test in the sealed chamber and evacuating, followed by flushing with a 5% CO_2, 10% H_2, and 85% N_2 (v/v) gas mixture. Palladium catalyst was placed in the chamber to remove oxygen. The chambers were placed below fluorescent strip lights (Philips daylight) at a constant distance to give a light intensity of 750 lux.

Optimal pH for growth was determined in filled bottles. The reference strains were grown in the medium of Pfennig (1965a) and the modified medium of van Niel (1931, 1944), at pH 7.5, 8.5, and 9.5. With the exception of E.mobilis and E.shaposnikovii (E.halophila and E.halochloris were not tested), none of the reference strains grew in either medium at pH 9.5. All the strains grew at pH 8.5 and 7.5, however, growth of E.mobilis and E.shaposnikovii, and the strains isolated from the Kenyan soda lakes was more prolific at pH 9.5 than at either pH 7.5 or 8.5, suggesting further similarities between the isolated strains and members of the genus Ectothiorhodospira. The salinity optima of E.mobilis, E.shaposnikovii, and the isolated strains were, therefore, determined at pH 9.5 in the presence of 0.1% (w/v) $Na_2S.9H_2O$ and 0.1% (w/v) sodium acetate. The isolates could be divided into two groups on the basis of their salinity requirement and optimum (table 8). Organisms similar to, and including strain B3 and NCL5 required little NaCl for growth, with optima between 0.1% and 2.0% (w/v) NaCl (table 8). Most of the strains were inhibited as the NaCl concentration was increased above 5.0-7.0% (w/v), although most were still capable of some growth at 10.0% (w/v) NaCl. Similar results were obtained for E.mobilis and E.shaposnikovii, the reported differences in NaCl optima were not

evident (Cherni et al., 1969; Pfennig & Trüper, 1974; Schmidt & Trüper, 1971). Strains E7, M2, M4, and similar organisms were characterised by their inability to grow in 0.1% (w/v) NaCl, and poor growth up to 3.0% (w/v) NaCl (table 8). The optimal NaCl concentration for growth of these strains was 5.0-6.0% (w/v) (table 8). At concentrations below 3.0% (w/v) NaCl, cells of strain M2 were extremely pleomorphic, and often formed long cells up to 20µm in length. Under optimal conditions the cells of strain M2 were small, regular cocco-bacilli. NaCl concentrations below 3.0% (w/v) have been shown to have a similar effect on cells of Chromatium buderi (Trüper & Jannasch, 1968). Most of the organisms similar to, and including strains E7, M2, and M4 grew well up to 9.0-10.0% (w/v) NaCl (table 8), but their NaCl optima were clearly lower than those reported for the extremely halophilic species, E.halophila, E.halochloris, and E.abd-el-malekii (Raymond & Sistrom, 1967; 1969; Imhoff & Trüper, 1977; personal communication). The results from the salinity optima (table 8) show that the majority of the organisms which had optima between 0.1 and 2.0% (w/v) NaCl were isolated from lakes where the NaCl concentration rarely exceeds 5.0% (w/v) (Talling & Talling, 1965; Ministry of Water Development, Kenya, unpublished), and most of those with optima between 5.0 and 6.0% (w/v) NaCl were isolated from the trona crust of Lake Magadi, where the NaCl concentration is high enough to support the growth of extremely halophilic bacteria resembling members of the Halobacteriaceae (Tindall et al., 1980). Although halophilic phototrophic organisms resembling E.halophila were observed, and have been isolated from enrichments for members of the Halobacteriaceae (Grant, Schofield, and Tindall, unpublished), they were not isolated from enrichments for phototrophic bacteria.

The pH optima of E.mobilis, E.shaposnikovii, and the isolated

Table 8 NaCl optimum for phototrophic bacteria

Strain no.	B2	B3*	B6	B7	E2	E3	E5	E10	E11	E13	E14	N1	N4	N5	N8	N10	N11	M1	NCL1	NCL2	NCL4	NCL6	E7*	N3	M5	M12	M17	M2*	M3	B5	DSM 239	DSM 243
NaCl (% w/v)																																
0.0	2	2	4	4	3	2	2	2	1	4	2	3	1	1	3	3	4	1	1	2	1	1	0	0	0	0	0	0	0	0	2	1
0.5	4	3	4	4	3	2	3	4	4	4	3	4	3	4	4	4	4	3	3	3	3	3	1	1	1	1	1	1	1	0	4	3
1.0	4	4	3	4	3	2	4	4	4	3	4	4	3	4	3	4	4	4	4	4	3	4	2	1	1	1	1	1	2	1	4	3
2.0	3	3	3	4	3	2	4	3	4	3	4	4	4	4	3	4	4	4	4	4	3	4	4	1	1	3	1	1	4	2	4	4
3.0	3	3	3	4	2	2	4	3	4	2	4	4	4	4	3	4	4	4	4	4	2	3	4	1	2	3	4	4	4	2	4	4
4.0	3	3	3	4	2	2	4	3	4	2	3	3	4	3	2	3	4	3	3	3	2	2	4	4	3	3	2	4	4	3	4	4
5.0	2	2	2	4	2	2	3	2	2	2	2	3	3	2	1	3	4	2	3	3	1	2	4	4	3	3	4	4	4	3	4	4
6.0	1	1	2	3	2	2	3	2	2	2	1	2	2	1	1	2	4	1	2	3	0	1	4	3	2	3	4	4	4	3	3	3
7.0	0	0	2	3	1	1	1	1	2	2	0	1	1	1	1	2	3	1	2	2	0	1	4	2	2	2	4	4	4	3	2	2
8.0	0	0	2	2	0	0	1	1	2	1	0	1	1	1	1	1	2	0	0	1	0	0	4	2	2	2	4	4	4	3	2	1
9.0	0	0	1	2	0	0	1	1	1	1	0	1	1	1	1	1	1	0	0	0	0	0	4	2	2	2	4	4	4	3	1	1
10.0	0	0	1	2	0	0	1	1	1	1	0	1	1	1	1	1	1	0	0	0	0	0	4	2	2	2	1	1	4	2	0	1

Strain no.	B1	B7	B8	B9	B10	E1	E4	E6	E8	E9	N2	N6	N7	N9	NCL3	NCL5*	M4*	M6	M7	M8	M9	M10	M11	M13	M14	M15	M16	M18	M19	M20	M21	G_1
NaCl (% w/v)																																
0.0	4	4	4	4	4	1	3	2	0	0	1	1	2	4	2	2	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1
0.5	4	4	4	4	4	2	4	2	2	4	3	4	4	4	4	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
1.0	4	4	4	4	4	2	4	2	2	3	4	4	4	3	4	4	1	2	1	1	2	2	1	1	1	1	1	1	1	1	1	3
2.0	4	4	4	4	4	2	3	2	3	3	3	4	4	2	4	4	4	4	4	3	4	4	4	3	4	4	3	1	1	1	4	3
3.0	3	4	4	4	4	2	3	2	3	1	3	4	4	2	3	3	4	4	4	3	4	4	4	3	4	4	4	4	3	4	4	2
4.0	3	4	4	4	4	2	1	2	3	1	2	4	3	2	2	3	4	4	4	3	4	4	4	3	4	4	4	4	3	3	4	2
5.0	2	4	4	4	4	2	1	2	2	1	2	4	3	2	1	3	4	4	4	3	4	4	4	3	4	4	4	4	1	2	4	1
6.0	1	3	4	4	3	2	1	2	1	1	2	3	3	2	1	2	4	4	4	3	4	4	4	3	4	4	4	4	1	1	4	1
7.0	0	3	4	3	3	1	0	2	1	0	1	2	3	1	0	1	4	4	2	3	4	4	4	3	4	4	4	4	1	1	4	1
8.0	0	2	2	3	3	1	0	1	1	0	0	2	3	1	0	0	4	2	2	3	4	4	4	3	4	4	4	4	1	1	4	1
9.0	0	2	2	3	2	1	0	1	1	0	0	1	3	1	0	0	4	2	1	2	4	4	4	2	3	4	4	4	1	1	4	1
10.0	0	2	2	3	1	1	0	1	1	0	0	1	1	1	0	0	4	1	1	2	4	4	4	2	3	4	2	1	1	1	2	1

type strains are denoted by *
growth was judged by comparison with the control, which had an optical density of 0.0 .
0- growth as control
1- optical density 0.2-0.5
2- optical density 0.6-0.9
3- optical density 1.0-1.3
4- optical density ≥1.4

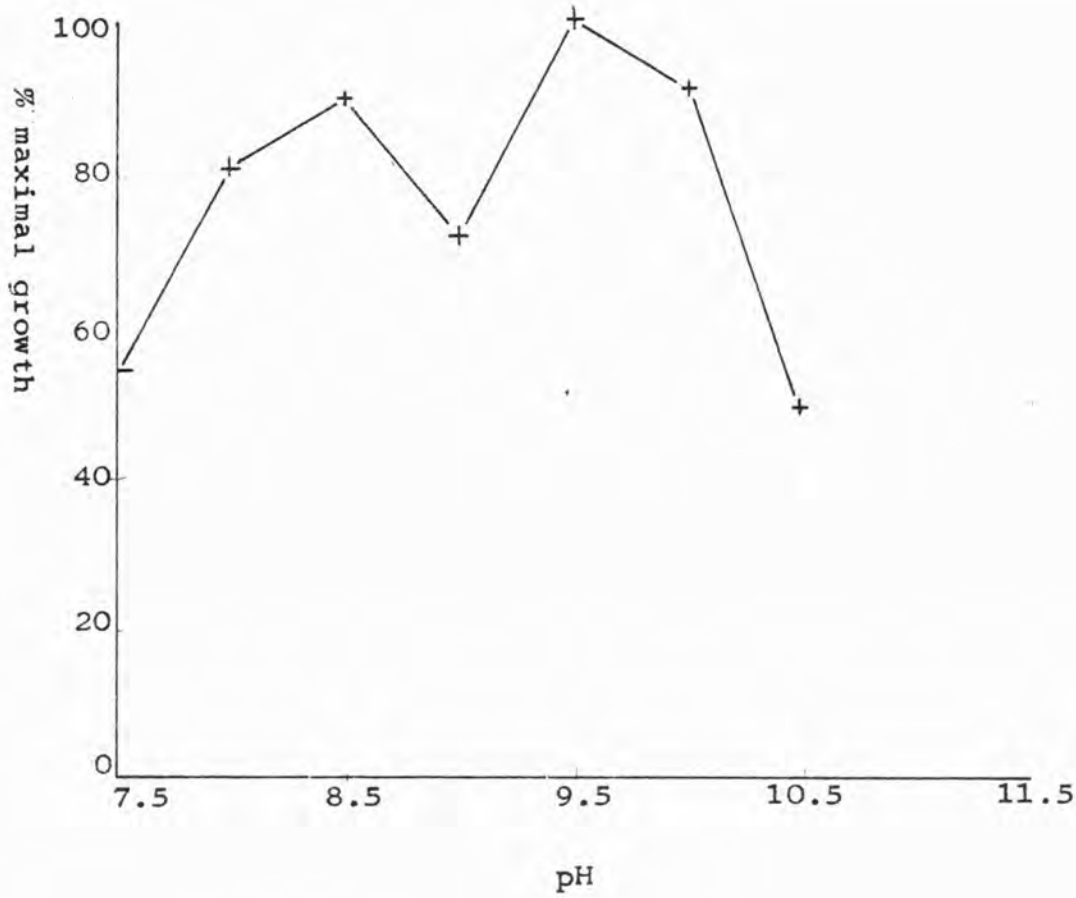
organisms were determined at the optimal salinity for growth, 1.5% (w/v) NaCl for strains B3, NCL5, and similar organisms, or 5.0% (w/v) NaCl for strains E7, M2, M4, and similar organisms. When grown in the presence of 0.1% (w/v) $Na_2S.9H_2O$ and 0.1% (w/v) sodium acetate all organisms showed two optima, at pH 8.5 and 9.5 (figure 61). The greatest optimum was at pH 9.5. Cultures grown in the presence of 0.1% (w/v) sodium acetate alone showed a single optimum at pH 9.5, while those grown in the presence of 0.1% (w/v) $Na_2S.9H_2O$ alone had a single optimum at pH 8.3 (figure 62). Gee et al. (1980) found that an alkaliphilic heterotrophic organism also showed two pH optima, but did not resolve them as being due to different constituents of the culture medium. Chesnokov and Shaposnikov (1936a) showed that for growth of E.mobile the pH optimum depended upon the redox potential of the compound used as an electron donor. The relationship between pH and redox potential, and its influence on the growth of bacteria is recorded for many species (Hewitt, 1957), although this effect has largely been ignored in studies on the pH optima of members of the Rhodospirillales. Van Niel (1931) found that the pH optimum for growth in the presence of sulphide was dependant upon its concentration. At the highest concentrations of sulphide tolerated by members of the purple sulphur bacteria the pH optimum was 8.5, but when the sulphide concentration was lowered, growth of a 'Pseudomonas' type, which deposited sulphur outside the cell, and was probably a member of the genus Ectothiorhodospira, occured over the range pH 8.5 to 10.5. Organisms depositing sulphur inside the cell, however, grew over the range pH 6.5 to 8.5, when the sulphide concentration was lowered. Chesnokov and Shaposnikov (1936a) also found that growth of E.mobile occured over the range pH 7.5 to 9.5, with an optimum in the range pH 8.5-9.0. The more alkaline pH optimum of members of the genus Ectothiorhodospira has been noted

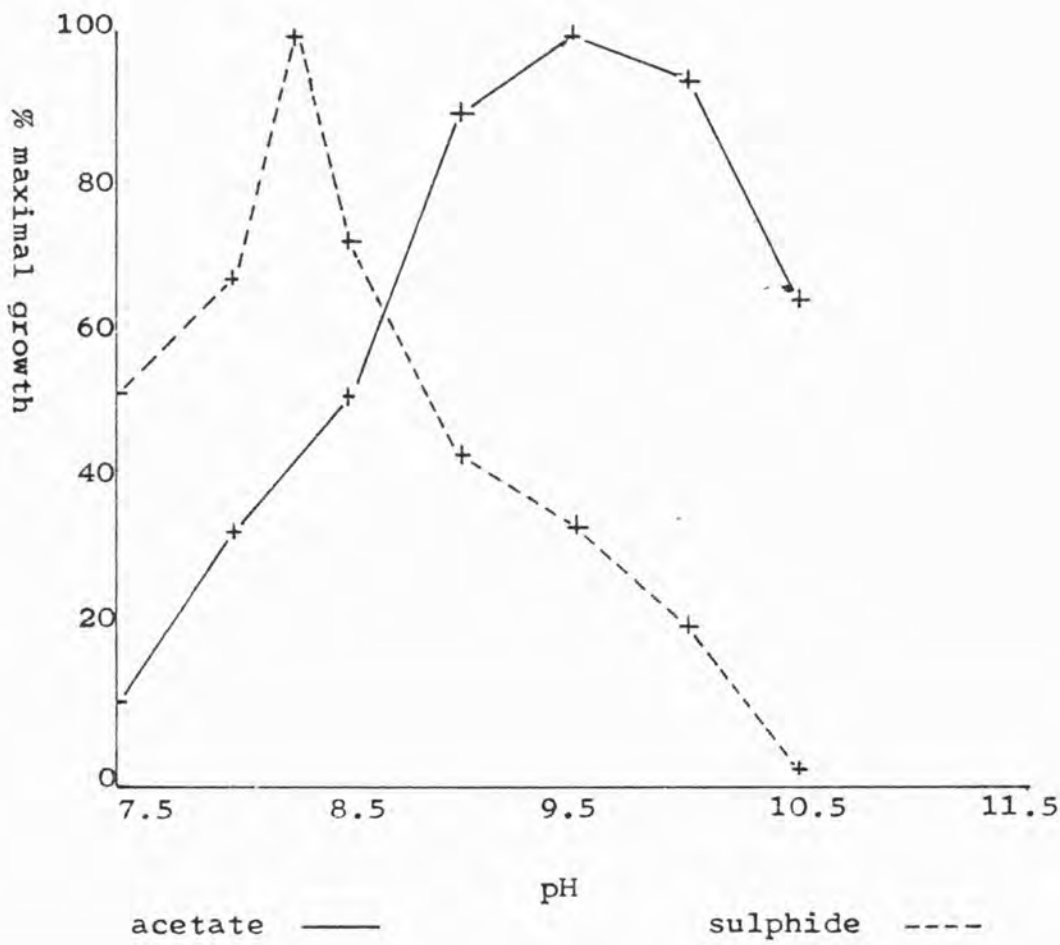
Figure 61. Optimal pH of the organisms isolated from the Kenya soda lakes.

The optimal pH of strain B3 was typical of all strains isolated from the Kenyan soda lakes, showing a bimodal distribution in the presence of sulphide and acetate. The presence of two pH optima was also found for E.mobilis and E.shaposnikovii grown under the same conditions.

Figure 62. Optimal pH of the organisms isolated from the Kenyan soda lakes.

The optimal pH of strain B3 in the presence of sulphide alone, or acetate alone showed single distinct optima. The results obtained for strain B3 were essentially similar to those obtained for all other strains isolated from the Kenyan soda lakes, and for E.mobilis and E.shaposnikovii.


100
80
60
40
20
0
% maximal growth
7.5
8.5
9.5
10.5
11.5
pH


100
80
60
40
20
0
% maximal growth
7.5
8.5
9.5
10.5
11.5
pH
acetate ——
sulphide ----

as a taxonomic feature (Trüper, 1978), and the pH optimum of E.halophila may be higher than that previously reported (Raymond & Sistrom, 1967; 1969; Biebl, unpublished). When organisms were grown in unbuffered medium containing 0.1% (w/v) $Na_2S.9H_2O$ the final pH of the medium was 8.5, whether the medium was initially inoculated with Ectothiorhodospira type organisms at pH 9.0-10.0, or with Chromatium type organisms at pH 7.0-8.0. Changes in the pH of the medium during growth of phototrophic bacteria in unbuffered medium was also recorded by van Niel (1931) and by Chesnokov and Shaposnikov (1936a). These results suggest that the growth of these organisms may, under certain conditions enhance a change in the pH towards the optimum pH for growth. The organisms isolated from the Kenyan soda lakes appear to have pH optima characteristic of the genus Ectothiorhodospira, but, since some of the organisms contain gas vacuoles, they may be regarded as a taxon similar to the genus Ectothiorhodospira.

Most members of the Chromatiaceae, and one species of the Rhodospirillaceae require vitamin B_{12} for growth (Pfennig, 1978b; Pfennig & Lippert, 1966; Thiele, 1968). All organisms isolated from the Kenyan soda lakes grew in medium containing 0.1% (w/v) yeast extract. Growth was tested in media without yeast extract or vitamin B_{12}, with vitamin B_{12}, or with yeast extract (table 9). Little growth was detected in media without yeast extract or vitamin B_{12}, either of which, alone, stimulated some growth (table 9). Growth in the medium containing yeast extract was, however, more prolific than in medium containing vitamin B_{12}. The more prolific growth in the presence of yeast extract may be due to its utilisation as a carbon source. In studies on an "autotrophic Rhodopseudomonas sp." (E.shaposnikovii), which did not require vitamin B_{12} for growth, yeast extract was shown to act as a carbon

source (Kondrat'eva, 1956; Upsenkaya & Kondrat'eva, 1962). Growth of E.mobilis is dependant on the presence of vitamin B_{12} (Trüper, 1968). However, both E.mobilis and E.shaposnikovii showed little growth in the absence of yeast extract and vitamin B_{12} at pH 9.5, and grew most prolifically in the presence of yeast extract (table 9), which was, therefore, routinely added to the medium.

The addition of yeast extract may also be important in providing a nitrogen source. Yang Hui-Fang (1962) found that yeast extract served as well as NH_4^+, urea, or peptone for the growth of "autotrophic Rhodopseudomonas sp." (Ectothiorhodospira sp.). During the course of growth experiments it was noticed that when medium was prepared at pH 9.5 there was a strong smell of ammonia, due to the effect of alkalinity on the equilibrium between NH_4^+ and NH_3. When 0.1% (w/v) NH_4Cl was added to the medium at pH 9.5 the final concentration of NH_4^+ in the medium, as determined by the method of Havilah et al. (1977), was found to be approximately 1.0mM, in contrast to the expected value of 55mM. It appeared that NH_4^+ might not, therefore, be capable of supporting the prolific growth observed in the cultures, and the role of NH_4^+, NO_3^-, and yeast extract in the nitrogen metabolism of the organisms was investigated. Growth of all strains, including E.mobilis and E.shaposnikovii, in media devoid of NH_4Cl and containing vitamin B_{12} was greatly stimulated by the presence of yeast extract (table 9), while NH_4Cl and KNO_3 did not support growth significantly greater than that of the controls, which were devoid of nitrogen sources other than gaseous nitrogen. Cultures containing NH_4Cl did show slightly better growth than cultures containing KNO_3, and presumably, the main factor in determining the utilisation of NH_4^+ appears to be the limited solubility of this radical at the pH of the growth medium. Yang Hui-Fang (1962) established that one of the

Table 9 Utilisation of vitamin B_{12} and nitrogenous compounds by phototrophic bacteria.

Strain no.	B2	B3*	B6	B7	E2	E3	E10	E11	E13	E14	N1	N4	N5	N8	N10	N11	M1	NCL1	NCL2	NCL4	NCL6
Vitamin B_{12} only	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vitamin B_{12} + 0.1% (w/v) NO_3^-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vitamin B_{12} + 0.1% (w/v) NH_4^+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Yeast extract, 0.1% (w/v)	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++

Strain no	B1	B7	B8	B9	B10	E1	E4	E6	E8	E9	N2	N6	N7	N9	NCL3	NCL5*	M2*	M3	B5	DSM 239	DSM 243
Vitamin B_{12} only	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vitamin B_{12} + 0.1% (w/v) NO_3^-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vitamin B_{12} + 0.1% (w/v) NH_4^+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Yeast extract, 0.1% (w/v)	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++

Strain no.	M4	M6	M7	M8	M9	M10	M11	M13	M14	M15	M16	M18	M19	M20	M21	E7*	N3	M5	M12	M17	G_1
Vitamin B_{12} only	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vitamin B_{12} + 0.1% (w/v) NO_3^-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vitamin B_{12} + 0.1% (w/v) NH_4^+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Yeast extract, 0.1% (w v)	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++

Ectothiorhodospira strains isolated from the marine environment was able to utilise a wide range of amino acids as nitrogen sources, while another strain showed a more restricted range of amino acid utilisation. In an environment where NH_4^+ is rapidly brought into equilibrium with the more labile NH_3, the ability to obtain nitrogen either from nitrogenous compounds, such as yeast extract and amino acids, or by direct fixation from the atmosphere would be advantageous. The stimulatory effect of yeast extract on the growth of these organisms appears to be due in part to its function as both nitrogen and carbon source.

Although members of the Rhodospirillales only grow phototrophically under anaerobic conditions, members of all the families have been shown to be able to utilise oxygen under certain conditions (Kiester, 1978; Pfennig, 1978b; Pierson & Castenholz, 1974a; 1978). Only some species have been shown to be capable of growing aerobically or microaerophilically in the dark (Gorlenko, 1974; Gusev et al., 1969; Kiester, 1978; Kondrat'eva, 1976; Kondrat'eva & Krasil'nikova, 1979; Kondrat'eva et al., 1976a; 1976b; Pfennig, 1978b; Pierson & Castenholz, 1974a; 1974b; Upsenkaya & Kondrat'eva, 1972). The ability of the isolated strains, E.mobilis and E.shaposnikovii to grow under a range of oxygen tensions was determined in soft agar tubes, placed either in the dark, or in the light. The position of growth in the tube indicated the organism's tolerance to oxygen. In the light, all strains, including E.mobilis and E.shaposnikovii, grew to within 5mm of the top of the agar, indicating that the organisms were tolerant of some oxygen when grown phototrophically (table 10). Results obtained from growth in the dark were less distinct, many of the strains failing to grow. In some of the non-gas vacuolate strains, including E.mobilis and E.shaposnikovii, growth occured as a sharp band 7-8mm below the top

Table 10 Growth of phototrophic bacteria in the dark.

growth 7-8mm from the top of the agar deep.

strains:	DSM 239	DSM 243	B8
	B9	B10	N6

growth throughout the tube to within 7-8mm from the top of the agar.

strains:	NCL1	NCL4	E2
	E3	E5	N1
	N5	N4	N10
	N11	M1	M6
	M7	M8	M9
	M14	M18	B6
	B7	E11	E12
	E10	E1	E6
	NCL6	B1	E9
	N7	N8	N9

no growth observed in the tubes.

strains:	B2	B3	B6
	B4	B5	E4
	E7	E8	N1
	N2	N3	NCL2
	NCL3	NCL5	M2
	M3	M4	M5
	M10	M11	M12
	M13	M15	M16
	M17	M19	M20
	M21		

of the tube (table 10). Other strains, which grew in the dark, including gas vacuolate forms, grew to within 7-8mm of the surface of the agar, and also grew throughout the rest of the tube. These results suggest that some of the strains are incapable of non-phototrophic growth, some grow non-phototrophically under microaerophilic conditions in the dark over a limited range of oxygen concentrations, and other strains are only inhibited by microaerophilic to aerobic conditions. None of the strains were able to grow under aerobic conditions in the dark or in the light. A response to oxygen concentration could also be observed in liquid cultures grown in the light. In strain B3 and similar organisms, grown under low light intensity, 200-300 lux, the cells possessed gas vacuoles, and accumulated at the top of the culture. When the culutres were opened, and left exposed to oxygen at low light intensity the organisms became motile, but did not lose their gas vacuoles. When sterile medium was aerated, and carefully layered above actively growing cultures, the organisms were observed to move away from the freshly added medium, eventually colonising the freshly added medium after 1-2 days additional growth.

The utilisation of carbon compounds was tested by the method of Thiele (1968) in the presence of carbon sources at either 0.1 or 0.05% (w/v) and 0.1% (w/v) $Na_2S.9H_2O$. Cultures were incubated in an anaerobic atmosphere containing 5.0% (v/v) CO_2, which caused the pH to fall during the course of growth of the organisms from pH 9.5 to 8.5. However, at the lower pH, growth was 90% of maximal growth at pH 9.5. Growth was recorded according to the criteria of Trüper (1968) and Imhoff and Trüper (1977; 1980). Due to the pH and inclusion of yeast extract, these results were not directly comparable with other studies (Kondrat'eva, 1956; Kondrat'eva & Krasnil'nikova, 1979; Trüper, 1968; Yang Hui-Fang, 1962), and

E.mobilis and E.shaposnikovii were, therefore, included in the growth experiments. Like small members of the genus Chromatium, all the organisms were able to utilise intermediates of the Krebs' cycle (table 11) (Imhoff & Trüper, 1980; Pfennig & Trüper, 1974; Thiele, 1968). Although growth in some carbon sources was apparently enhanced in the presence of yeast extract, E.mobilis and E.shaposnikovii showed no other significant differences between the results obtained in this study and those obtained previously (Kondrat'eva, 1956; Trüper, 1968; Yang Hui-Fang, 1962). Some degree of variation between different strains of the same species is to be expected (Thiele, 1968), and has been reported for strains of Ectothiorhodospira isolated from the same environment (Matheron & Bauliage, 1972; Trüper, 1968; Yang Hui-Fang, 1962). Most of the strains were capable of utilising lactate, glycerol, and fructose (table 11). Formate did not support growth of any of the organisms, and ethanol, butyrate, and benzoate supported the growth of a few strains only (table 11). Some differences were noted between the various designated groups. Strain E7 and similar organisms did not utilise glucose, fructose, or glycerol; glucose and glycerol were not utilised by strain M2 and similar organisms; strain M4 and similar organisms could be divided into two groups on their ability or inability to utilise the carbon sources glucose, fructose, and glycerol (table 11). Within the groups represented by the strains B3 and NCL5 there was a much greater degree of variation in the ability to utilise glucose, fructose, and glycerol, and no characteristic pattern of utilisation was evident. Casamino acids and citrate were not utilised by the majority of the strains, as reported for other strains of Ectothiorhodospira (Grant et al., 1979; Matheron & Bauliage, 1972; Kondrat'eva, 1956; Trüper, 1968; Yang Hui-Fang, 1962). Kondrat'eva (1956) found that the growth of

Table 11. Utilisation of carbon compounds by phototrophic bacteria.

Strain no.		B2	B3*	B6	B7	E2	E3	E5	E10	E11	E13	E1	N1	N4	N5	N8	N10	M1	NCL1	NCL2	NCL4	NCL6	E7*	N3	M5	M12	M17	M2*	M3	B5	M4*	M6	M7	M8	M9	M10	M11	M13	M14	M15	M16	M18	M19	M20	M2
Carbon source (% w/v).																																													
acetate	0.1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
pyruvate	0.1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
propionate	0.05	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
succinate	0.1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
malate	0.1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
fumarate	0.1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
glucose	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	1	1	0	1	1	1
fructose	0.1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	1	0	0	0	0	0	1	1	0	1	1	1
glycerol	0.1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	0	1	1	1	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	1	1	1	1	1	1
lactate	0.05	0	0	1	0	1	1	1	0	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1
oxalate	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0	0	0	1	0	1	1	1	0	1	1	1	0	1	1	0	1	0	1	1	1	1	1	1
casamino acids	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	1	1	0	0	0	0
citrate	0.1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
formate	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ethanol	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
benzoate	0.05	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
butyrate	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

E.shaposnikovii in the presence of some carbon compounds was dependant on the addition of bicarbonate or a reduced sulphur compound. The concentration of the reduced sulphur compound in the medium has also been shown to influence the final biomass in cultures grown photoorganotrophically (Kondrat'eva & Krasil'nikova, 1979). The results show that there are many minor differences between the strains, but the general pattern observed for carbon compound utilisation was not significantly different from results obtained previously (Grant et al., 1979; Kondrat'eva, 1956; Matheron & Bauliage, 1972; Trüper, 1968; Yang Hui-Fang, 1962). Most members of the genus Ectothiorhodospira do not utilise ethanol, formate, benzoate, citrate, or casamino acids, as is also typical of other small members of the Chromatiaceae (Imhoff & Trüper, 1980; Matheron & Bauliage, 1972; Thiele, 1968). Ethanol and casamino acids are more widely utilised by members of the Rhodospirillaceae (Pfennig, 1978b), The variation in the ability to utilise glucose and fructose has been observed in strains isolated from the same environment (Trüper, 1968), and in otherwise similar strains (Kondrat'eva, 1956; Matheron & Bauliage, 1972; Osnitskaya, 1954; Scardovi, 1950). There are few differences between the recognised species, E.mobilis and E.shaposnikovii in their carbon source utilisation (Kondrat'eva, 1956; Trüper, 1968). However, a noticable feature of the organisms isolated from the Kenyan soda lakes was the more restricted range of carbon compounds utilised by some of the strains requiring 3.0% (w/v) NaCl for growth, represented by the strains E7, M2, and M4. The extremely halophilic members of the genus Ectothiorhodospira also show a more restricted range of carbon source utilisation (Imhoff & Trüper, 1977; Raymond & Sistrom, 1967; 1969), compared to the freshwater or marine isolates. However, within the group represented by strain M4 there are several strains

which utilise a wider range of carbon sources than the low salinity strains. The division of the isolates into groups on the basis of salinity requirement does not, therefore, correlate with all the divisions based on the range of carbon sources utilised. The strains represented by strains B3 and NCL5 are very similar in their carbon source utilisation, and these two groups, which have similar NaCl optima were only distinguishable by their morphology and the presence of gas vacuoles in strain B3. Similarly, the strains E7, M2, and many of the M4 type strains showed a restricted range of carbon source utilisation, and were only distinguishable by cellular morphology and the absence of gas vacuoles in strain E7.

The ability of the organisms to photooxidise sulphur compounds was tested in media containing 0.1% (w/v) sodium thiosulphate, sulphite, sulphide, or elemental sulphur as sole electron donors. All the strains tested were capable of growth in the presence of sulphide or elemental sulphur (table 12). The formation and utilisation of polysulphides by all strains during the oxidation of sulphide to sulphate was indicated by the yellow colour of the medium prior to sulphur deposition, when the medium turned milky (van Niel, 1931). Strains M2 and M4 grew more slowly in the presence of sulphur, and elemental sulphur deposited outside the cell during the photooxidation of sulphide accumulated more than in cultures of E.mobilis, E.shaposnikovii, strains E7, NCL5, and B3. Sulphite was not utilised by organisms belonging to the groups represented by the type strains M2, M4, and E7, but was utilised by all other strains. Thiosulphate was utilised by strains E7, B3, and NCL5, and by some of the strains similar to strain M4, but was not utilised by strain M2 and similar organisms (table 12). The inability to utilise sulphite or thiosulphate was restricted to the strains requiring a minimum of 3.0% (w/v) NaCl for growth.

Table 12 Utilisation of sulphur compounds by phototrophic bacteria.

Strain no.		B2	B3*	B6	B7	E2	E3	E10	E11	E13	E14	N1	N4	N5	N8	N10	N11	M1	NCL1	NCL2	NCL4	NCL6
Sulphur compound (% w/v)																						
sulphide	0.1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
thiosulphate	0.1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
sulphite	0.1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
sulphur	0.1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Strain no.		B1	B7	B8	B9	B10	E1	E4	E6	E8	E9	N2	N6	N7	N9	NCL3	NCL5	M2*	M3	B5	DSM 239	DSM 243
Sulphur compound (%w/v)																						
sulphide	0.1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
thiosulphate	0.1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+
sulphite	0.1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+
sulphur	0.1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Strain no.		M4*	M6	M7	M8	M9	M10	M11	M13	M14	M15	M16	M18	M19	M20	M21	E7*	N3	M5	M12	M17	G_1
Sulphur compound (% w/v)																						
sulphide	0.1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
thiosulphate	0.1	+	–	–	+	+	+	–	+	–	–	+	–	+	+	+	+	+	–	–	+	+
sulphite	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
sulphur	0.1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Strains which were not capable of utilising thiosulphate and sulphite were generally characterised by their restricted range of carbon source utilisation, but some of the strains showed a wider range of carbon source utilisation, and no direct correlation could be made between the limitation in carbon and sulphur source utilisation. The utilisation of reduced sulphur compounds, including sulphide, thiosulphate, sulphite, and sulphur serves to differentiate the taxa of the Rhodospirillales. This may include the differentiation of distinct species (Pfennig & Trüper, 1974). Within the genus Ectothiorhodospira, sulphide is invariably oxidised to sulphate, with elemental sulphur being deposited extracellularly (Pfennig & Trüper, 1974; Trüper, 1968; Trüper & Pfennig, 1978; Trüper, 1978). A number of strains of Ectothiorhodospira have been isolated which were capable of utilising thiosulphate and sulphite (Kondrat'eva & Krasil'nikova, 1979; Matheron & Bauliage, 1972; Raymond & Sistrom, 1967; 1969; Trüper, 1968; Yang Hui-Fang, 1962), while other strains have been shown to lack the ability to utilise either thiosulphate or sulphite (Kondrat'eva, 1956; Scardovi, 1950; Yang Hui-Fang, 1962). One species, E.halophila, also lacks an assimilatory sulphate reduction pathway (Raymond & Sistrom, 1967; 1969). Although the utilisation of sulphur compounds may be used in the differentiation of the taxa, these criteria are insufficient to justify new taxa on this basis alone. Variation has been shown to occur in a number of species of phototrophic bacteria with relation to sulphur compound utilisation, including E.shaposnikovii (Pfennig & Trüper, 1974). The two strains of autotrophic Rhodopseudomonas sp. isolated by Yang Hui-Fang (1962) showed different salt tolerances, which could be correlated with differences in the utilisation of sulphur compounds. Whether these represent two different species was not determined, but their salt tolerances were similar to those reported for

E.mobilis and E.shaposnikovii (Cherni et al., 1969; Trüper, 1968). No differences were observed between the two species obtained from the DSM, in either salt tolerance or utilisation of sulphur compounds. In these cases the differences may be due to strain variation within a species, whereas, in some of the isolates from the Kenyan soda lakes, other data, including salt optima, carbon source utilisation, and morphology indicate that the differences in sulphur compound utilisation may be of taxonomic significance.

c. GC base content.

The GC base content of the strains B3, E7, NCL5, and M2 were determined to examine the relationship between these organisms and the members of the genus Ectothiorhodospira. The GC base content of strain B3 was 61.4 ± 1.0 mol% (T_m) and 61.9 ± 1.0 mol% (buoyant density), and that of strain NCL5 61.9 ± 1.0 mol% (T_m). These values were not significantly different to those obtained for E.shaposnikovii (Mandel et al., 1971; Imhoff & Trüper, 1977; Pfennig & Trüper, 1974), more recent determinations of E.mobilis (Trüper, personal communication), and for the Ectothiorhodospira sp. described by Grant et al. (1979). The GC base content of strain E7 was 63.5 ± 1.0 mol% (T_m), and that of strain M2 was 58.8 ± 1.0 mol% (bouyant density). The GC base content of some strains in species of Rhodospirillaceae cover a wide range, with values varying by as much as 5.0 mol%, and in Chr.minus, 10.0 mol% (Mandel et al., 1971). Differentiation of members of the Rhodospirillineae on the basis of the GC base content of the DNA is seldom possible, since with few exceptions the GC base content of the forty species examined are between 55.0 and 70.0 mol% (Mandel et al., 1971; Trüper & Pfennig, 1978). Species are, therefore, recognised on the basis of morphological and physiological, as well as GC base content differences. Taken together, the results suggest that the strains B3 and NCL5 are similar to the organisms

E.mobilis and E.shaposnikovii, with the exception that the strain B3 produces gas vacuoles, and may be regarded as a member of a taxon closely related to these two species. The other gas vacuolate strain, M2 has a lower GC content than that of strain B3, and may be regarded as a member of the same genus as strain B3, but possibly a different species, when morphology and physiology of the two strains are also compared. A similar relationship may also exist between the non-gas vacuolate strains NCL5 and E7, both of which appear to be members of the genus Ectothiorhodospira.

d. Phototrophic pigments.

The absorption maxima of all strains showed that they all contained bacteriochlorophyll a (figures 63-68). The absorption maxima for the strains B3, E7, and NCL5 were at 375, 585, 795, 855, with a shoulder at 895nm (bacteriochlorophyll a), and at 455, 485, 520, with a shoulder at 545nm (carotenoids) (figures 63, 64, 65). These were identical to the absorption maxima of E.mobilis and E.shaposnikovii grown under the same conditions (figure 63).

Strains M2 and M4 had similar, but not identical absorption spectra, maxima for strain M2 being at 375, 585, 775, 795, 827, and 890nm (bacteriochlorophyll a), and at 485, 507, and 545nm (carotenoids) (figure 67), while the absorption maxima of strain M4 were at 375, 590, 795, 845, and 890nm (bacteriochlorophyll a), and at 485, 505, with a shoulder at 545nm (carotenoids) (figure 68), Differences in the absorption maxima of bacteriochlorophyll in vivo, in the infra red region, have been attributed to a variety of factors, such as the age of the culture, strain of the organism, and the cultural conditions (Biebl & Drews, 1969; Thornber et al., 1978), which cause changes in the bacteriochlorophyll-bacteriochlorophyll and bacteriochlorophyll-protein interactions. The previously reported absorption maxima are different in some cases to those obtained for

Figure 63. Absorption specrtum of <u>Ectothiorhodospira shaposnikovii</u>

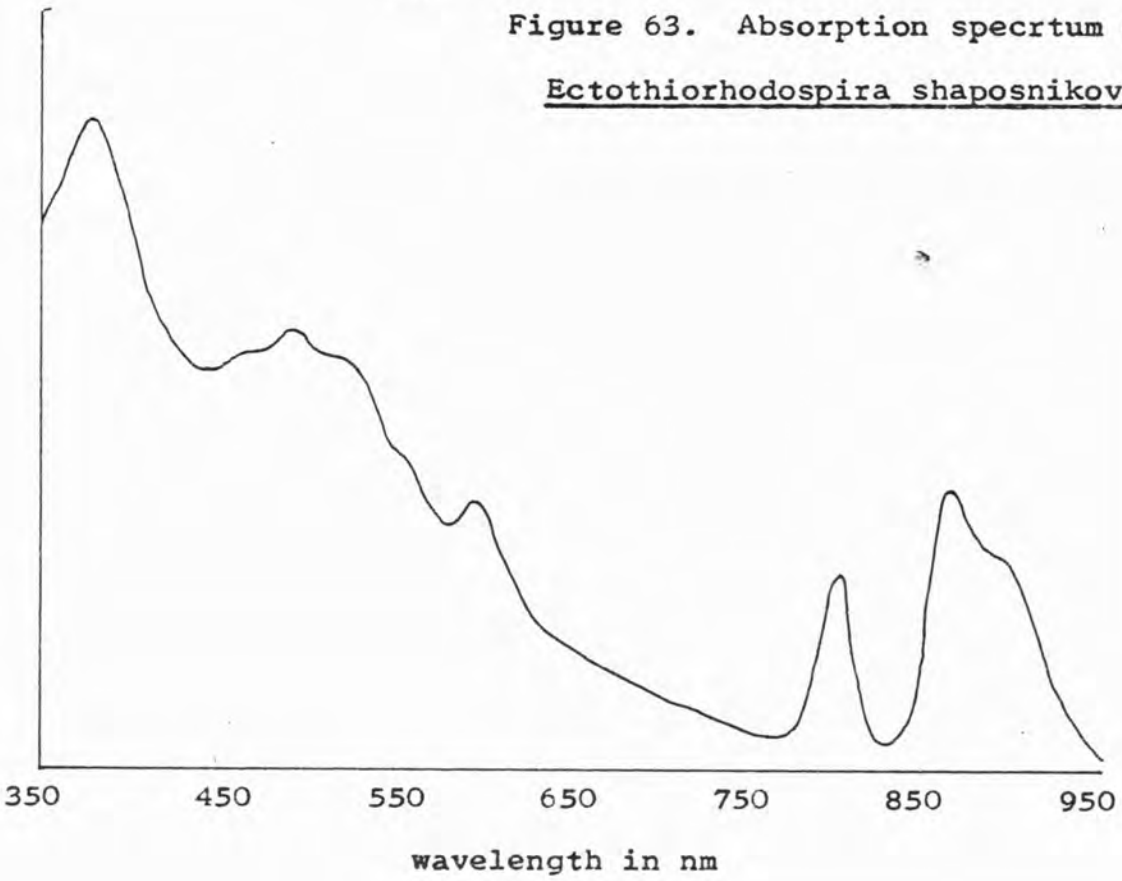
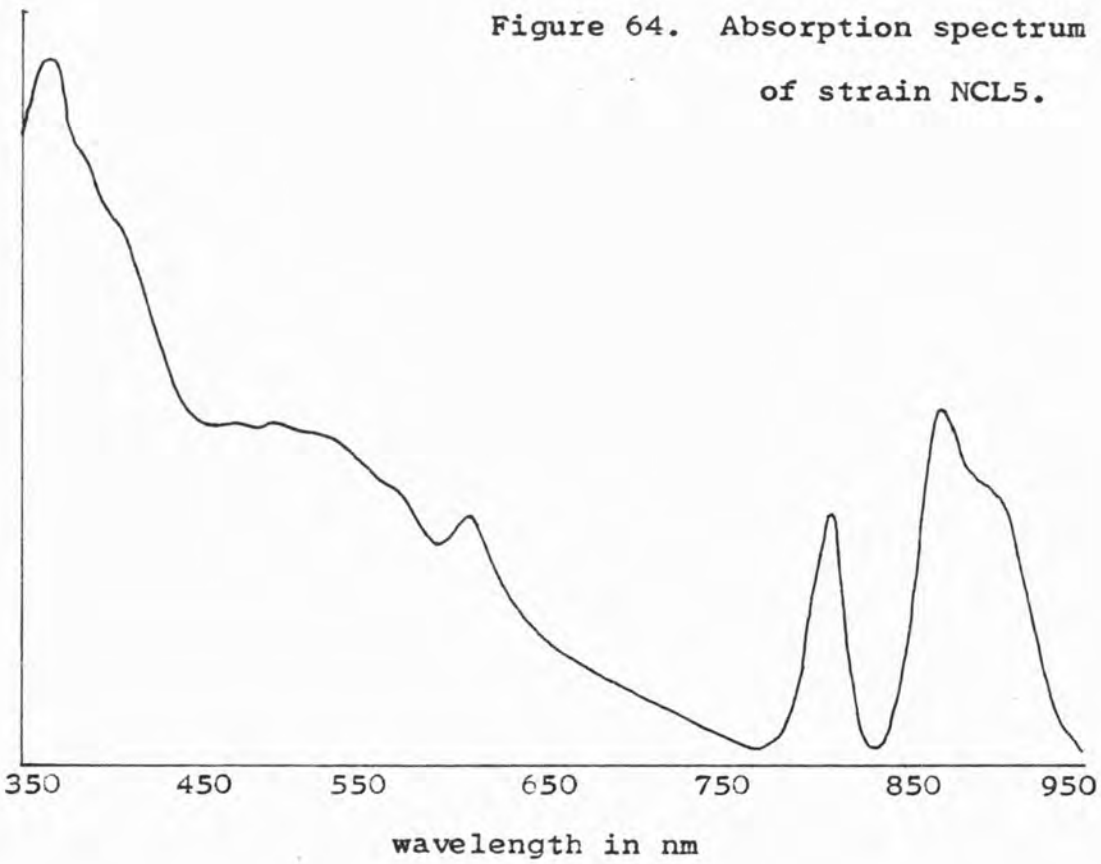


Figure 64. Absorption spectrum of strain NCL5.



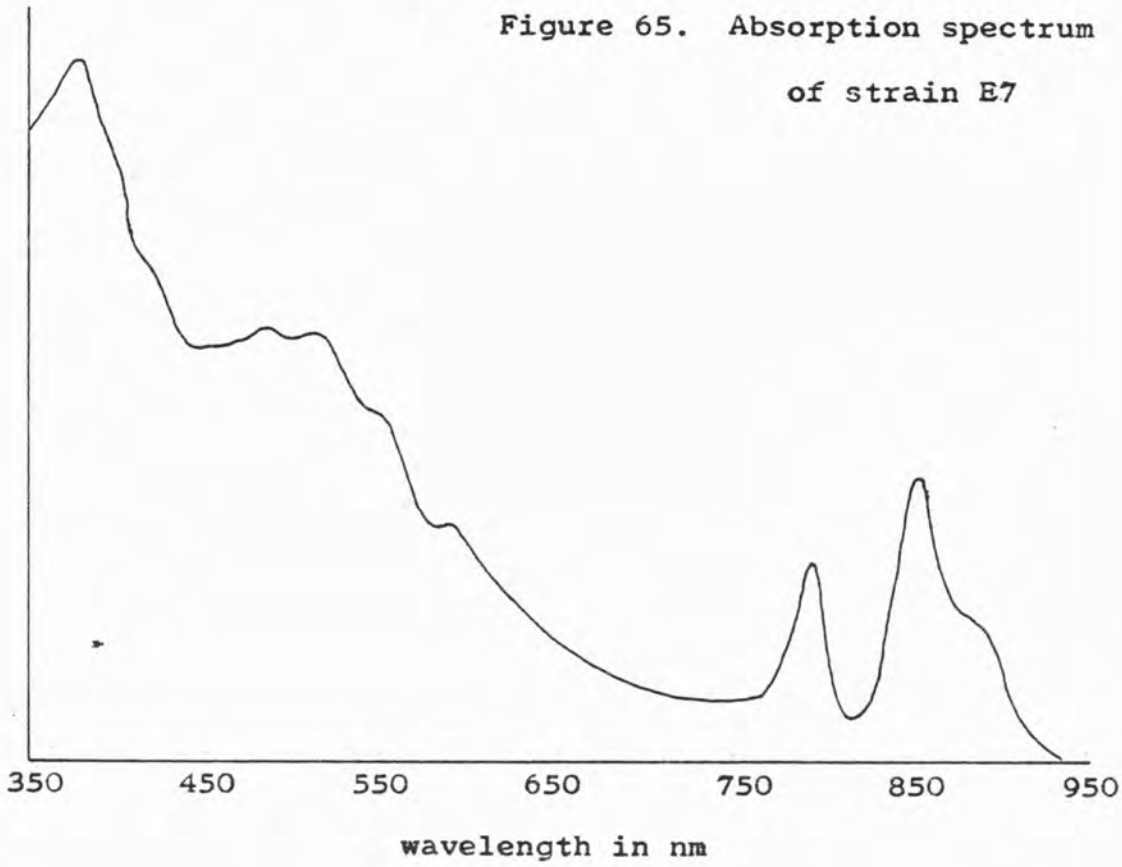


Figure 65. Absorption spectrum of strain E7

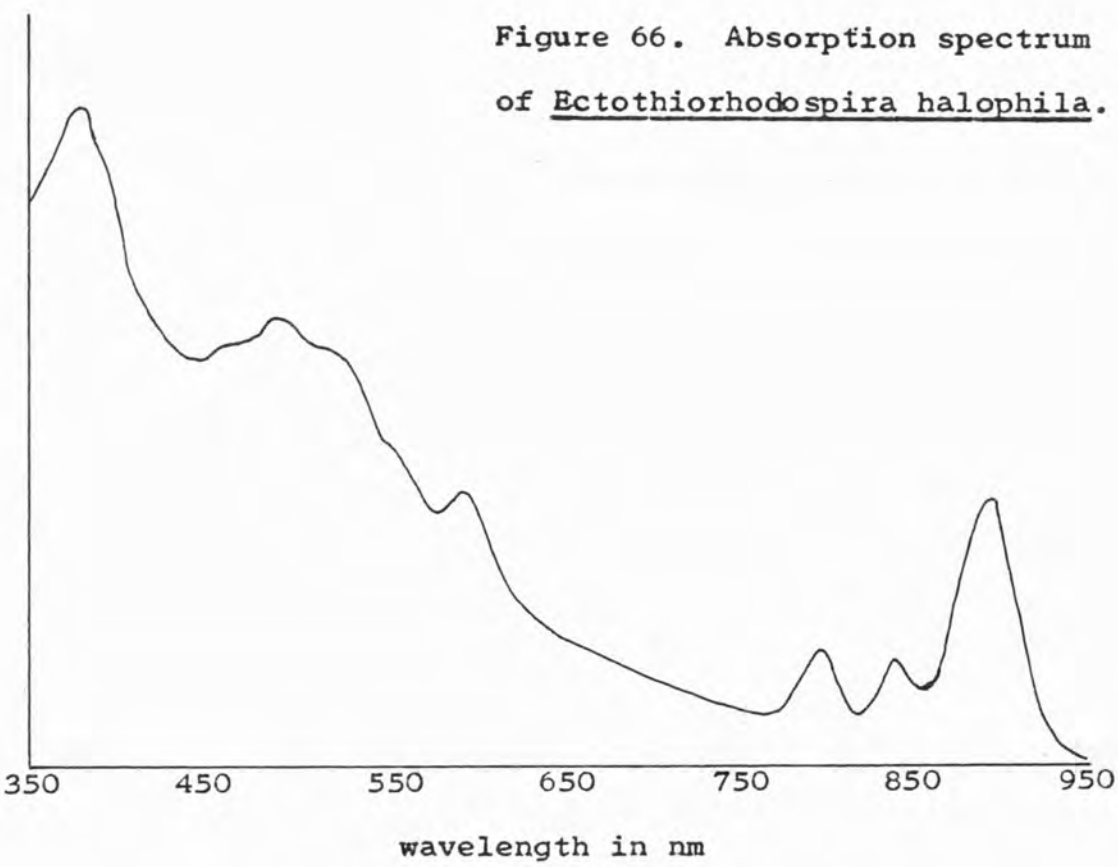


Figure 66. Absorption spectrum of <u>Ectothiorhodospira halophila</u>.

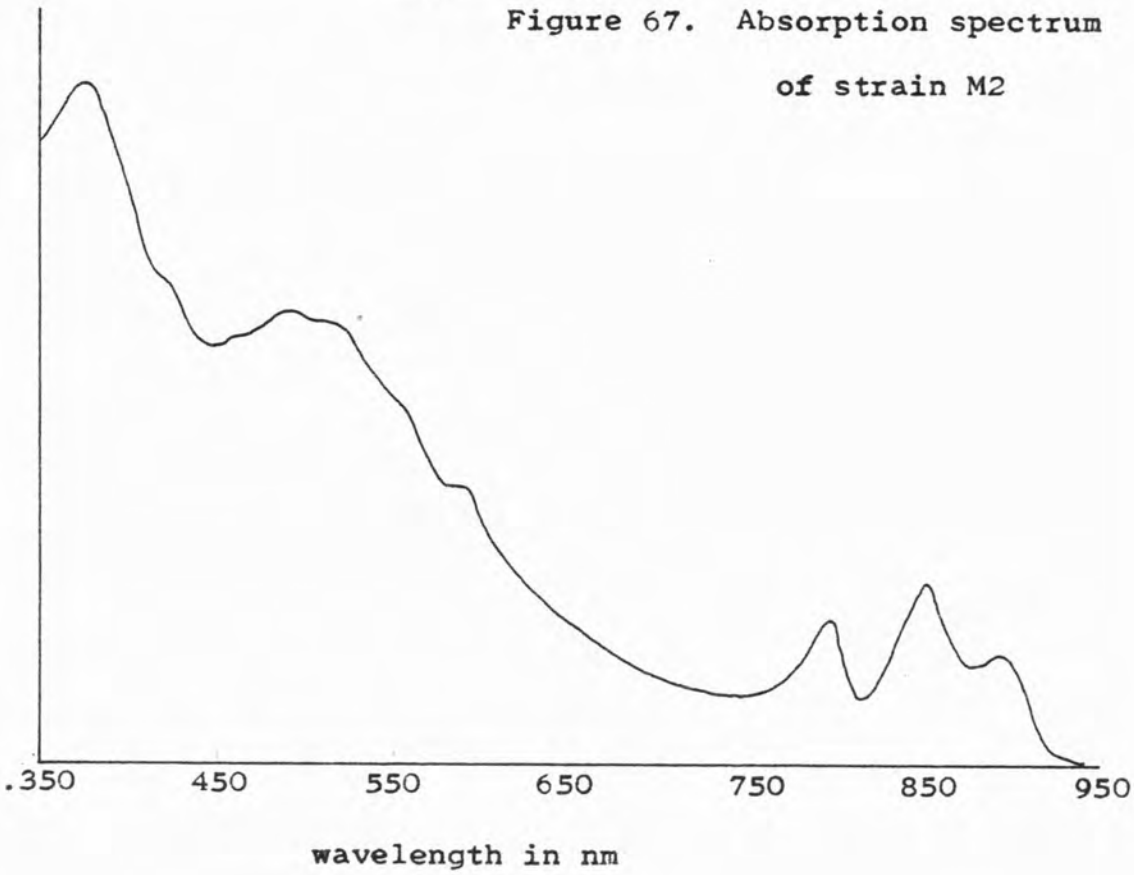


Figure 67. Absorption spectrum of strain M2

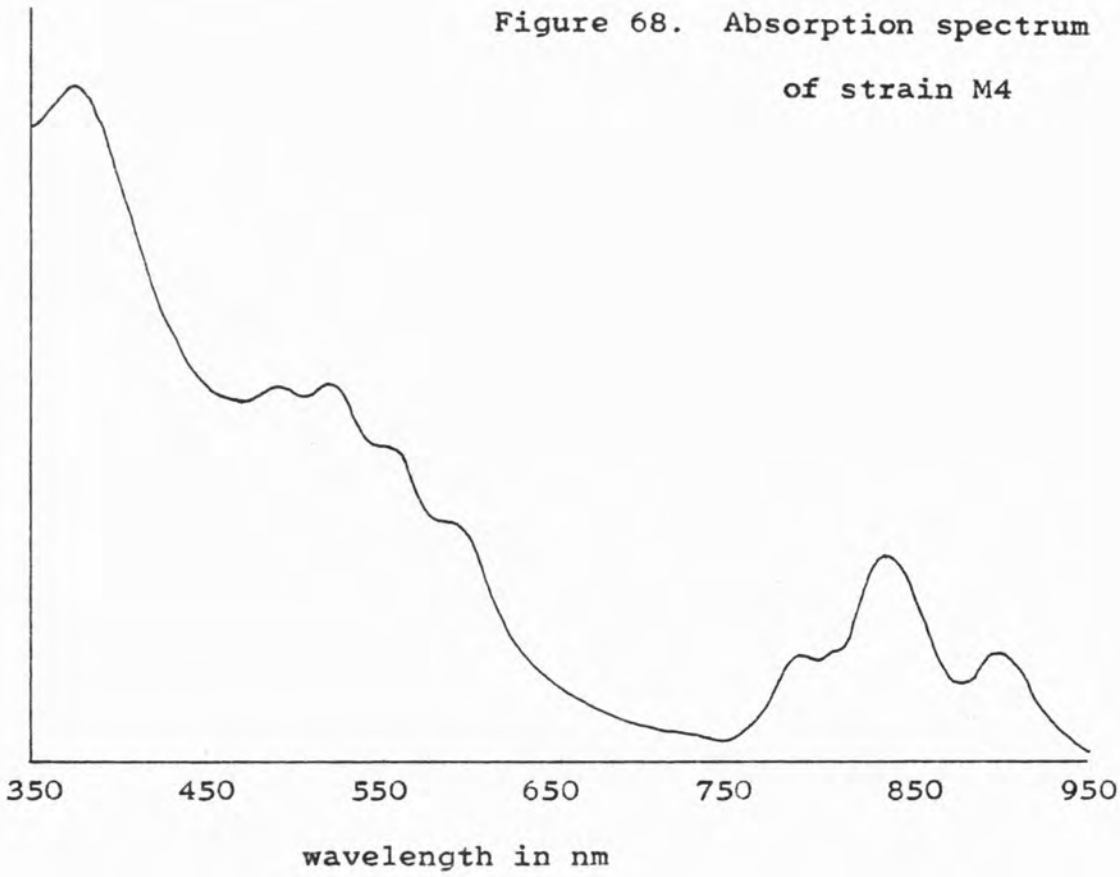


Figure 68. Absorption spectrum of strain M4

the type strains of Ectothiorhodospira. Raymond and Sistrom (1967) recorded the absorption maxima of E.halophila in the infra red region as maxima at 798, 885, and a shoulder at 850nm, there being no other differences in the spectra (figure 66). The major differences between the absorption maxima of E.mobilis obtained previously and that observed were also in the infra red region, Trüper (1968) recording the maxima at 805, 875, and a shoulder at 900nm. There were no differences between the spectra previously reported for E.shaposnikovii (Kondrat'eva & Malofeeva, 1964; Upsenkaya & Kondrat'eva, 1972). The differences between the previously reported spectra of E.mobilis and E.halophila, and those reported here, may be due to the nature of the light source, or the culture media. Differences in the spectra of any one strain were evident when grown photolithotrophically, photoorganotrophically, or photomixotrophically. All spectra were recorded from cells grown photomixotrophically, in the presence of 0.1% (w/v) $Na_2S.9H_2O$ and 0.1% (w/v) sodium acetate. The similarities between the absorption spectra of strains M2, M4, and E.halophila in the infra red region of the spectrum may suggest that in part the salinity requirement of the organisms may be important in affecting changes in the spectra associated with bacteriochlorophyll interactions. However, strain E7 has a similar absorption spectrum to that of E.mobilis and E.shaposnikovii, but has a NaCl requirement similar to that of strain M2 and M4.

Differences in the absorption spectra between 400 and 550nm are due to the carotenoid content of the strains, which may be influenced by cultural conditions (Pfennig et al., 1968; Schlegel & Pfennig, 1961; Schmidt, 1978; Schmidt & Trüper, 1971; Schmidt et al., 1965; Trüper, 1968). The presence of maxima at 485, 505-515 and 545nm in all strains were indicative of the presence of group I carotenoids, the normal spirilloxanthin series (Biebl & Drews, 1969;

Kondrat'eva & Malofeeva, 1964; Schlegel & Pfennig, 1961; Trüper, 1968). Typically cultures of organisms containing carotenoids of the normal spirilloxanthin series are red to brown-red in colour, although gas vacuolate strains may be pink-red (Pfennig & Trüper, 1974; Trüper & Pfennig, 1978). Cell suspensions of strains E7, B3, and NCL5 were red or pink in colour, but the colour of cell suspensions of strain M4 were similar to that of Chr.minus, which contains okenone as the major carotenoid. Cell suspensions of strain M2 were also distinctive in colour, being red-violet. The carotenoids of the type strains were subsequently extracted and compared with carotenoids obtained from the reference strains, by elution from aluminium oxide columns, co-chromatography, and absorption spectra. Strains B3, E7, and NCL5 contained carotenoids of the normal spirilloxanthin series, and were indistinguishable from E.mobilis and E.shaposnikovii. Strains M2 and M4 were distinctive in that they contained fewer carotenoids. Strain M2 contained only two carotenoids, which were identified as spirilloxanthin and rhodopin. Strain M4 contained a single carotenoid, which was identified as spirilloxanthin. Although strain M4 was similar in colour to cell suspensions of okenone containing organisms, the in vivo absorption spectrum and carotenoid analysis did not show that the carotenoid okenone was present. Although organisms producing carotenoids of the normal spirilloxanthin series usually contain a number of carotenoids of this group, some strains are known in which spirilloxanthin is the main carotenoid, or there may be only one or two additional carotenoids present (Schmidt, 1978; Schmidt et al., 1965). The strains M2 and M4 are not atypical, although members of the genus Ectothiorhodospira, which they closely resemble usually contain a number of carotenoids of the normal spirilloxanthin series. In E.halophila, however, the major carotenoid present is

spirilloxanthin, with no other carotenoids present in appreciable amounts (Schmidt & Trüper, 1971). The range of colour differences observed between the strains, and their carotenoid content illustrated the difficulty and subjective nature of assigning a colour description to the various species, and its use as an indicator of carotenoid content (Trüper & Pfennig, 1978).

e. Lipid analyses.

Analysis of the lipid composition of strains B3, M2, M4, NCL5, and E7 were carried out by comparison with lipids obtained from Sigma and total lipid extracts from Chr.minus (BN 1211 and DSM 178), Chr.minutissimum, Chr.vinosum (strain D), Rhsp.rubrum, Rhm.vannielii, E.mobilis, E.shaposnikovii, E.halophila, and E.halochloris. The lipid composition of the strains B3, NCL5, E7, M2, and M4 were essentially similar, and could not be distinguished from the lipid composition of E.mobilis and E.shaposnikovii. The major lipids present in these organisms were phospholipids (figures 69, 70), glycolipids were not detected in the lipid extracts. The polar lipid composition was essentially similar to that reported by Oyewole and Holt (1976) for E.mobilis, the phospholipids, phosphatidyl ethanolamine (PE), phosphatidyl glycerol (PG), phosphatidyl choline (PC), and diphosphatidyl glycerol (DPG) were present. In addition an unidentified phospholipid was present, which had an Rf similar to that of PC in both solvent systems (figures 69, 70). In the first solvent, chloroform:methanol:water (65:25:4), a lipid was present with an Rf slightly greater than that of PE, but it was not a phospholipid, and it may have been either a quinone or an isoprenoid neutral lipid (figure 69).

The lipid composition of the extremely halophilic E.halophila and E.halochloris were distinguishable from the other members of the genus Ectothiorhodospira examined. The major polar lipids were

phospholipids, and no glycolipids were detected in the extracts of either species (figures 69, 70). DPG was apparently present in both species, as was reported for E.halophila (Raymond unpublished, in Kenyon, 1978), but PE was not present. An amino-lipid was present in both species, which had an Rf similar to that of PE in the second solvent (figure 70), chloroform:methanol:acetic acid:water (80:12:15:4), but PE was clearly absent from the lipid extracts developed in the first solvent (figure 69). PG was the only other lipid present in E.halophila, which was identified. The lipid compositions of the species E.halophila and E.halochloris were similar to one another, and both are distinguishable from other members of the genus Ectothiorhodospira examined. Comparison with the lipids of Halobacterium salinarium indicated that the lipids of E.halophila and E.halochloris were not ether linked, confirming previous reports (Raymond unpublished, in Kenyon, 1978). These two species are not, therefore, members of the archaebacteria (Woese et al., 1978; Oro et al., 1978), but the distinctive lipid composition may be suggestive of a taxonomic division within the presently described genus Ectothiorhodospira.

The lipid composition of the members of the genus Ectothiorhodospira, and the isolates from the Kenyan soda lakes were compared with the polar lipid composition of members of the Chromatiaceae and Rhodospirillaceae. The lipid composition of Chr.minus was similar to that of E.mobilis and E.shaposnikovii, with the exception that glycolipids were occassionally detected in Chr.minus (figures 69, 70). PC could not be detected in Chr.minutissimum or Chr.vinosum, but in other respects the lipid composition was similar to that of Chr.minus. In the first solvent system a non-phosphate positive lipid was detected in Chr.minus, Chr.minutissimum, and Chr.vinosum, which had an Rf identical to the non-phosphate positive lipid

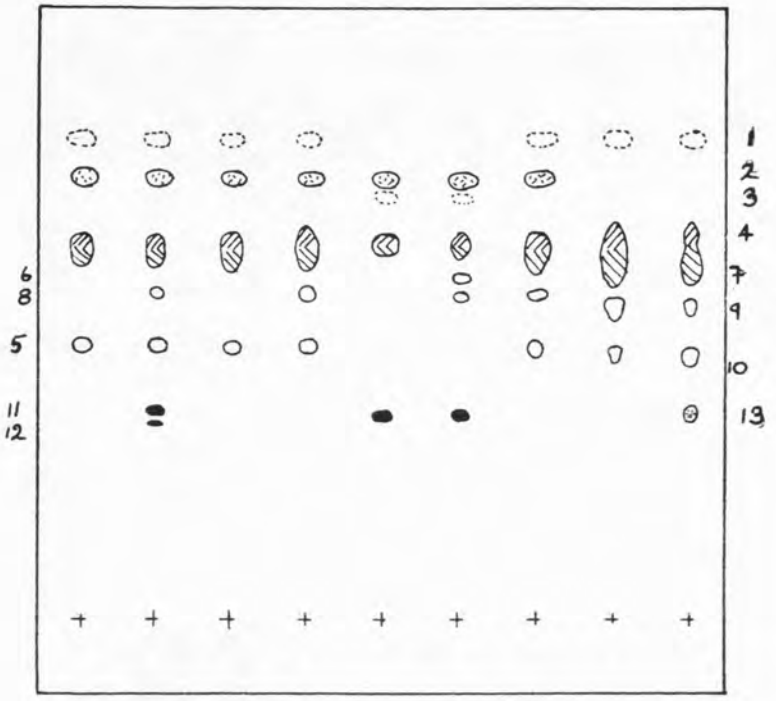
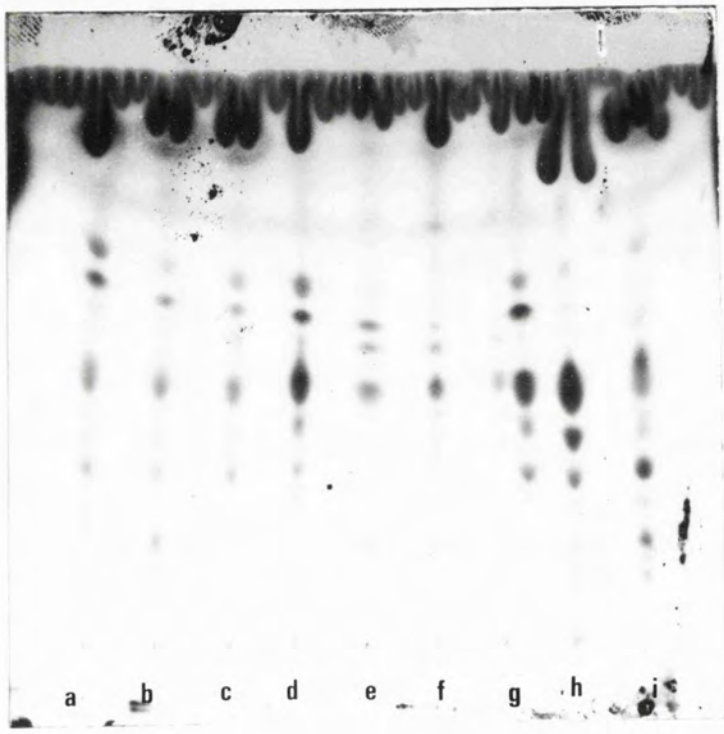


Figure 69. Thin layer chromatograph of the polar lipids of phototrophic bacteria.

Thin layer chromatograph developed in the solvent chloroform: methanol:water (65:25:4). Staining reactions are represented by: ○ phosphate positive; ◌ phosphate negative; ⊘ periodate-Schiff positive immediately; ⊘ periodate-Schiff positive after 24h; ⊙ ninhydrin positive; ● glycolipids.

The lipids were identified as: 1. non-phosphate positive lipid; 2. PE; 3. non-phosphate positive lipid; 4. PG and DPG; 5. PC; 6 and 7. amino-lipids; 8,9, and 10. unidentified phospholipid; 11 and 12. glycolipids; 13. unidentified amino-lipid.



a. Chr.minutissimum

b. Chr.minus (BN 1211)

c. Chr.vinosum (D)

d. Chr.minus (DSM 178)

e. Rhsp.rubrum

f. Rhm.vannielii

g. E.mobilis

h. E.halophila

i. E.halochloris

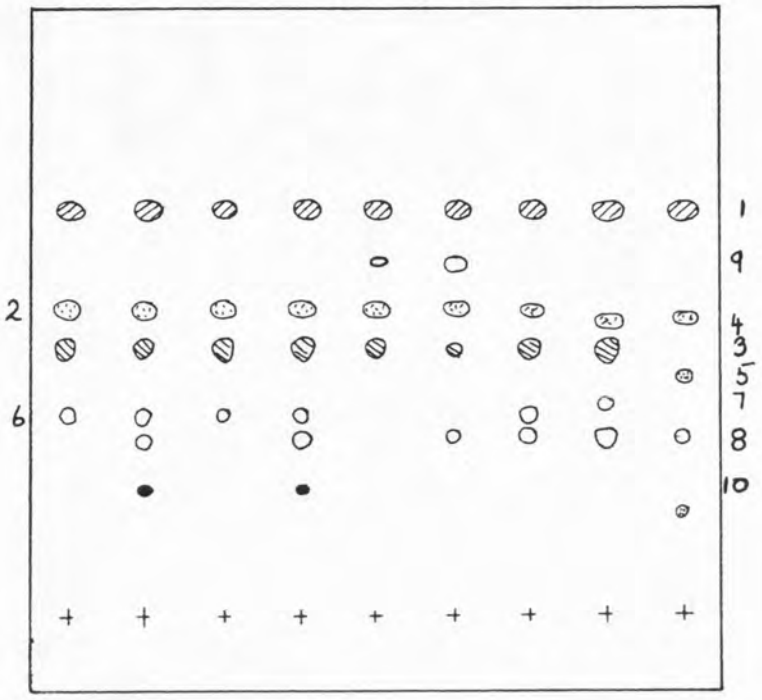


Figure 70. Thin layer chromatograph of the polar lipids of phototrophic bacteria.

Thin layer chromatograph developed in the solvent chloroform: methanol:acetic acid: water (80:12:15:4). Staining reactions are represented by: phosphate positive; phosphate negative; periodate-Schiff positive after 24h; periodate-Schiff positive immediately; ninhydrin positive; glycolipids.

The lipids were identified as: 1. DPG; 2. PE; 3. PG; 4. amino-lipid; 5. amino-lipid; 6. PC; 7.and 8. unidentified amino-lipid; 10. glycolipid; 9. PA

a. Chr.minutissimum

b. Chr.minus (BN 1211)

c. Chr.vinosum (D)

d. Chr.minus (DSM 178)

e. Rhsp.rubrum

f. Rhm.vannielii

g. E.mobilis

h. E.halophila

i. E.halochloris

detected in members of the genus Ectothiorhodospira (figure 69). The lipid composition of Chr.vinosum was similar to that previously reported (Steiner et al., 1969), and these results suggest that the polar lipid composition of the small members of the genus Chromatium is similar to that of E.mobilis and E.shaposnikovii. In Rhsp.rubrum and Rhm.vannielii the polar lipid composition was similar to that described previously (Kenyon, 1978), containing DPG, PG, PE, and PC as the major phospholipids (figures 69, 70). Phosphatidic acid was detected in Rhm.vannielii, but not in Rhsp.rubrum. The major differences between these two species and the other organisms tested was in the presence of amino-lipids, which were probably the ornithine lipids identified in previous studies (Kenyon, 1978). In the first solvent there was also present a non-phosphate positive lipid, with an Rf less than that of PE, and also allowed the members of the Rhodospirillaceae to be distinguished from the members of the Chromatiaceae (figure 69), including the genus Ectothiorhodospira. The identity of the non-phosphate positive lipids was not investigated in either the members of the Rhodospirillaceae or Chromatiaceae tested. However, they may be neutral lipids, such as squalenes or hopanoids. This would be consistent with the finding that members of the Rhodospirillaceae contain hopanoids, while members of the Chromatiaceae contain squalenes (Ourisson et al., 1979; Rohmer et al., 1979).

The lipid patterns of members of the Rhodospirillaceae and Chromatiaceae can be distinguished from one another as illustrated here and in previous work (Kenyon, 1978). The similarity between the polar lipid composition of members of the genus Chromatium and E.mobilis and E.shaposnikovii suggests that these two species, and the strains B3, E7, NCL5, M2, and M4 may be more closely related to the genus Chromatium. The reported similarity between Chr.vinosum

and Th.roseopercina in their lipid composition (Kenyon, 1978; Steiner et al., 1969; Takacs & Holt, 1971) suggest that this type of lipid composition may be characteristic of members of the Chromatiaceae, and E.mobilis and E.shaposnikovii are, therefore, more closely related to this family than to the family Rhodospirillaceae by the polar lipid composition. The distinctive lipid composition of the extremely halophilic E.halophila and E.halochloris shows some similarities to both the members of the Chromatiaceae and Rhodospirillaceae, but is sufficiently different from either of these families to indicate the investigation of these two species (and E.abd-el-malekii) as a novel taxon. The environment has been shown to have a significant effect on the composition of the cell envelope of bacteria (Ellwood & Tempest, 1972; Hiramatsu et al., 1978; Koyama & Nosoh, 1976; Novitsky & Kushner, 1976), however, the organisms have usually only been tolerant of the extreme conditions, and conclusions drawn from these studies cannot be directly applied to organisms for which the so-called extreme environment is the natural environment, as in the extreme halophiles. The differences in the lipid composition of E.halophila and E.halochloris may be a consequence of the highly saline environment, but such changes may not be reversible at low salinity, which could account for their dependence upon high salinity for growth. The significance of such changes in the chemistry of the cell wall in the taxonomy of these species must be considered in conjunction with other criteria, such as physiology, morphology, and genetics.

4. ISOLATION AND CHARACTERISATION OF EXTREMELY HALOPHILIC BACTERIA.

4.1. Enrichment and isolation of cultures.

Samples of saltern liquor, obtained in 1976 and 1977 by Dr.A.A. Mills and from the Magadi Soda Co.Ltd., contained large numbers of bacteria, which were initially isolated in a medium of the following composition (%w/v): NaCl, 20; $MgCl_2$, 0.1; NH_4Cl, 0.1; KCl, 0.1; $Na_2CO_3.10H_2O$, 20; yeast extract, 0.1; pH 10.0 (Grant & Tindall, 1980). The organisms, which grew slowly were subsequently shown to grow better in the modified medium of Brown (1963) described by Tindall et al. (1980). Samples of saltern liquor or trona crust collected in 1978 from Lake Magadi were inoculated into the modified medium of Brown (1963), and incubated at 37°C for 3-4 weeks. The cultures were claret in colour, and like the saltern liquor, the absorption spectrum revealed the presence of both carotenoids and bacteriochlorophyll (figures 71, 72). Single colonies were obtained by sub-culturing the enrichments on to medium solidified with 2.0% (w/v) agar. Tests showed that the organisms grew more prolifically on the medium of Payne et al. (1960), modified as described by Tindall et al. (1980), and all growth tests were subsequently carried out using this medium. The strains were purified by three transfers on this medium after growth at 37°C for 3-4 weeks.

4.2. Characterisation of pure cultures.

a. Morphology.

Although 36 strains were initially isolated (table 13), which each showed subtle differences from one another, three major morphological types were evident, and three type strains were designated SP1, SP2, and MS3. Strain SP1 was isolated from saltern liquor sent from Kenya in 1977, and similar organisms were isolated from the saltern liquor and lake trona in 1978. The organism was a Gram-negative rod, 0.7-1.0µm wide by 4.0-6.0µm long (figure 73).

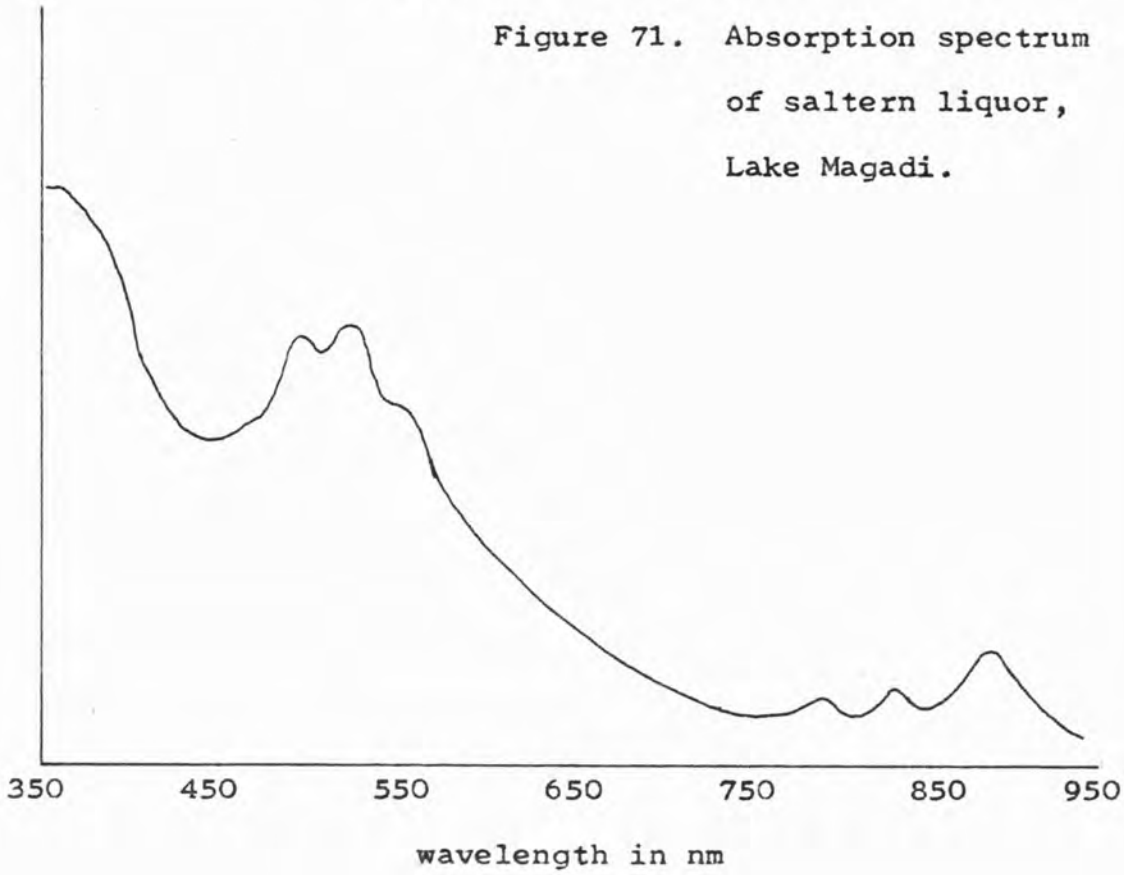


Figure 71. Absorption spectrum of saltern liquor, Lake Magadi.

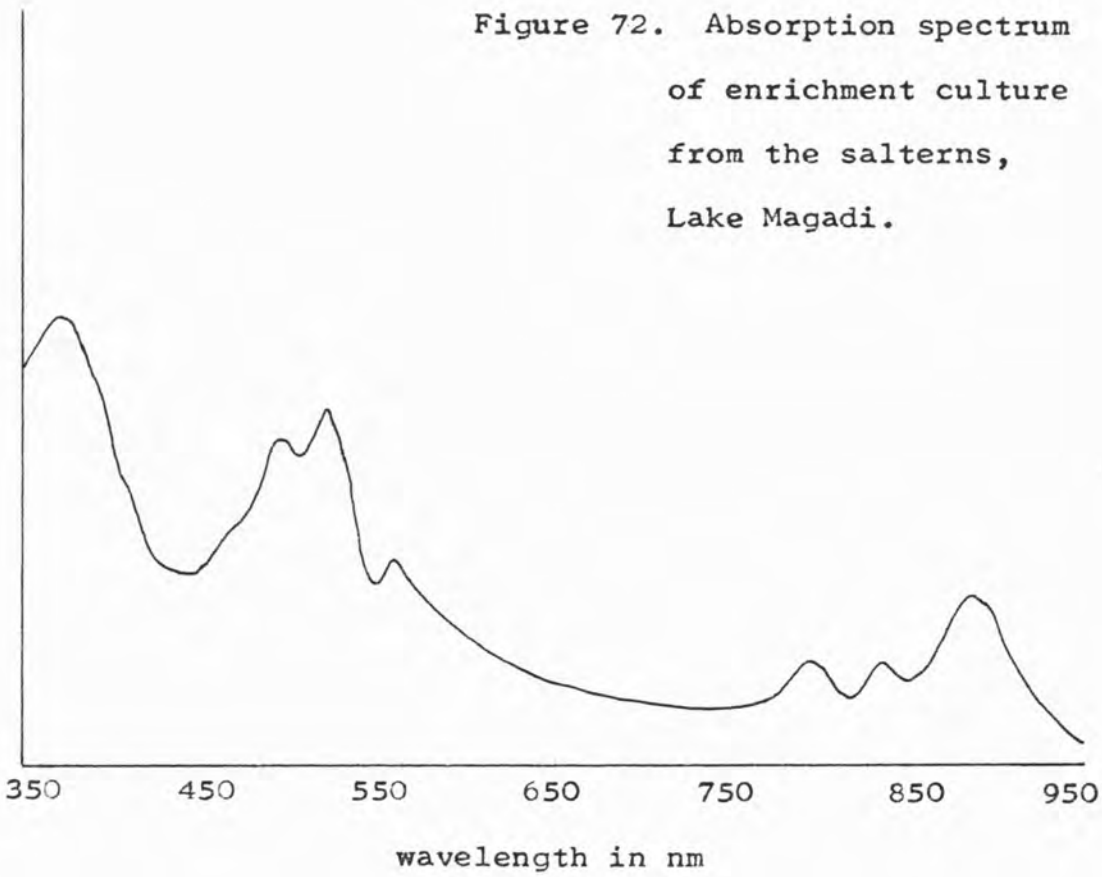


Figure 72. Absorption spectrum of enrichment culture from the salterns, Lake Magadi.

Table 13 List of strains of extremely halophilic bacteria isolated from Lake Magadi and the solar salterns.

Strain	Isolated from:
SP1*	Salterns, Lake Magadi
SP4	Salterns, Lake Magadi
MS1	Trona, Lake Magadi
MS4	Trona, Lake Magadi
MS6	Trona, Lake Magadi
MS7	Trona, Lake Magadi
MS8	Trona, Lake Magadi
MS9	Trona, Lake Magadi
MS10	Trona, Lake Magadi
MS11	Trona, Lake Magadi
MS12	Trona, Lake Magadi
MS14	Trona, Lake Magadi
MS15	Trona, Lake Magadi
MS17	Trona, Lake Magadi
MS21	Trona, Lake Magadi
MS23	Trona, Lake Magadi
MS24	Trona, Lake Magadi
MS25	Trona, Lake Magadi
SP2*	Salterns, Lake Magadi
SP3	Salterns, Lake Magadi
SP3	Salterns, Lake Magadi
SP5	Salterns, Lake Magadi
SP6	Salterns, Lake Magadi
SP7	Salterns, Lake Magadi

Table 13 (cont.)

Strain	Isolated from:
MS3*	Trona, Lake Magadi
MS5	Trona, Lake Magadi
MS13	Trona, Lake Magadi
MS16	Trona, Lake Magadi
MS18	Trona, Lake Magadi
MS19	Trona, Lake Magadi
MS20	Trona, Lake Magadi
MS26	Trona, Lake Magadi
MS27	Trona, Lake Magadi

All strains were Gram-negative.

(the type strains of the extremely halophilic bacteria isolated are marked *).

After 2 weeks incubation at 37°C the colonies were orange-red, convex with an entire edge, 3-4mm in diameter, translucent and mucoid (figure 74). Cells of strain SP1 were motile. A preliminary report on strain SP1 has already appeared (Tindall et al., 1980).

Strain SP2 was isolated in 1978 only from the saltern liqour, and was a Gram-negative coccus 1.5µm in diameter when grown on plates. This strain was initially identified as a Halococcus type organism, but when it was grown in liquid culture the organism was a long, fine, filamentous rod, 0.5-0.7µm wide by 10-15µm long (figure 75). The orange colonies were 5-6mm in diameter after 2 weeks growth at 37°C (figure 76). Colonies were opaque, friable, with a crenated surface. The opacity of the culture was indicative of the presence of gas vacuoles, although they were not observed in the cells. Strain SP2 could not be shown conclusively to be motile, although a slow tumbling motion was detected in liquid culture. In liquid culture a pellicle sometimes formed, and the cells formed a viscous sediment in old cultures.

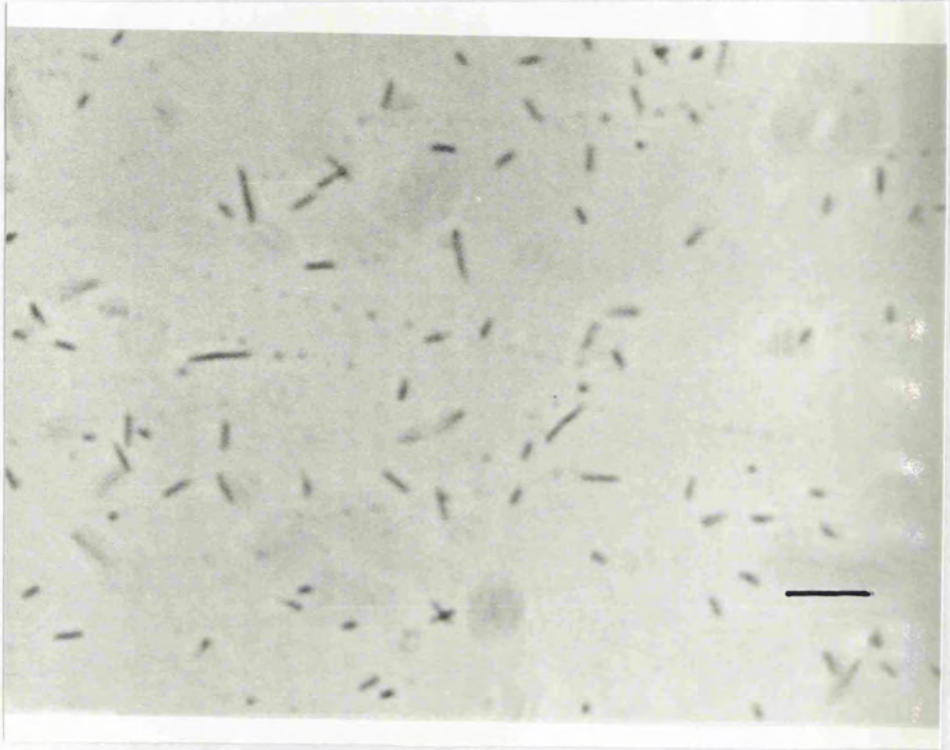
Strain MS3 was isolated, in 1978, from the trona crust of the lake, and was not observed, or isolated from the saltern liquor. The colonies were initially grey and translucent, but the mucoid colonies changed to a salmon-pink colour, and became friable and opaque, 4-5mm in diameter after 2 weeks growth at 37°C (figure 78). Cells were Gram-negative, motile rods, 0.7-1.0µm wide by 2.0-4.0µm long (figure 77). Colonies were convex with an entire edge. The small size of typical Halobacterium sp., less than 0.2mm in diameter, colonies is shown in figure 79 for comparison.

b. Physiology.

The absorption spectrum of whole cells of the three strains showed maxima at 475, 505, and 545nm (figure 80), characteristic of bacterioruberins found in members of the genus Halobacterium and

Figure 73. Cells of strain SP1, x400, bar indicates 10µm.

Figure 74. Colonies of strain SP1 after 3 weeks growth, bar indicates 10mm



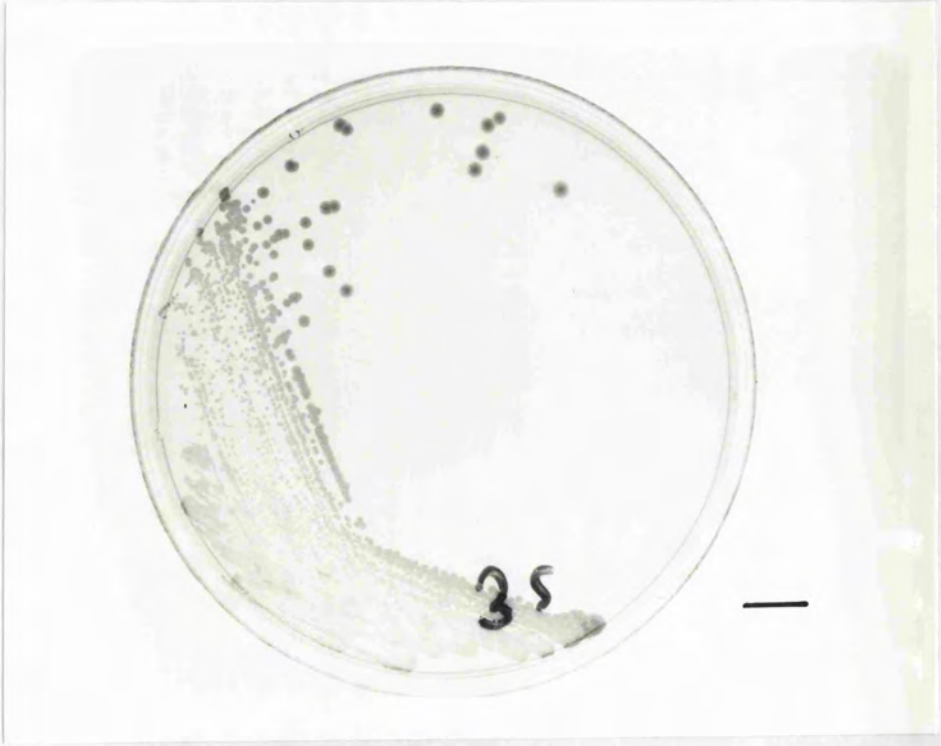
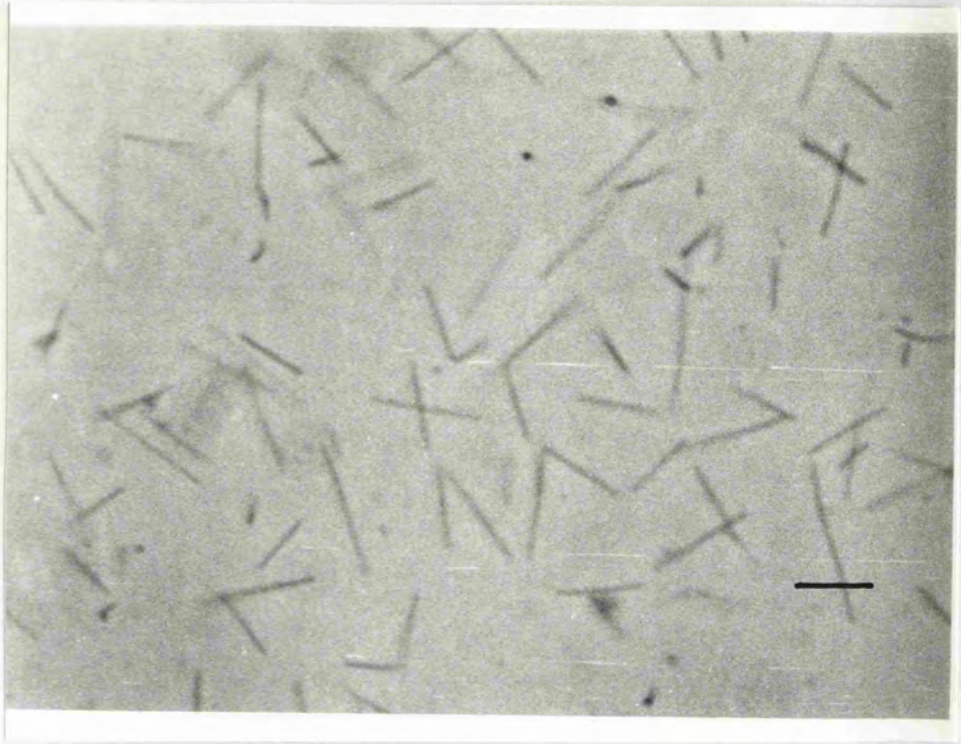

35

Figure 75. Cells of strain SP2, x400, bar indicates 10µm.

Figure 76. Colonies of strain SP2 after 3 weeks growth, bar indicates 10mm.



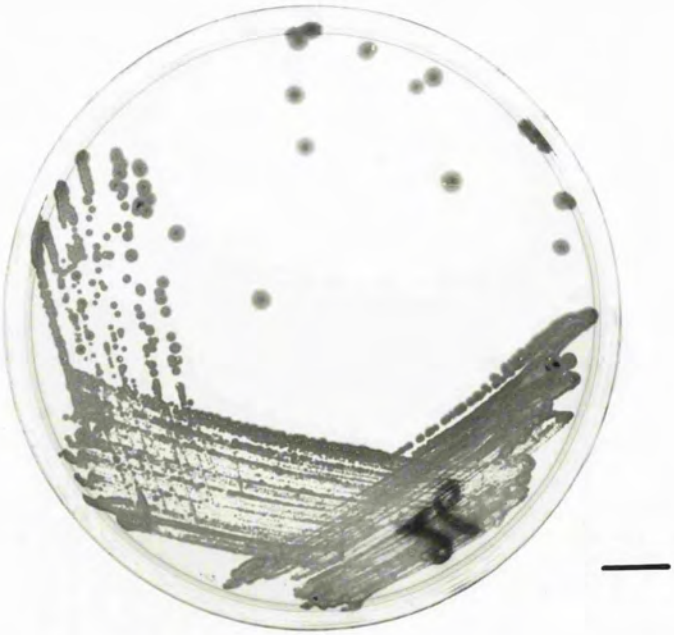
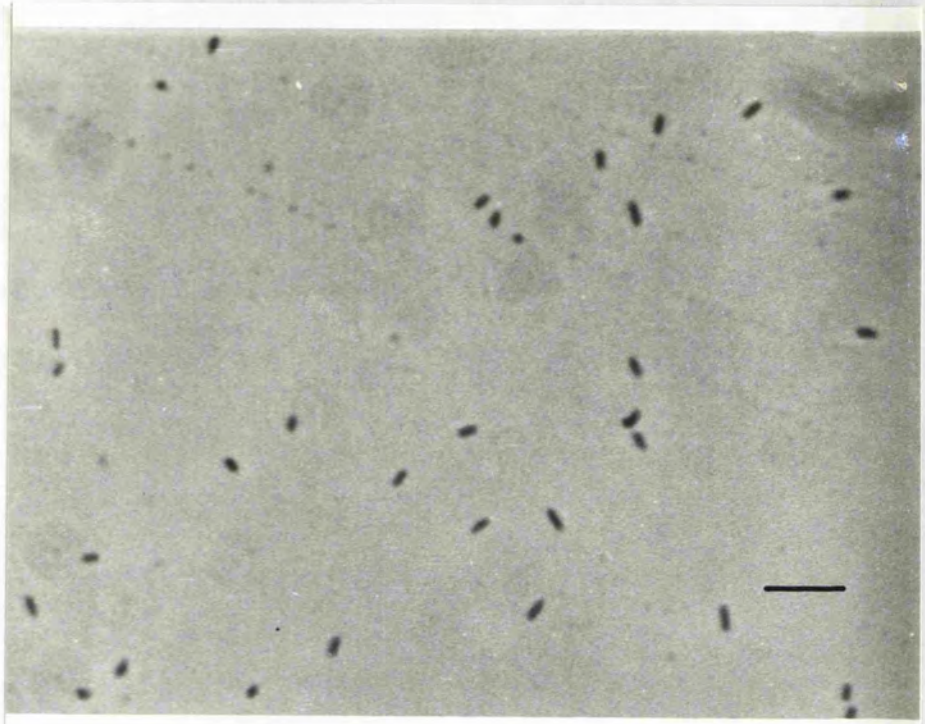


Figure 77. Cells of strain MS3, x400, bar indicates 10µm.

Figure 78. Colonies of strain MS3 after 3 weeks growth, bar indicates 10mm.



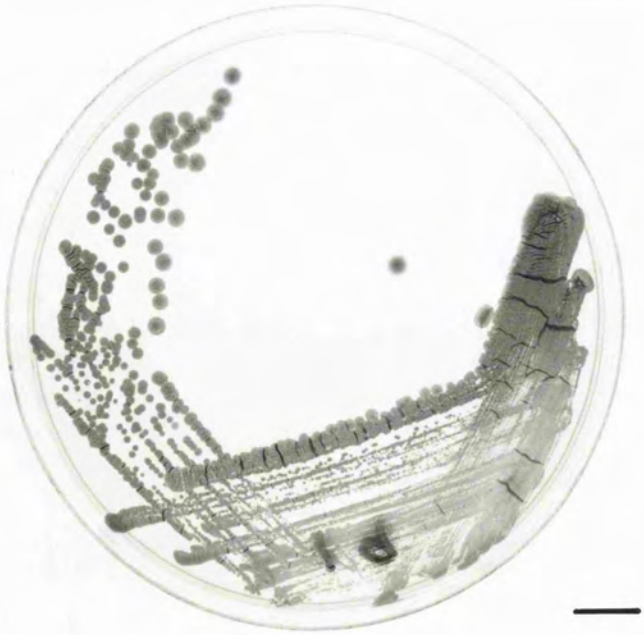
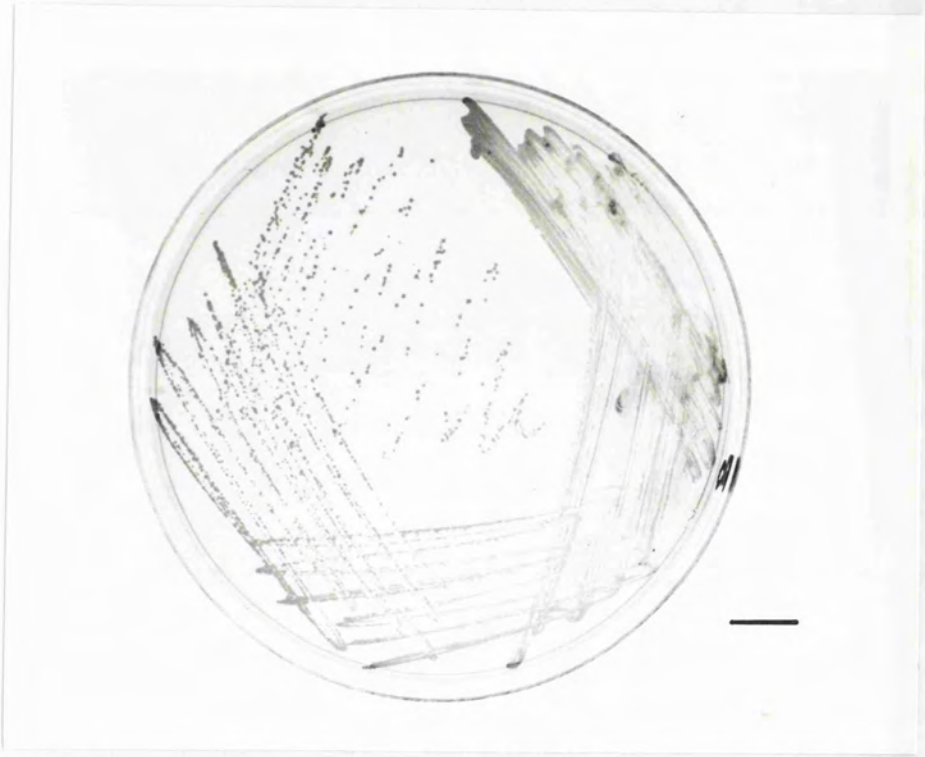


Figure 79. Colonies of Halobacterium halobium after 3 weeks growth, bar indicates 10mm.



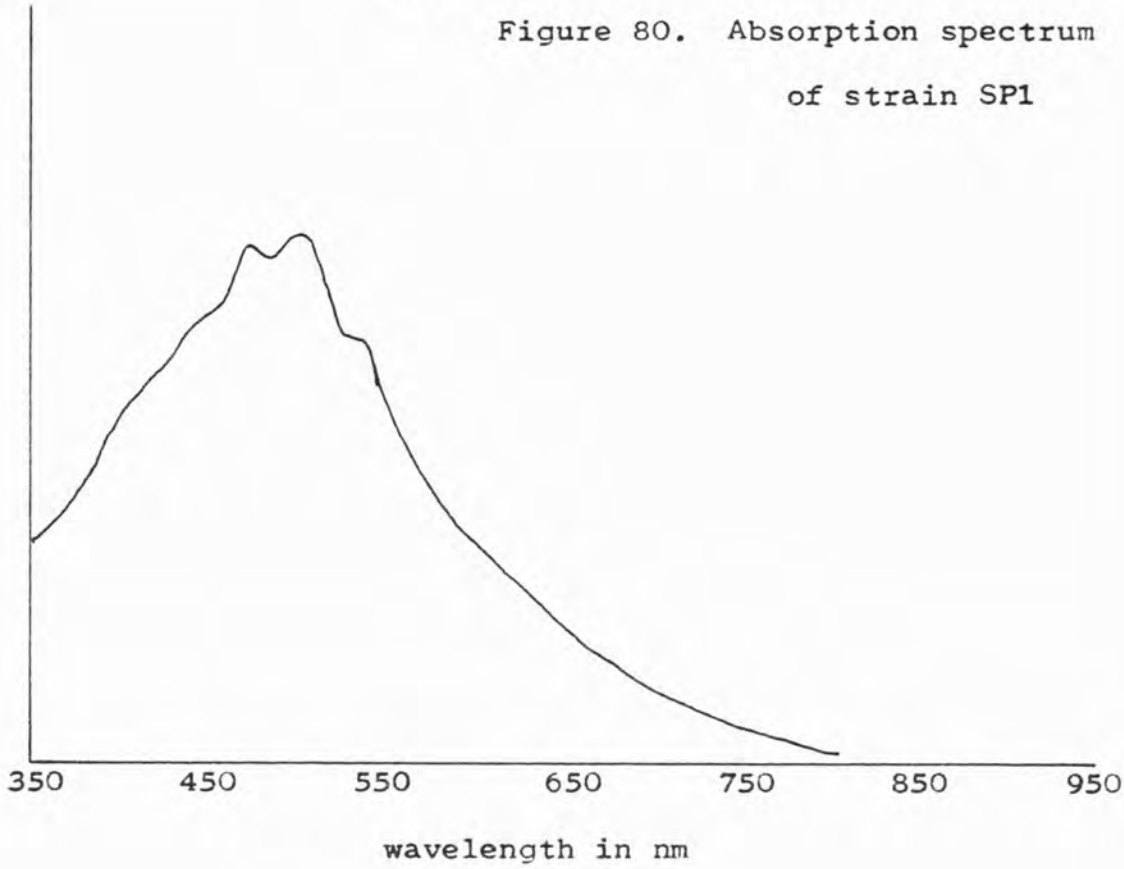


Figure 80. Absorption spectrum of strain SP1

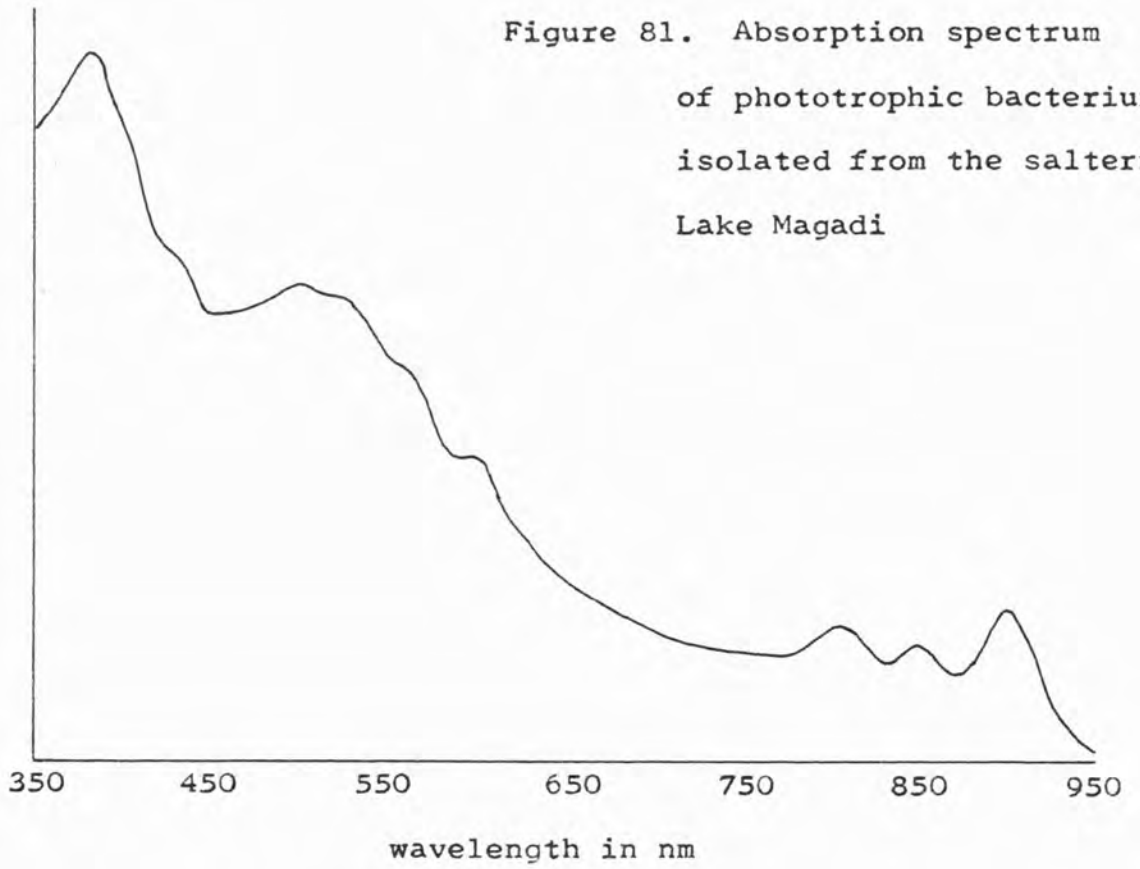


Figure 81. Absorption spectrum of phototrophic bacterium isolated from the salterns Lake Magadi

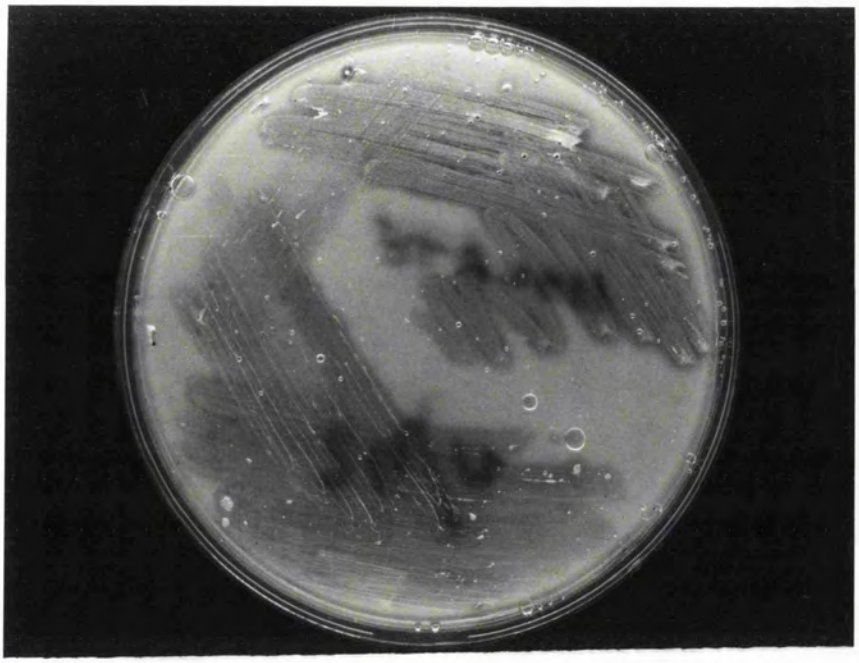
Halococcus (Gibbons, 1974). Bacteriochlorophyll present in the saltern liquor was found to be present as bacteriochlorophyll a, in a halophilic purple bacterium (figure 81), resembling members of the genus Ectothiorhodospira (Grant, Schofield, and Tindall, unpublished). Cells of all three strains of extremely halophilic bacteria became spherical when suspended in media containing 1.0M NaCl, and some of the cells of strain SP2 in the filamentous form lysed. Cells of all strains lysed in distilled water, although the coccoid cells of strain SP2 grown on plates were the least sensitive to low ionic strength. Similarities to members of the genus Halobacterium were suggested, and the organisms were, therefore, compared with type strains of the genus Halobacterium.

Growth of the reference strains, and strains SP1, SP2, and MS3 was tested at pH 7.5, 8.5, and 9.5, in the medium of Payne et al. (1960), in which $MgSO_4.7H_2O$ was ommitted, or added to give a final concentration of 0.1 or 2.0% (w/v). Strains SP1, SP2, and MS3 grew well at pH 9.5 in the presence of 0.1% (w/v) $MgSO_4.7H_2O$, or without additional Mg^{2+}. With 2.0% (w/v) $MgSO_4.7H_2O$ at pH 9.5, some inhibition of strains SP1, SP2, and MS3 occured, the cells of strain SP1 and MS3 became coccoid in morphology. All recognised species of Halobacterium showed good growth in media containing 2.0% (w/v) $MgSO_4.7H_2O$ at pH 7.5, but grew poorly at this pH with 0.1% (w/v) $MgSO_4.7H_2O$, and not at all without additional $MgSO_4.7H_2O$. Strains SP1, SP2, and MS3 grew well at pH 9.0-10.0, but not below pH 8.5, irrespective of the Mg^{2+} concentration. At pH 8.5 cells of strains SP1 and MS3 became elongated. All three strains survived inoculation into medium of pH 11.0, although the pH dropped to pH 10.0-9.5 as growth occured. The lack of growth at pH 7.5, irrespective of the Mg^{2+} concentration indicated that the requirement for high pH was not a consequence of the Mg^{2+} concentration. Growth of the known

species of Halobacterium was good in 2.0% (w/v) $MgSO_4.7H_2O$ at pH 7.5, but was poor and variable at pH 8.5, no growth occured at pH 9.5. It was not possible to separate the effects of high pH and Mg^{2+} concentration in this case, since high pH and high Mg^{2+} concentration are mutually incompatible. The concentration of Mg^{2+} present in a soluble form was determined by atomic absorption flame spectrophotometer. At pH 9.5 media to which $MgSO_4.7H_2O$ was added precipitated $MgCO_3$ in large quantities. The soluble Mg^{2+} concentration of media to which no $MgSO_4.7H_2O$ was added was found to be less than 0.05mM, while the concentrations in media containing 0.1 and 2.0% (w/v) $MgSO_4.7H_2O$ were found to be 0.1mM and 10.0mM respectively. The soluble Mg^{2+} optima of the strains SP1, SP2, and MS3 were between 0.1mM and 2.0mM, although growth was not significantly impared in media containing less than 0.05mM soluble Mg^{2+}. The formation of coccoid cells in many species of Halobacterium usually occurs under conditions of Mg^{2+} limitation (Brown & Gibbons, 1955), whereas in strains SP1 and MS3 the cells became elongated at low concentrations of Mg^{2+} at pH 8.5. The effects of Mg^{2+} concentration and pH appear to have a coupled effect. Colwell et al. (1979) reported that 75% of the strains similar to H.cutirubrum tested grew at pH 9.0, but only 13% of strains similar to H.salinarium grew at this pH. No reference was made to the method of controlling pH, nor was the effect of pH on the solubility of Mg^{2+} mentioned, and interpretation of this data requires caution. The organisms isolated from Lake Magadi seem, therefore, to either have a low requirement for Mg^{2+}, or they have a high affinity for it, which enables them to survive in an environment low in Mg^{2+}. During the course of early enrichments and isolation of the strains, in media containing 2.0% (w/v) $MgSO_4.7H_2O$, at pH 9.5, it was noticed that plates containing a precipitate of $MgCO_3$ were cleared

Figure 82. Clearing of plates by Lake Magadi isolates.

When plates were prepared, containing 2.0% (w/v) $MgSO_4.7H_2O$, a precipitate of $MgCO_3$ formed, which disappeared from the plates directly below the growing colonies of the Lake Magadi isolates of extremely halophilic bacteria.



of the precipitate below the growing colonies (figure 82), indicating that the organisms may have a high affinity for Mg^{2+}. The strains isolated from Lake Magadi, thus seem to differ from the reference strains of Halobacterium in their ability to grow at high pH, in media almost devoid of Mg^{2+}.

The optimal salinity for growth was investigated at 37°C and 26°C to establish whether the high salinity requirement was affected by the growth temperature. The optimal salinity was determined in media containing K_2CO_3 or Na_2CO_3 to adjust the pH to 9.5. Strain SP1 grew optimally in 4.0M NaCl at 37°C, and 4.5M at 26°C, growth did not occur significantly below 3.0M and 2.0M NaCl at 37°C and 26°C respectively (figure 83). Strains SP2 and MS3 grew optimally in 3.0M and 3.5M NaCl at 37°C and 26°C respectively (figures 84, 85). The minimum NaCl concentrations for growth were 1.5M for strain SP2 and 2.0M for strain MS3 at both growth temperatures. Although the effect of temperature on the salinity optima of halobacteria has been little studied, the work with halotolerant organisms and H.volcanii shows that the salinity requirement usually increases as the temperature increases (Matheron et al., 1976; Mullakhanbhai & Larsen, 1975; Novitsky & Kushner, 1975). The increase of the salinity optima by strains SP1, SP2, and MS3 with lowering temperature is the converse of these previous studies, but shows that the extremely halophilic nature of the organisms is not lost by lowering of the growth temperature. The organisms were able to grow in saturated saline solution (about 5.0M NaCl in the presence of Na_2CO_3). The salinity optima were not affected by the substitution of K_2CO_3 for Na_2CO_3. The high salinity optima of the organisms suggest further similarities to members of the genus Halobacterium.

The work of a number of authors have shown that there are some biochemical tests which are typical of the family Halobacteriaceae

Figure 83. NaCl optimum of strain SP1.

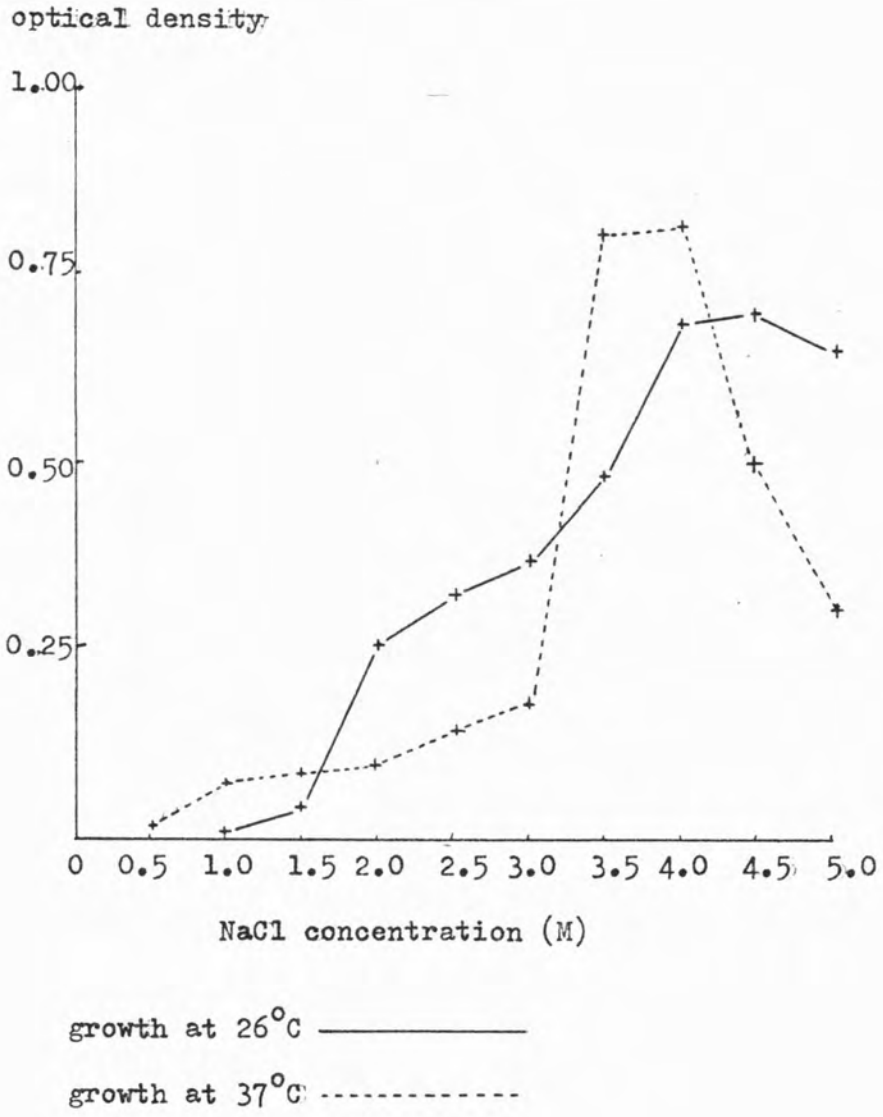
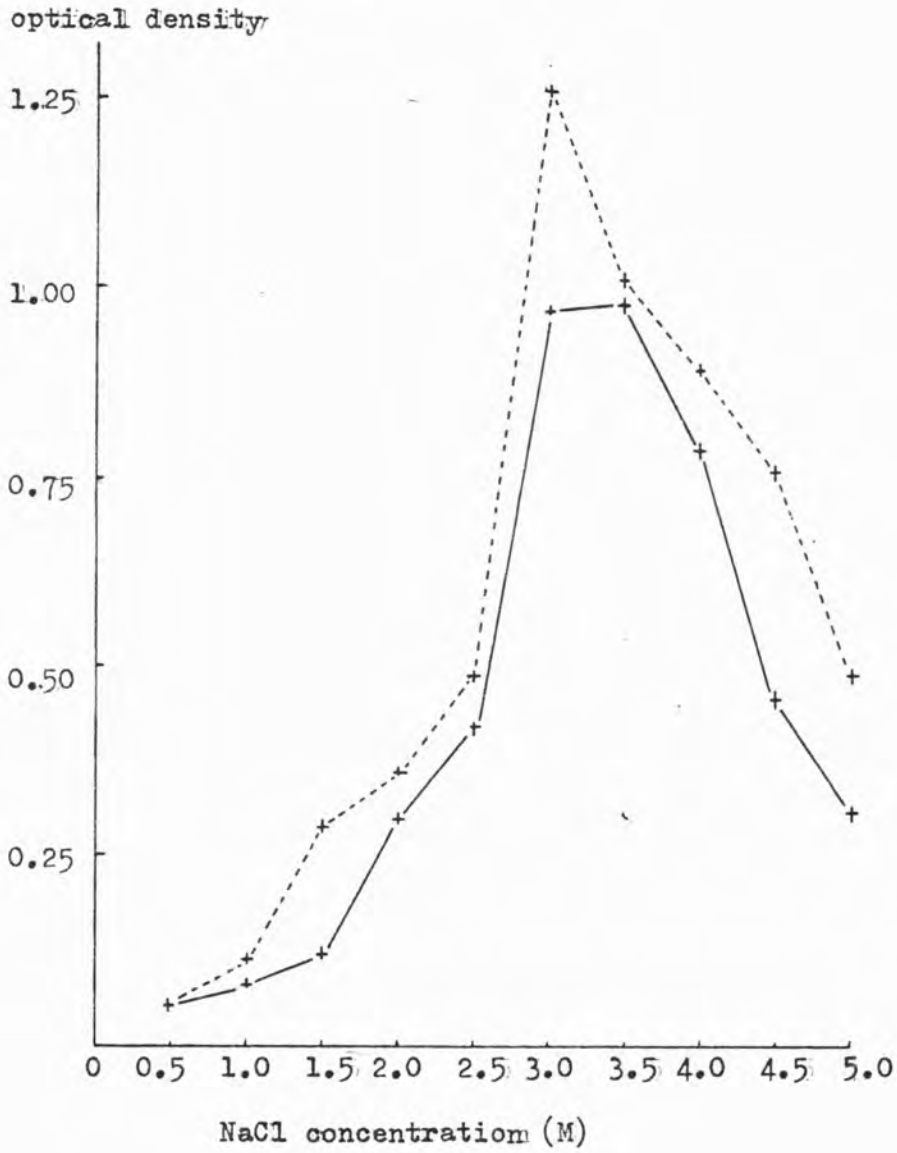


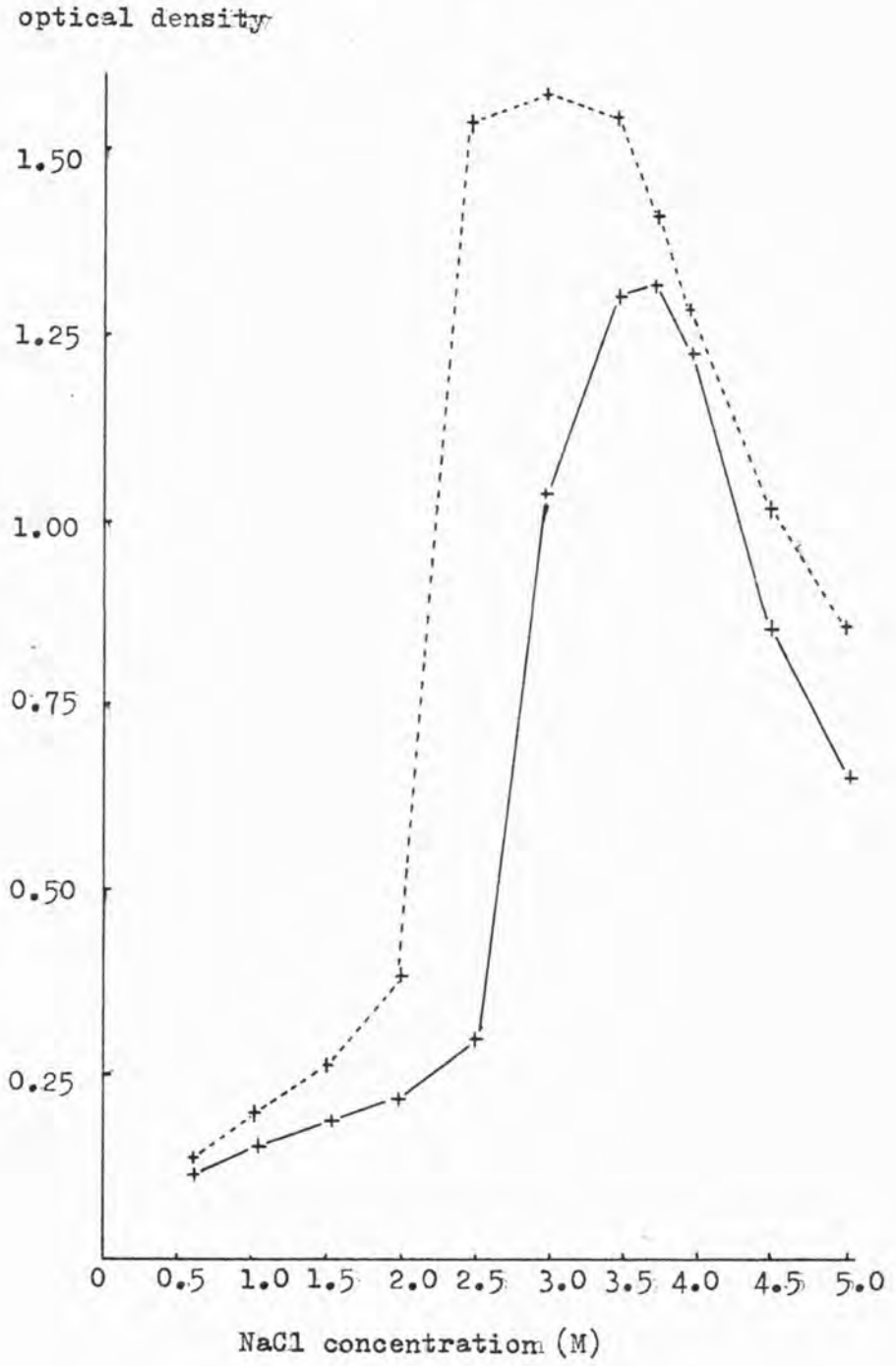
Figure 84. NaCl optimum of strain SP2.



growth at 26°C ————————

growth at 37°C ----------

Figure 85. NaCl optimum of strain MS3



growth at 26°C ————————

growth at 37°C - - - - - - - - - - - - -

(Colwell et al., 1979; Gibbons, 1957; 1974; Gonzalez et al., 1978; Kocur & Hodgkiss, 1973; Tomlinson & Hochstein, 1976). All tests were carried out at 4.3M NaCl to conform to the criteria of Gibbons (1957), and the results are presented in table 14. Gelatinase activity was detected in most of the Halobacterium sp. tested after 2-3 weeks. Strain SP2 and MS3 were also positive for gelatinase activity after 2 weeks, as were other strains similar to these type strains. Strain SP1 was negative for gelatinase activity after 4 weeks, after which time the controls containing Na_2CO_3 showed a positive reaction. Some strains similar to strain SP1 were positive after 4 weeks incubation, but not after 3 weeks, and this may also have been due to the alkaline nature of the medium. The controls at high pH never showed a positive gelatinase activity until after 3-4 weeks incubation. All strains of Halobacterium sp., strains SP1, SP2, and MS3 were amylase negative, but all strains produced sulphide from thiosulphate (table 14). Reduction of nitrate or nitrite was not detected in any of the strains tested, although the salinity interfered with the detection of nitrate. Gas was not seen to be produced from cultures containing either nitrate or nitrite. The detection of caseinase activity was not possible at high pH, due to the degradation of the casein under these conditions. The production of indole was detected from tryptone with Halobacterium sp., but this was not possible for strains SP1, SP2, and MS3, due to a false positive reaction for indole in the control at high pH.

Sensitivity to antibiotics was tested by the method of Colwell et al. (1979). All strains tested were sensitive to $HgCl_2$ and novobiocin, which are non-specific in their action, and were insensitive to penicillin G, ampicillin, streptomycin, tetracyclin, and polymixin B. Insensitivity to these antibiotics at these concentrations is typical, although some Halobacterium spp. have

Table 14. Characterisation of the extremely halophilic bacteria from Lake Magadi.

antibiotic sensitivity	strain		
	SPI	SP2	MS3
0/129	+	++	++
penicillin G	-	-	-
novobiocin	++	++	++
polymixin b	-	-	-
ampicillin	-	-	-
$HgCl_2$	++	++	++
tetracyclin	-	-	-
dehydrostreptomycin	-	-	-

(- insensitive; + some inhibition of growth; ++ total inhibition of growth).

	SP1	SP2	MS3
gelatinase	- 28 days	+ 14 days	+ 14 days
catalase	+	+	+
oxidase	+	+	+
reduction of NO_3^-	-	-	-
reduction of NO_2^-	-	-	-
starch hydrolysis	-	-	-
S^{2-} from thiosulphate	+	+	+
GC base content T_m (mol.%)	64.5	65.0	63.0
bouyant density	62.14/51.4	63.2	60.99/49.53
Glucosamine/mg protein	15µg	1.0µg	12µg

Figure 86. Sensitivity of extreme halophiles to antibiotics.

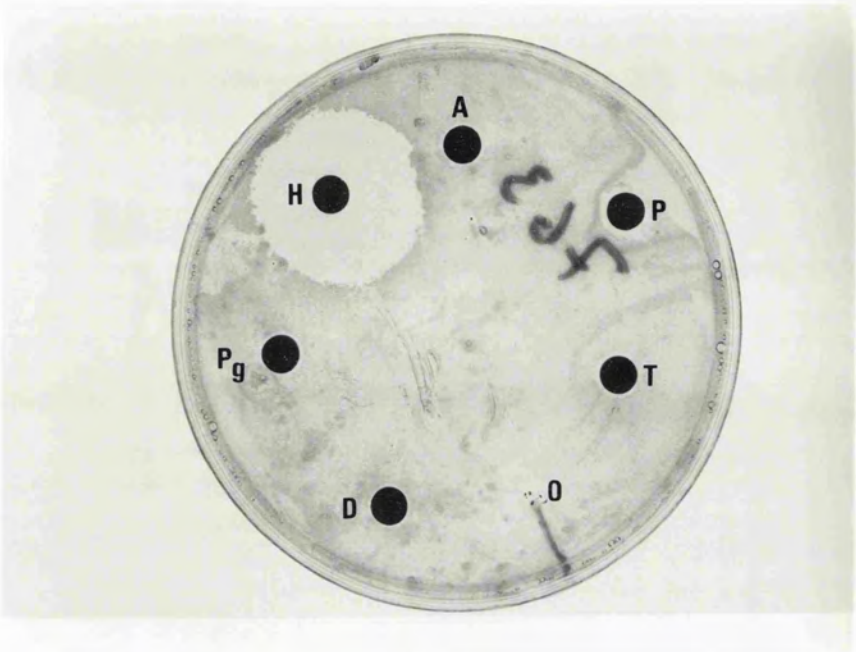
Sensitivity of strain SP1 and the reference strains of Halobacterium to O/129 and other antibiotics. These strains are insensitive to most antibiotics, including inhibitors of murein synthesis. The large zone of clearing is caused by $HgCl_2$, while O/129 only slightly reduces growth.

Key: A-ampicillin; D-dehydrostreptomycin; H-$HgCl_2$; O-O/129; P-polymixin b; Pg-penicillin G; T-tetracyclin

Figure 87. Sensitivity of extreme halophiles to antibiotics.

Sensitivity of strains SP2 and MS3 to O/129 and other antibiotics. These organisms are also insensitive to many antibiotics, but differ from strain SP1 and the type strains of Halobacterium in being sensitive to O/129, which causes extensive clearing of the plates. Sensitivity to $HgCl_2$ is obscured by the adjacent zone of clearing caused by O/129.

Key as figure 86.


A
H
P
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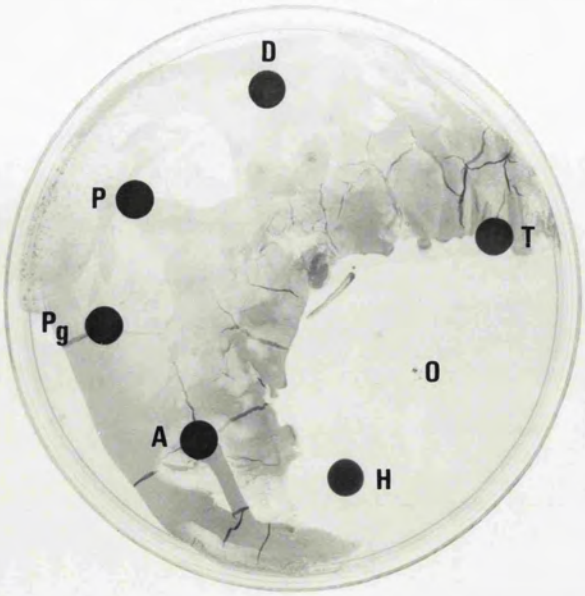

D
P
T
Pg
O
A
H

been reported as sensitive to polymixin B (Colwell et al., 1979; Gibbons, 1974; Mescher & Strominger, 1975). Most of the strains were also sensitive to the vibriostat 0/129, but this was evident at two levels of response. Strain SP1 and sensitive Halobacterium sp. were not initially clearly sensitive to 0/129, but after more than two weeks growth the strains showed sensitivity as a zone of poor growth, without a clear zone of total inhibition of growth (figure 86). Strains SP2 and MS3 in contrast, were semsitive to 0/129, which was evident after a few days growth, as a large zone of total inhibition of growth (figure 87). These differences in sensitivity to 0/129 also occur in other genera, such as Aeromonas and Vibrio, and the two levels of sensitivity may be related to the rate or mechanism of uptake (Colwell, personal communication). It was possible, therefore, to distinguish organisms similar to strains SP2 and MS3 from strain SP1 and similar organisms by the different 0/129 sensitivity.

Stimulation or inhibition of growth by the various carbon sources on solid media was not observed, but utilisation of carbon sources in liquid media showed clear differences between the three type strains SP1, SP2, and MS3. The results are presented graphically (figures 88, 89, 90). Strain SP1 did not appear to be capable of utilising any of the carbon sources provided, but ribose and glycerol appeared to inhibit growth. Similar results were also obtained with strain MS3, with the exception that glycerol did not inhibit growth, and acetate was stimulatory. Lactose and glycerol were capable of some stimulation of growth in strain MS3, but not to the same magnitude as acetate (figure 90). Gas was not detected in any of the cultures, and the final pH of the medium did not alter significantly after 10 days growth (table 15). Strain SP2 grown under the same conditions did not utilise lactose, but

Figure 88. Utilisation of carbon sources by strain SP1.

Graph no.

1. glucose, 0.1% ——— control ------
2. fructose, 0.1% ——— control --------
3. ribose, 0.1% ——— control --------
4. lactose, 0.1% ——— control ---------
5. mannitol, 0.1% ——— control --------
6. glycerol, 0.1% ——— control --------
7. sucrose, 0.1% ——— control -------
8. acetate, 0.1% ——— control --------

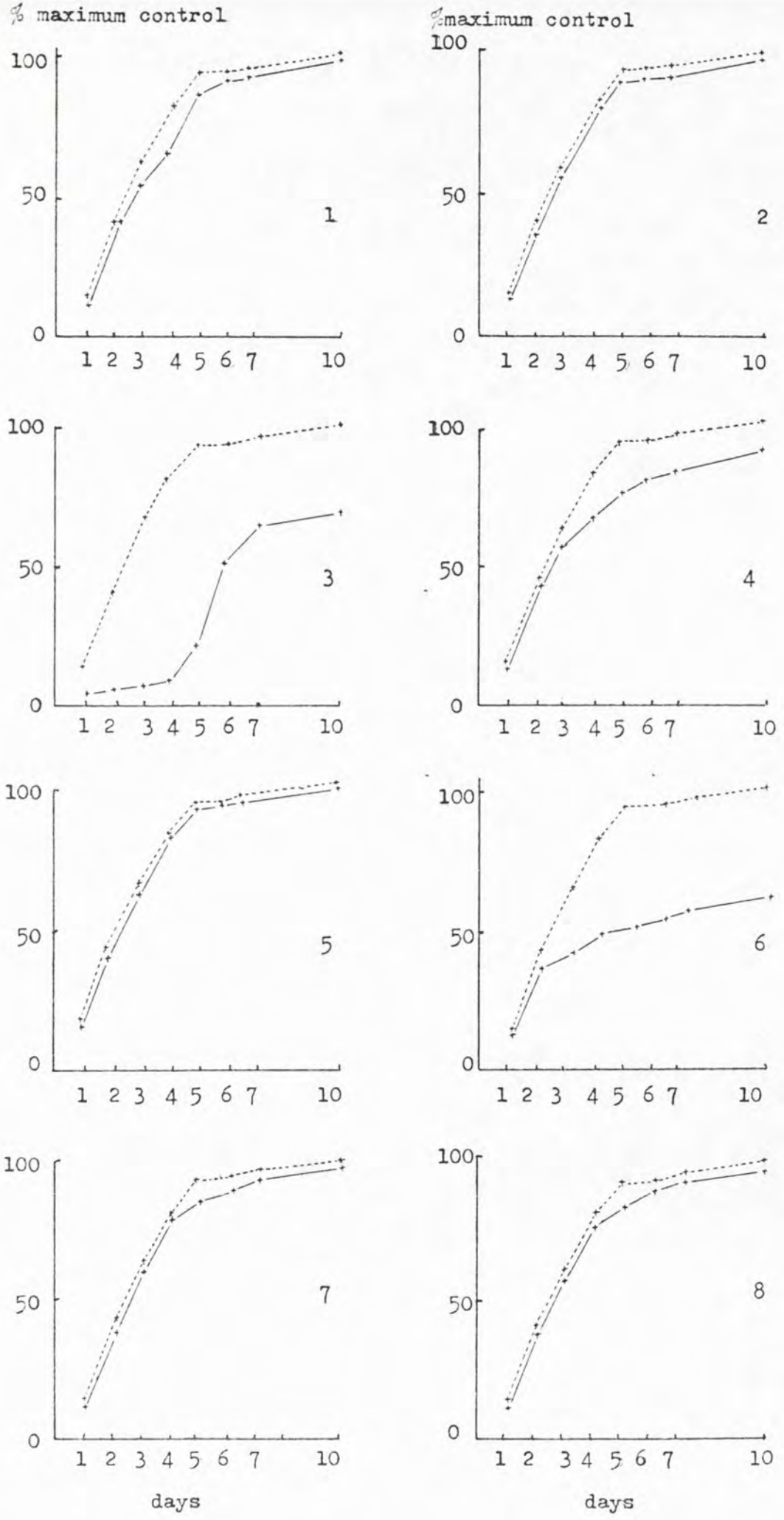

% maximum control
100
50
0
1
2
3
4
5
6
7
10
days

Figure 89. Utilisation of carbon sources by strain SP2.

Graph no.

1. glucose, 0.1% ——— control ------
2. fructose, 0.1% ——— control -------
3. ribose, 0.1% ——— control -------
4. lactose, 0.1% ——— control -------
5. mannitol, 0.1% ——— control -------
6. glycerol, 0.1% ——— control --------
7. sucrose, 0.1% ——— control -------
8. acetate, 0.1% ——— control -------

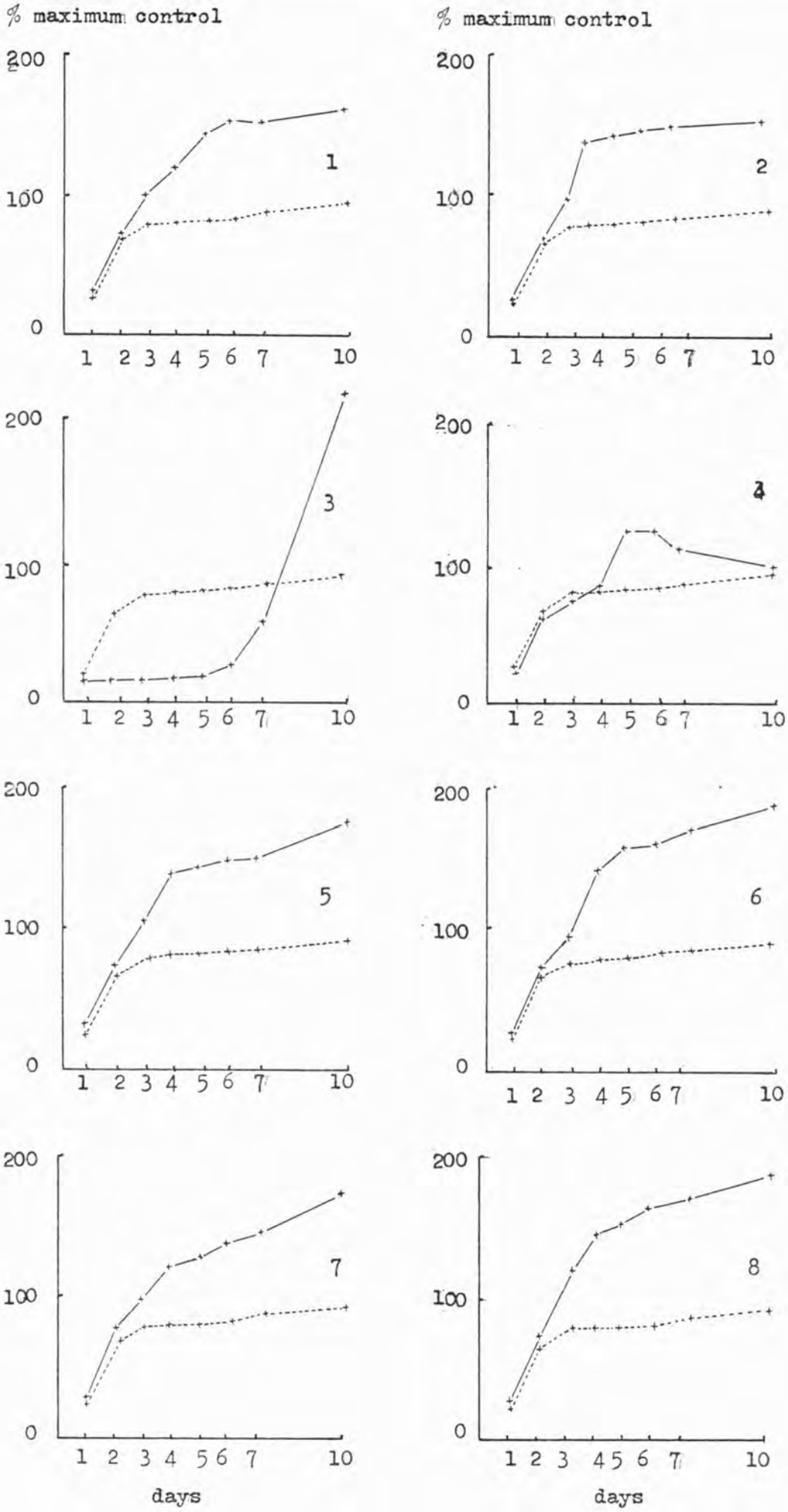

% maximum control
% maximum control
200
100
0
1
2
3
4
5
6
7
8
1 2 3 4 5 6 7 10
days
days

Figure 90. Utilisation of carbon sources by strain MS3.

Graph no.

1. ribose, 0.1% ——— control ------
2. fructose, 0.1% ——— control -------
3. glycerol, 0.1% ——— control -------
4. lactose, 0.1% ——— control -------
5. mannitol, 0.1% ——— control ------
6. glucose, 0.1% ——— control -------
7. sucrose, 0.1% ——— control -------
8. acetate, 0.1% ——— control -------

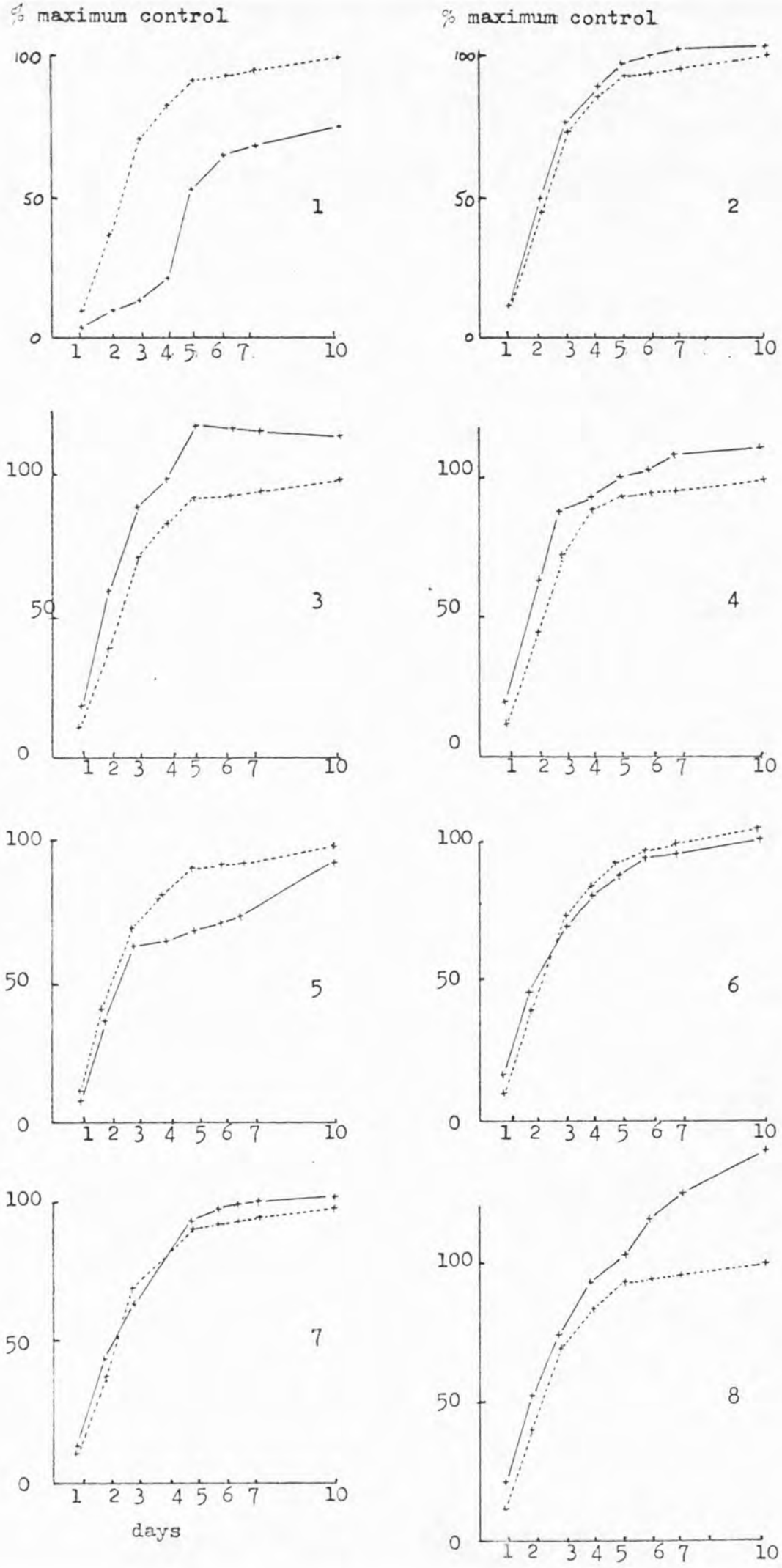

% maximum control
% maximum control
100
50
0
1
2
3
4
5
6
7
8
1 2 3 4 5 6 7 10
days

Table 15. Effect of growth of strains SP1, SP2, and MS3 on the pH of the medium.

Carbon compound	strain SP1		strain SP2		strain MS3	
	initial pH	final pH	initial pH	final pH	initial pH	final pH
glucose	9.5	8.9	9.5	8.8	9.5	8.9
fructose	9.5	9.0	9.5	8.7	9.5	9.0
ribose	9.5	8.9	9.5	8.9	9.5	8.8
lactose	9.5	9.0	9.5	8.8	9.5	9.0
mannitol	9.5	9.2	9.5	9.0	9.5	9.1
glycerol	9.5	9.0	9.5	9.1	9.5	9.1
sucrose	9.5	9.0	9.5	9.0	9.5	9.1
acetate	9.5	8.9	9.5	9.0	9.5	8.9
control	9.5	9.1	9.5	9.0	9.5	9.0

all other carbon sources significantly increased the optical density of the cultures, indicating that this strain was capable of utilising a number of carbon sources for growth (figure 89). Gas production was not detected and the pH of the medium did not drop significantly in cultures where the carbon source was utilised by the organism (table 15). The absence of gas may be attributed to the high solubility of carbon dioxide at high pH, which is the usual product of fermentative metabolism, and the acid produced may not have been sufficient to cause a drop in the pH in view of the Na_2CO_3 content of the medium. It has been assumed that halobacteria are either incapable of utilising carbon sources, or of utilising them only via a fermentative pathway (Gonzalez et al., 1978; Hochstein, 1978; Stoeckenius et al., 1979; Tomlinson & Hochstein, 1972; 1976). Recently Larsen (personal communication) has found that H.volcanii was capable of metabolising a number of sugars via an oxidative pathway. It is, therefore, possible that strain SP2 may not utilise the carbon sources via a fermentative pathway, and the possiblity of an oxidative pathway should be investigated further. The ability of various species to utilise carbon sources, such as sugars for growth has been used for the taxonomic differentiation of the members of the genus Halobacterium (Elazari-Volcani, 1957), and this criterion may be used in future in the taxonomy of the extremely halophilic bacteria (Dundas, 1978; Gonzalez et al., 1978; Tomlinson & Hochstein, 1976). Although sugars and glycerol do not significantly stimulate the growth of species such as H.cutirubrum, they have been shown to influence the formation of neutral lipids, including carotenoids and retinal (Gochnauer et al., 1972; Kushwaha & Kates, 1979a; 1979b).

The ability of the strains SP1, SP2, and MS3 to grow under anaerobic conditions was tested in agar stabs, and on plates incubated

in anaerobic jars. Although sparse growth of all three strains was observed on plates incubated in an anaerobic atmosphere, no growth was observed below the surface of the agar in stabs. Growth on plates was probably due to traces of oxygen in the plates or in the anaerobic atmosphere, indicating that the strains SP1, SP2, and MS3 would grow in the presence of low concentrations of oxygen, but not under anoxic conditions.

c. Isolation and characterisation of cell membranes.

Typically, halobacteria which are incapable of utilising sugars by fermentative pathways, produce a specialised membrane in the cell membrane, the purple membrane (Ginzburg, 1978; Stoeckenius, personal communication; Stoeckenius et al., 1979; Tomlinson & Hochstein, 1976) The purple membrane, which contains retinal and an opsin-like protein bound together as bacteriorhodopsin, allows the organism to obtain energy from sunlight under low oxygen concentrations. Although cells of H.halobium reportedly fix CO_2 under these conditions (Danon & Caplan, 1977), the mechanism remains to be fully elucidated (Kerscher & Oesterhelt, 1977; Lanyi, 1978). The remainder of the cell membrane consist of a red membrane, which contains the carotenoids. The two membrane fractions may be separated from one another by density gradient centrifugation (Becher & Cassim, 1975; Oesterhelt & Stoeckenius, 1971).

The presence of retinal and purple membrane was investigated by growth of the cultures under conditions known to stimulate production of the purple membrane (Kushwaha et al., 1975a; 1976) in Halobacterium spp. H.halobium strain R_1 has a deficient bacterioruberin synthesis, and was used as a source of purple membrane. Purple membrane was also isolated from cells of H.halobium (NCMB 736) and H.cutirubrum by density gradient centrifugation by the proceedures of Oesterhelt and Stoeckenius (1974) and Kushwaha

et al. (1975a; 1976) as described in Materials and Methods (3.9d), using sucrose density gradient centrifugation. Samples of purple membrane obtained by this method were then used to test the suitability of Percoll in the isolation of purple membrane. Initial tests showed that the purple membrane formed a discrete band in Percoll gradients under suitable conditions. Subsequently it was shown that the purple and red membranes from H.halobium and H.cutirubrum could be isolated using Percoll. Purple and red membrane fractions isolated from cells lysed and centrifuged in Percoll banded at a density of 1.045g/l and 1.020g/l respectively, somewhat lower than those values obtained in sucrose, of 1.18g/l and 1.16g/l for the purple and red membranes respectively. Purple membrane isolated in sucrose and subsequently centrifuged in Percoll also banded at a density of 1.045g/l, indicating that the purple membrane was unaffected by treatment with sucrose (figure 91). Presumably the purple and red membranes are in a dehydrated state in sucrose, due to its osmotic effect, whereas in Percoll dehydration apparently does not occur, which results in the lower densities of the membranes. SDS polyacrylamide gel electrophoresis of the purple membranes isolated using Percoll showed that the purity of this fraction was comparable to that obtained using sucrose. In pure purple membranes SDS polyacrylamide gel electrophoresis showed a single protein band of molecular weight 26,000d, typical of the opsin-like protein found in bacteriorhodopsin (Becher & Cassim, 1975; Kushwaha et al., 1975a; 1976; Oesterhelt & Stoeckenius, 1974). Contamination of the purple membrane with red membranes was found to occur when large amonuts of red membrane were synthesised by the organism. SDS polyacrylamide gel electrophoresis revealed the presence of red membranes by the presence of 12-20 additional bands. The degree of red membrane contamination could be determined in the purple

Figure 91. Density gradient centrifugation of the purple membrane.

The purple membrane from Halobacterium halobium strain R_1 isolated using Percoll density gradients. The gradient has been overloaded in order to show the banding of the purple membrane in this density gradient medium. Adjusting the conditions under which the gradients are run allows both the purple and red membranes of the halobacteria to be isolated.

membrane more easily by measuring the absorption maxima between 400 and 600nm (figure 92, 93, 94). Strain R_1 was found to be the most suitable for the isolation of purple membrane as found previously (Stoeckenius, 1978). Purple membrane was not isolated from H.trapanicum. The advantages of Percoll were the relatively short time to generate the gradient at fairly low speeds (20,000g for 30min). Using the proceedures worked out for the isolation of purple membrane and red membrane from known Halobacterium sp., the membrane content of the strains SP1, SP2, and MS3 was investigated using Percoll gradients. A single membrane fraction was obtained from strain SP2, which was red-orange in colour. The density of the membrane was 1.020g/l in Percoll, and had an absorption spectrum typical of the red membrane (figure 93), with maxima at 475, 505, and 545nm (Oesterhelt & Stoeckenius, 1974). No purple membrane could be detected in the density gradient by spectroscopy. Strains MS3 and SP1 yielded two membrane fractions, which banded at densities of 1.020g/l and 1.040g/l, in Percoll. The lighter membrane fraction was red-orange in colour, and had an absorption spectrum typical of the red membrane of halobacteria, with maxima at 475, 505, and 545nm, and was almost identical to that obtained for the single membrane fraction from strain SP2 (figure 93). The heavier membrane fraction, in both cases, always contained substantial amounts of red membrane, as indicated by the reddish colour of the band, and by its absorption spectrum (figure 94), but was of a purplish hue. Continuous spectra of the heavy membrane fraction showed a very slight shoulder about 600nm, but not of sufficient magnitude to confirm the presence of purple membrane. The shoulder could be resolved into a peak by difference spectroscopy, using red membrane as a reference, thus elliminating it from the heavy membrane absorption spectrum (figure 92). The difference spectrum obtained indicated that the

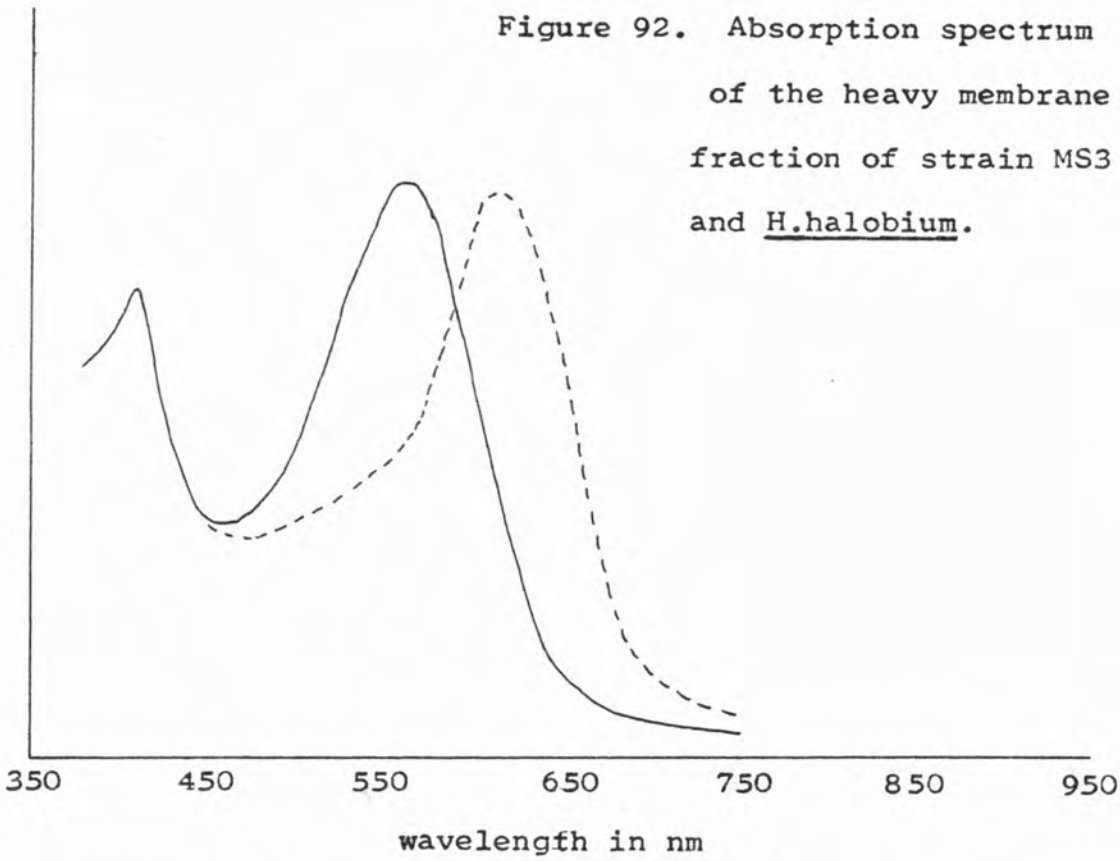


Figure 92. Absorption spectrum of the heavy membrane fraction of strain MS3 and H.halobium.

--------- strain MS3

———— strain R_1, H.halobium purple membrane

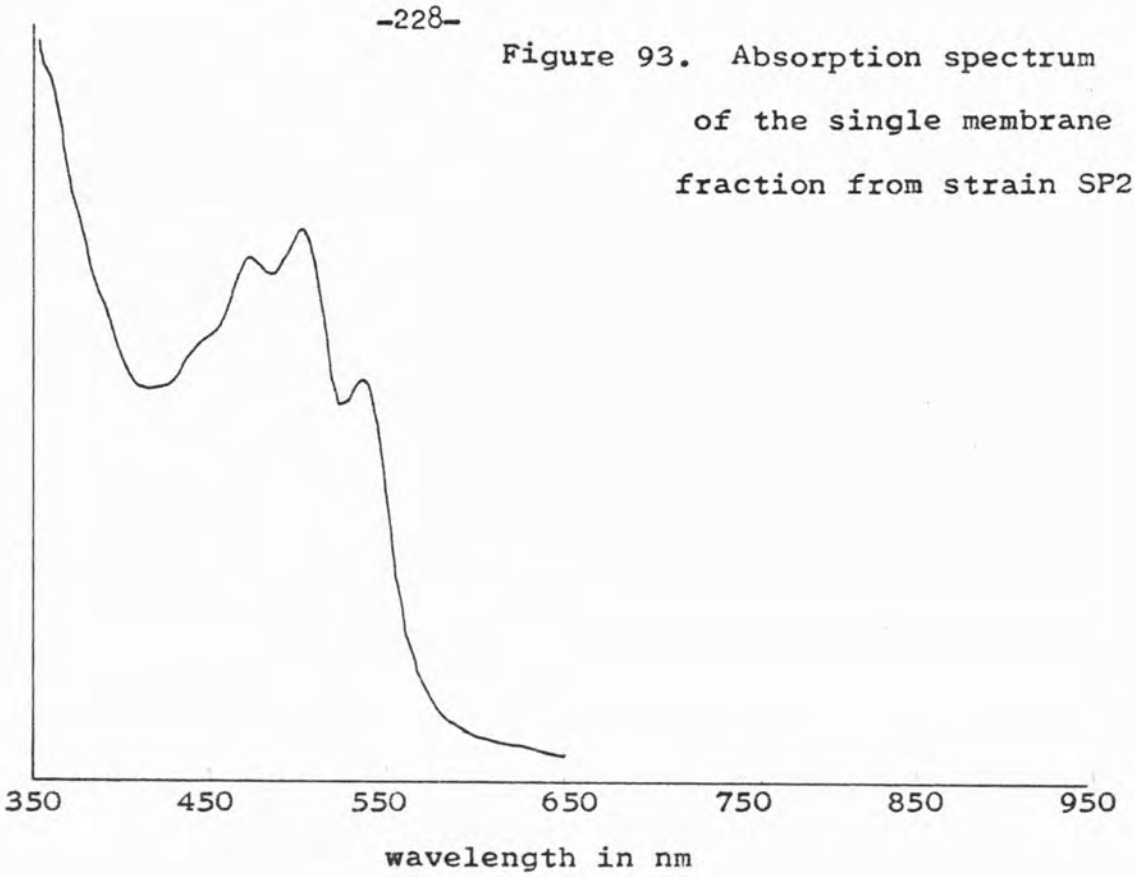


Figure 93. Absorption spectrum of the single membrane fraction from strain SP2

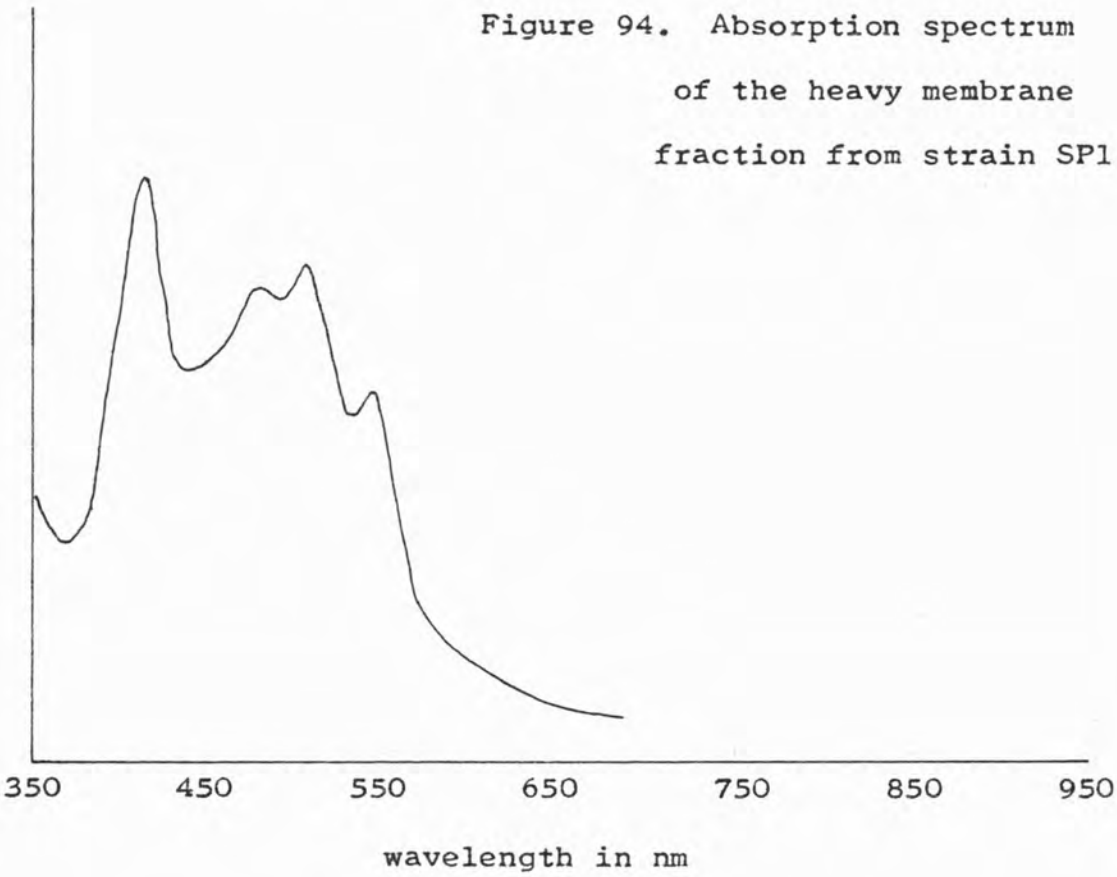


Figure 94. Absorption spectrum of the heavy membrane fraction from strain SP1

peak absorbance was at 625nm, rather than the typical 570nm peak found in the purple membrane (Kushwaha et al., 1975a; 1976; Oesterhelt & Stoeckenius, 1974; 1971). A second absorption maxima was also visible at 400nm in the difference spectrum, and in the absorption spectrum of the heavy membrane fraction, and is also found in preparations of purple membrane (figure 92, 94) (Oesterhelt & Stoeckenius, 1974). Experiments with isolated purple membrane have produced a number of spectral forms, usually under extreme conditions, such as low temperature, high or low pH, with absorption maxima ranging from 400 to 640nm (Stoeckenius et al., 1979), and the maximum at 625nm in the heavy membrane fractions of strains SP1 and MS3 seen here may be a reflection of the high pH experienced by the organisms. Attempts to isolate retinal from cells of strains SP1, SP2, and MS3 by the proceedures of Oesterhelt et al. (1973), or from membrane fractions were not successful, although retinal could be detected in cells and membrane fractions of Halobacterium sp. which contained purple membrane. The results indicate that a membrane system similar to, but not identical to the purple membrane found in Halobacterium sp. may exist in strains SP1 and MS3. The absence of purple membrane-like fractions from strain SP2 is not unusual, since so far purple membrane has not been isolated from any of the halobacteria which are capable of utilising sugars for growth (Ginzburg, 1978; Stoeckenius, personal communication; Stoeckenius et al., 1979; Tomlinson & Hochstein, 1976; Larsen, personal communication; Weber & Mevarech, 1978a).

d. Cell wall analysis.

To determine whether the organisms were similar to members of the archaebacterial Halobacteriaceae, the nature of the cell wall was investigated by chemical analysis. Insensitivity to antibiotics which interfer with peptidoglycan synthesis has been taken to

indicate an atypical cell wall (Jones J et al., 1977; Mescher & Strominger, 1975; 1978). The major components lacking in the cell wall of the halobacteria are the sugar amines of the peptidoglycan murein (Larsen, 1967; Mullakhanbhai & Larsen, 1975). Although their presence is not necessarily indicative of the presence of murein, their absence indicates that there is no murein in the cell wall. The analyses of the cell walls of strains SP1, SP2, and MS3, by the method of Mullakhanbhai and Larsen (1975), showed that no muramic acid was present, but there were traces of glucosamine (table 14). In strains SP1 and MS3 the concentration of glucosamine present in the cell wall was comparable to the concentrations of 10μg glucosamine/mg protein found in H.halobium, H.cutirubrum, and H.salinarium (Bayley & Morton, 1978), but in strain SP2 the concentration of glucosamine was much lower, and comparable to the value of ·6μg glucosamine/mg protein reported for H.volcanii (table 14) (Mullakhanbhai & Larsen, 1975). These differences in the cell wall composition between the strains SP1, SP2, and MS3 were also reflected in the SDS polacrylamide gel electrophoresis of the cell envelopes. Strains SP1 and MS3 contained a carbohydrate positive band of molecular weight similar to that found in H.salinarium, and was probably a glycoprotein (figure 95). Strain SP2 did not have a carbohydrate positive band, and the major protein in the cell wall appeared to be that having a molecular weight of 50,000d (figure 95). Strains SP1 and MS3 were similar to one another in their protein content, and also resembled the protein content of H.salinarium. The differences between H.salinarium and strains SP1 and MS3 may be partly explained by the high pH at which the isolates from Lake Magadi grow, since alkaline pH has been shown to have an effect on the protein content of a Bacillus sp. (Koyama & Nosoh, 1976). In the Bacillus sp. examined (Koyama & Nosoh, 1976) alkaline pH had the

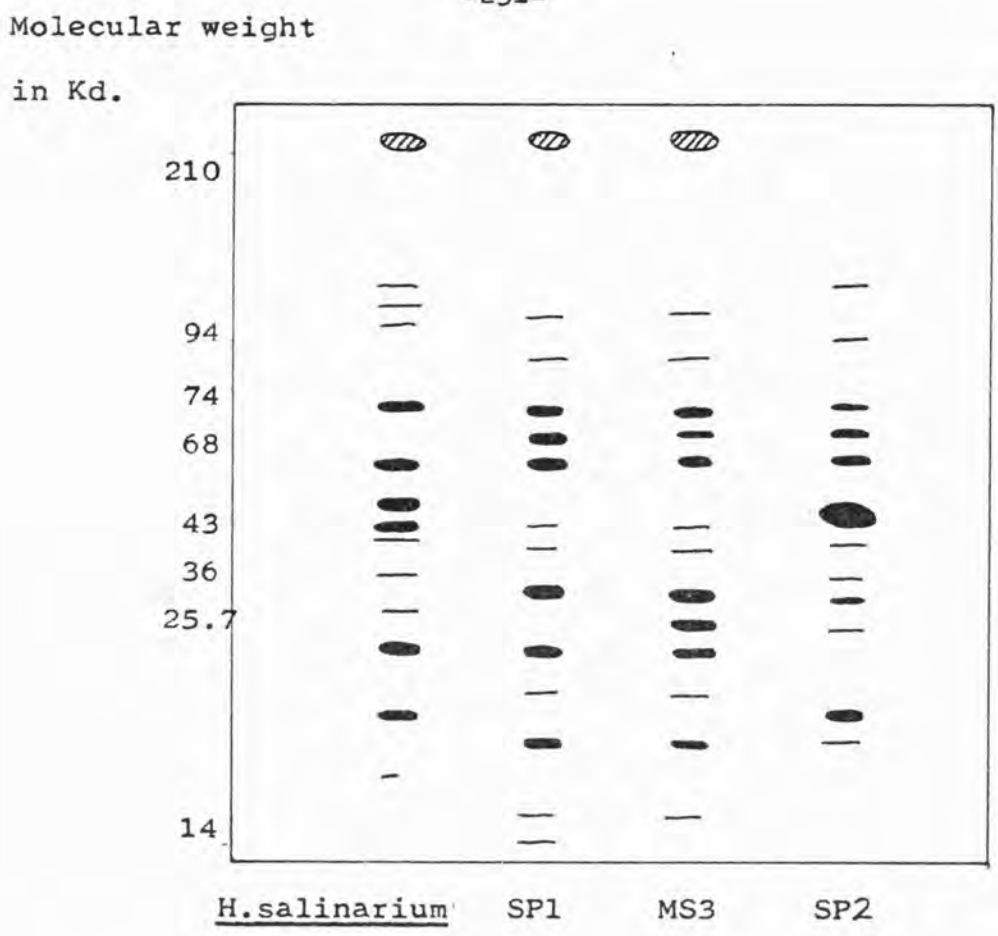


Figure 95. SDS polyacrylamide gel electrophoresis of the envelope protein from extremely halophilic bacteria.

The envelope protein from H.salinarium, and strains SP1, SP2, and MS3 were run in the same gels and stained for protein with Coomassie Brilliant Blue , and for carbohydrate, using periodate-Schiff stain . Strains SP1, MS3, and H.salinarium contained a glycoprotein of similar molecular weight to that reported previously (Mescher & Strominger, 1978) in halobacteria. The strain SP2 was unusual in that it did not contain a carbohydrate positive band, and the major protein present appeared to be that with a molecular weight of 50,000d

effect of increasing the number of low molecular weight proteins, an effect also observed in the strains SP1 and MS3 (figure 95). The absence of a carbohydrate positive band in strain SP2 may indicate that there is no glycoprotein in the cell wall of this strain. The molecular weight of the major protein band is, however, close to that of 5 ,000d reported for the large molecular weight glycopeptide, which is a subunit of the glycoprotein in H.salinarium (Mescher & Strominger, 1976a; 1976b; 1978). The absence of glycoprotein in the cell wall of strain SP2 may also be reflected in the cells being extremely fragile to mechanical disruption, and their sensitivity to heat and the lowering of the pH, both of which caused the cells to become spherical. The spherical nature of the cells on agar plates may be due to surface tension effects on the fragile cell wall, although this seems unlikely as this may also be expected to occur in liquid cultures. A glycoprotein has not been observed in H.volcanii (Mullakhanbhai & Larsen, 1975), which is also an extremely fragile organism, and contains glucosamine at a concentration comparable to that found in strain SP2.

Analysis of the bulk protein, when compared with the analysis of the bulk protein of H.halobium shows that the values for all strains were similar, with an excess acidic amino acid composition (table 16). The values for the strains SP1, SP2, and MS3 excess amino acid are all slightly greater than that recorded for H.halobium (table 16). The major differences are in the relative concentrations of aspartic acid, glutamic acid, and valine. The values for aspartic acid are lower in H.halobium than in the three isolated strains, whereas the values for glutamic acid are similar. In H.halobium there is an excess of glutamic acid over aspartic acid, as there is in strain SP1, but in strains SP2 and MS3 there is an excess of aspartic acid over glutamic acid (table 16). The greatest excess

Table 16 The amino acid composition of the bulk protein of strains SP1, SP2, and MS3, and Halobacterium halobium.

amino acid	Mol % content of amino acid			
	SP1	SP2	MS3	H.halobium
aspartic	14.06	16.01	14.69	10.69
threonine	5.86	6.65	6.32	8.07
serine	4.69	4.87	4.91	4.82
glutamic	15.76	13.86	13.66	14.88
glycine	8.72	7.94	9.13	8.07
alanine	8.33	7.70	9.59	9.85
valine	10.16	7.86	7.12	9.54
methionine	1.95	2.62	2.11	2.41
iso-leucine	4.56	5.24	5.02	4.09
leucine	7.03	8.99	9.10	7.65
tyrosine	2.86	3.96	3.50	2.52
phenylalanine	4.30	3.96	4.55	2.41
histidine	2.60	2.38	2.78	2.73
lysine	2.73	2.70	2.31	2.52
arginine	6.38	5.08	4.21	5.35
total acidic	29.82	29.87	28.35	26.84
total basic	11.71	10.16	10.30	11.11

in aspatic acid over glutamic acid was in strain SP2 and similar organisms. Strains SP1 and MS3 show greatest similarity between the values for aspartic acid, whereas strains SP2 and MS3 show greatest similarity between the glutamic acid values (table 16). The significance of these values is unclear, since differences between the strains are indicated, but values previously reported for members of the genus Halobacterium vary with the same species, although differences between species were also evident (Brown, 1963; Mullakhanbhai & Larsen, 1975; Kushner & Onishi, 1966; Reistad, 1970; Steensland & Larsen, 1969). The general pattern is characteristic of members of the genus Halobacterium.

e. Lipid analyses.

Analysis of the lipid content of strains SP1, SP2, and MS3 were carried out with H.salinarium as a standard. As indicated by the absorption spectrum of whole cells, bacterioruberins were the major carotenoids present. The bacterioruberins were identified by the chromatographic method of Kushwaha et al. (1975b). The bacterioruberins present in H.salinarium were the same as those found in H.cutirubrum, and were identified as isomers of bacterioruberin (R_1 and R_2), mono-anhydrobacterioruberin (R_3 and R_4), bisanhydro-bacterioruberin (R_5), and an unidentified carotenoid (R_6) (Kushwaha & Kates, 1979a; 1979b; Kushwaha et al., 1976). Strains SP1 and SP2 contained the carotenoids R_1 to R_6 inclusive, and were similar to H.salinarium in their bacterioruberin content, but in strain MS3 bacterioruberins R_1 and R_2 were the only identifiable bacterioruberins present. A yellow carotenoid was present with an Rf similar to R_6 in all strains, and was probably β-carotene or lycopene. The cultural conditions may affect the synthesis of the carotenoids (Gochnauer et al., 1972; Kushwaha & Kates, 1979a; 1979b), thus differences between strains, although consistent, may reflect

different tolerances of the growth conditions, or since strain MS3 appears to synthesise bacterioruberins at a later stage of growth than strains SP1 and SP2, these differences may be due to the rate of production of carotenoids. Mutants have also been isolated from the bacterioruberin producing species H.halobium and H.salinarium which are deficient in carotenoid synthesis (Dundas & Larsen, 1962; Stoeckenius, 1978), and differences in bacterioruberin synthesis alone are not sufficient to justify the designation of separate species.

Squalenes were present in all of the strains, including H.salinarium, and depending upon the cultural conditions, squalene, dihydrosqualene, and tetrahydrosqualene could be identified in all of the strains. Squalenes are thought to be involved in the stability of the cell membrane, and may functionally replace sterol found in eukaryotes, which serves a similar purpose (Lanyi & Silverman, 1972; Ourisson et al., 1979; Rohmer et al., 1979). The degree of unsaturation of the squalene molecule does not appear to affect the stability of the cell membrane, and the fully saturated molecule, squalane may replace squalene in the cell membrane of some members of the methanogenic bacteria (Tornabene et al., 1979). In H.cutirubrum the degree of aeration of the culture affects the relative compostion of the molecules squalene, dihydrosqualene, and tetrahydrosqualene (Tornabene, 1979), and it seems likely that variations between different cultures of the same strain of the isolated organisms are due to this effect.

Menaquinones were identified in the neutral lipid fraction of all the strains by thin layer chromatography (Collins et al.,1980) as MK-8, with variable amounts of either MK-9 or MK-8H_2. Mass spectroscopy confirmed the identity of MK-8, and established that the only other menaquinone present was MK-8H_2 (Collins, personal communication). Although Tornabene et al. (1969) and Kushwaha et al.

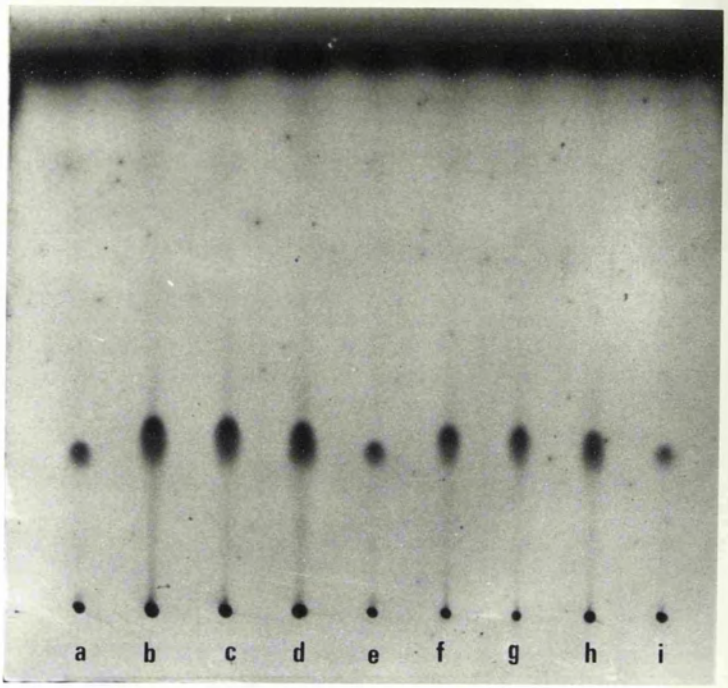
(1974) did not identify menaquinones other than MK-8, they recognised that others were probably present, and MK-8 and MK-$8H_2$ were subsequently found in H.volcanii (Mullakhanbhai & Larsen, 1975), and both appear to be present in variable amounts in all halobacteria (Collins et al., submitted). The variable amounts of the two menaquinones may be related to the cultural conditions in the same way that lowering the rate of aeration of a culture favours the hydrogenation of unsaturated isoprenoids, like squalene (Tornabene, 1979). Thus, hydrogenation of MK-8 may result in the formation of MK-$8H_2$ at concentrations dependant upon the degree of aeration of the culture.

Polar phospholipids and glycolipids of H.salinarium were identified by chromatography in the solvent systems described by Kates (1978), and by spray reagents. The polar lipid composition of the three strains and H.salinarium were then compared in the four solvent systems described (Materials and Methods, 3.5b). The total polar lipid composition of H.salinarium was similar to that described by Kates et al. (1966). The lipids were shown to be ether linked by the release of only minor amounts of fatty acids on acid methanolysis, and by chromatographic behaviour of the major product (figure 96). The presence of an absorption maximum at 1100-1110nm, in the infra-red, indicated the presence of typical C-O-C ether bonds. Acid methanolysis of cells of strains SP1, SP2, and MS3 also released only minor amounts of fatty acids, and the major product, which had an Rf similar to that of the ether lipid from H.salinarium (figure 96), and was also shown to contain ether bonds by infra-red spectroscopy. The minor differences between the Rf values of the ether lipid products of acid methanolysis of strains SP1, SP2, MS3, and H.salinarium may be indicative of structural changes in the lipids of the isolates. The polar lipids of

Figure 96. Products of acid methanolysis of cells of Halobacterium salinarium and the strains SP1, SP2, and MS3.

The products of acid methanolysis, obtained by the method of Minnikin et al.,(1975), were chromatographed in the solvent hexane:diethyl ether (85:15). The products were identified as: 1. traces of fatty acids; 2. ether lipid; 3. diether lipid, 2,3-di-O-phytanyl glycerol.

The strains are: a,e, and i. H. salinarium; b and f. strain SP1; c and g. strain SP2; d and h. strain MS3.



a,e,i. H.salinarium

b,f. strain SP1

c,g. strain SP2

d,h. strain MS3

H.salinarium were identified as derivatives of 2,3-di-O-phytanyl glycerol. The main phospholipids were ether analogues of phosphatidyl glycerol, phosphatidyl glycerophosphate, and phosphatidyl glycerosulphate (figures 97, 98, 16). Three glycolipids were also present, which were probably glycolipid derivatives of 2,3-di-O-phytanyl glycerol isolated previously, identified as a glycolipid sulphate, triglycosyl diether, and a glycolipid (figures 97, 98) (Kates, 1978; Kates et al., 1966; Kushwaha et al., 1976).

The polar lipids of strains SP1, SP2, and MS3 showed some distinct differences in composition compared to H.salinarium. The major lipids were all phospholipids, and there were no traces of glycolipids (figures 97, 98). The lipid composition of the three strains were, therefore, easily distinguishable from that of H.salinarium (figures 97, 98), and other members of the Halobacteriaceae examined (Kates et al., 1966). The major phospholipids found in the strains SP1, SP2, and MS3 were identical, and some of them had Rf values similar to the phospholipids found in H.salinarium (figures 97, 98). The major phospholipids found in the three strains had an Rf similar to that of the diether analogue of phosphatidyl glycerophosphate, and showed staining properties similar to this phospholipid, suggesting that it was structurally analogous to this lipid in some of its functional side groups. Two other phospholipids were present with Rf values close to that of the diether analogue of phosphatidyl glycerol (figures 97, 98), and were difficult to distinguish from one another in periodate-Schiff stain, and it is possible that the possession of the same functional side groups to this phospholipid means that both are structurally analogous to the diether analogue of phosphatidyl glycerol. The fourth phospholipid could not be identified by any specific spray reagent, other than the modified Zindzadze reagent of Vaskovsky and Kostetsky (1968),

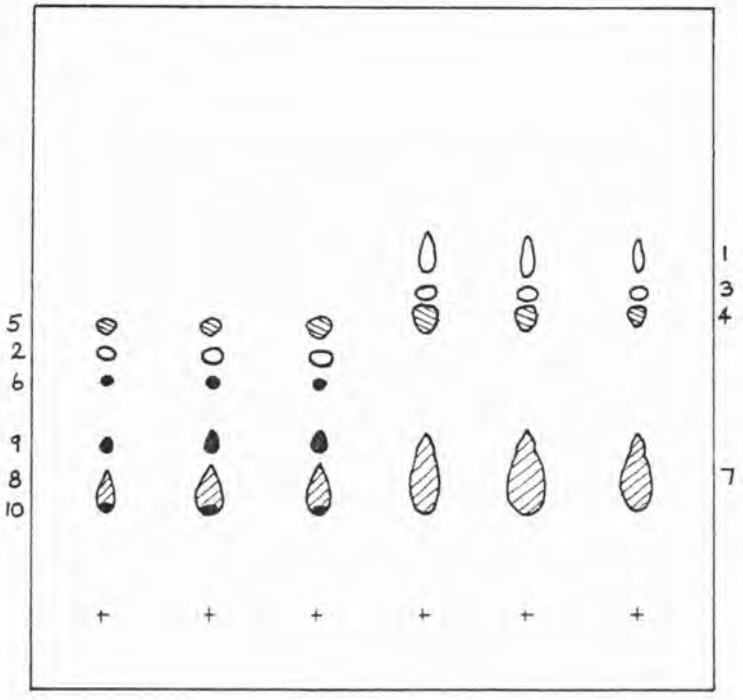
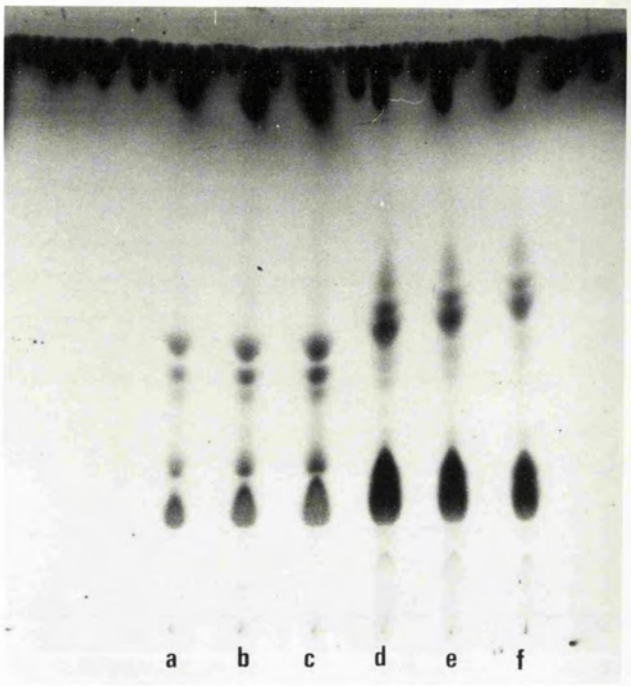


Figure 97. Thin layer chromatograph of the polar lipids of extremely halophilic bacteria.

Chromatograph developed in the solvent chloroform: methanol:water (65:25:4). The staining reactions are represented by: ○ phosphate positive; ⊘ periodate-Schiff positive immediately; ⊘ periodate-Schiff positive after 24h; ● glycolipids.

The lipids were identified as: 1. phospholipid ether; 2. phosphatidyl glycerosulphate diether; 3. phospholipid ether, possibly periodate-Schiff positive; 4. periodate-Schiff positive phospholipid ether; 5. phosphatidyl glycerol diether; 6. glycolipid; 7. slow periodate-Schiff positive phospholipid; 8. phosphatidyl glycerophosphate diether; 9. glycolipid, possibly triglycosyl diether; 10. glycolipid, possibly glycolipid sulphate.



a,b,c. H.salinarium

d. strain SP1

e. strain SP2

f. strain MS3

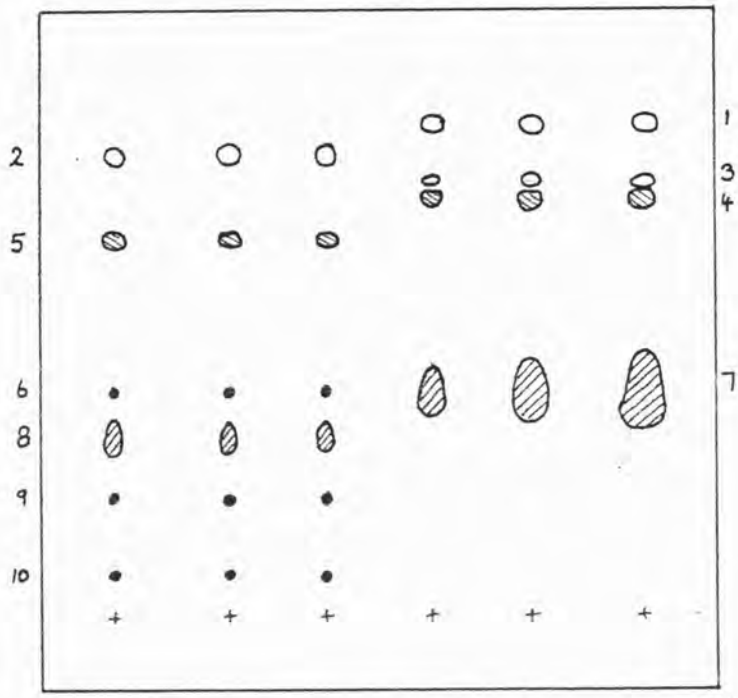
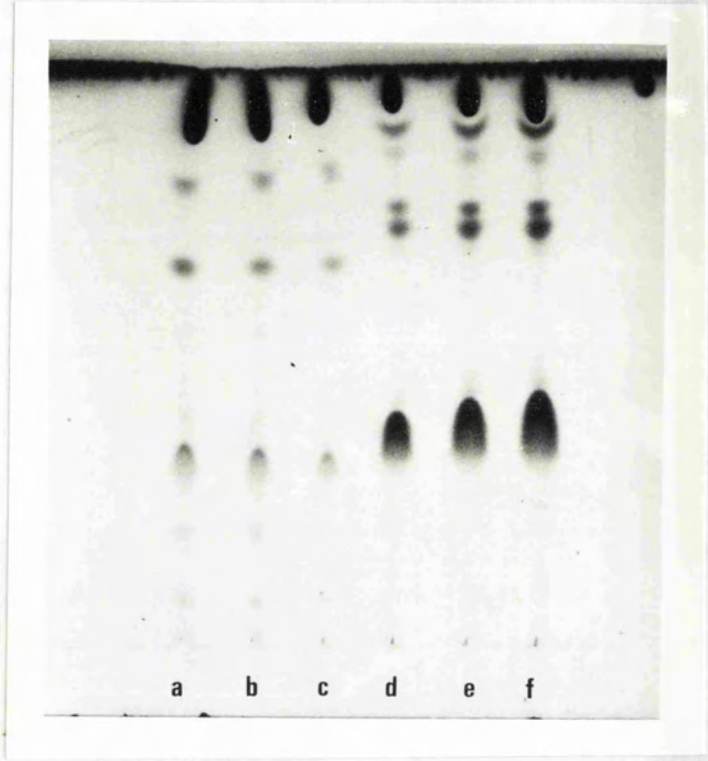


Figure 98. Thin layer chromatograph of the polar lipids of extremely halophilic bacteria.

Chromatograph developed in the solvent chloroform: methanol:acetic acid:water (80:12:15:4). The staining reactions are represented by: ○ phosphate positive; ◍ periodate-Schiff positive immediately; ⊘ periodate-Schiff positive after 24h; ● glycolipids.

The lipids were identified as: 1. phospholipid ether; 2. phosphatidyl glycerosulphate diether; 3. phospholipid diether; possibly periodate-Schiff positive; 4. periodate-Schiff positive phospholipid ether; 5. phosphatidyl glycerol diether; 6. glycolipid; 7. slow periodate-Schiff positive phospholipid ether; 8. phosphatidyl glycerophosphate diether; 9. glycolipid, possibly triglycosyl diether; 10. glycolipid, possibly glycolipid sulphate.



a,b,c. H.salinarium

d. strain SP1

e. strain SP2

f. strain MS3

and remains to be investigated further. The differences in the Rf values of the phospholipids found in the strains SP1, SP2, and MS3, and H.salinarium may be attributed to either differences in the ether lipids from which they are derived, or to changes in the structure of the functional side groups. This does not, however, preclude that both such changes may occur.

The present data suggests that the strains SP1, SP2, and MS3 differ from typical members of the family Halobacteriaceae, in that although they contain ether lipids, and show many physiological properties similar to the halobacteria, the polar lipid composition of the three strains are distinctive from that of typical halobacteria. Whatever the nature of the ether lipids in strains SP1, SP2, and MS3, the differences from H.salinarium suggest that these organisms may constitute a distinct taxonomic group of the halobacteria.

f. GC base content.

The GC base content of the DNA of the strains SP1, SP2, and MS3 was determined by thermal denaturation and buoyant density, using standards of Micrococcus lysodiekticus and Escherichia coli strain K_{12} DNA. Melting point determination showed that the values were lower than those previously reported for members of the family Halobacteriaceae, with the exception of H.volcanii (table 17) (Joshi et al., 1962; Moore & McCarthy, 1966a; Mullakhanbhai & Larsen, 1975). The GC base content of strain SP1 was 64.5 $\pm$ 1.0 mol% (T_m), 65.0 $\pm$ 1.0 mol% (T_m) for strain SP2, and that of strain MS3 was 63.0 $\pm$ 1.0 mol% (T_m) (table 17). Satellite bands were not evident using this method, but buoyant density determinations of the GC base content showed there was a satellite band present in both strains SP1 and MS3, which accounted for about 10% of the DNA. No satellite band was apparent in strain SP2 (table 17). The DNA satellite band in strain SP1 occured at a value of 51.4 $\pm$ 1.0 mol% (buoyant

density), compared to 62.1 ± 1.0 mol% for the main band, and in strain MS3 the satellite band had a value of 49.5 ± 1.0 mol% (buoyant density), with a main band at 61.0 ± 1.0 mol%. The satellite bands of the strains SP1 and MS3 occured at values of 9.3 mol% and 11.5 mol% lower than the respective main bands, the satellite bands in members of the Halobacteriaceae usually occurs at values about 10.0 mol% lower than that of the main band (Joshi et al., 1962; Moore & McCarthy, 1966a). The strains SP1 and MS3 do not seem to be unusual in the possession of a satellite band as organisms similar to members of the Halobacteriaceae. The satellite band in H.salinarium strain 5 and H.halobium has been shown to be involved in the synthesis of gas vesicles, and there is some evidence that the satellite band may also be involved in the synthesis of purple membrane (Simon, 1978; Weidinger et al., 1979). The absence of a satellite band in strain SP2 is unusual, but may be taken as an indication that the presence of a satellite band of DNA is not essential to the survival of these organisms in the highly saline environment. Unfortunately, at present there is little data on the GC base content of some of the new species (Gonzalez et al., 1978; Hochstein, personal communication; Tomlinson & Hochstein, 1976), or on those at present considered as species incertae sedis, therefore, it cannot be concluded that the absence of a satellite DNA band is restricted to strain SP2 within the extremely halophilic bacteria.

DISCUSSION

PHOTOTROPHIC BACTERIA.

The soda lakes of the East African Rift Valley represent a group of unique alkaline, saline lake waters. Despite similarities to other soda lakes in Africa and on other continents, their water chemistry allows them to be identified as distinct from other soda lakes. The predominant feature of soda lakes is the associated lack of divalent cations in solution, which are essential for the growth of many organisms. It is surprising, therefore, that these environments are characterised by their large numbers of phototrophic organisms, which cause colouration of the water (Imhoff et al., 1978b; Isachenko, 1934; Jenkin, 1932; Melack, 1978). Previous studies have shown that primary productivity by algae is higher in these alkaline, carbonate rich waters (Hecky & Kilham, 1973; Talling et al., 1973), than in any other lakes, and recent studies have shown that large numbers of phototrophic bacteria as well as cyanobacteria and algae may occur in these lakes (Grant et al., 1979; Imhoff et al., 1978a; 1978b). Phototrophic bacteria isolated from soda lakes investigated previously include only members of the genus Ectothiorhodospira (Grant et al., 1979; Imhoff & Trüper, 1977; 1978; Imhoff et al., 1978a; 1978b). The organisms isolated from the Kenyan soda lakes, in this study, represent a group of organisms which were biochemically and physiologically similar to the members of the genus Ectothiorhodospira, and it appears that organisms of this type are characteristic of this type of environment. Other phototrophic bacteria have been observed in the Kenyan soda lakes and lakes of the Wadi Natrun (Imhoff et al., 1978a; 1978b), but not in large numbers. The preponderance of members of the genus Ectothiorhodospira and similar organisms in alkaline, saline lakes is also evident from the occurence of blooms of these organisms seen at Lake Nakuru during 1978, and in stored samples from Lake Magadi, in 1978. The

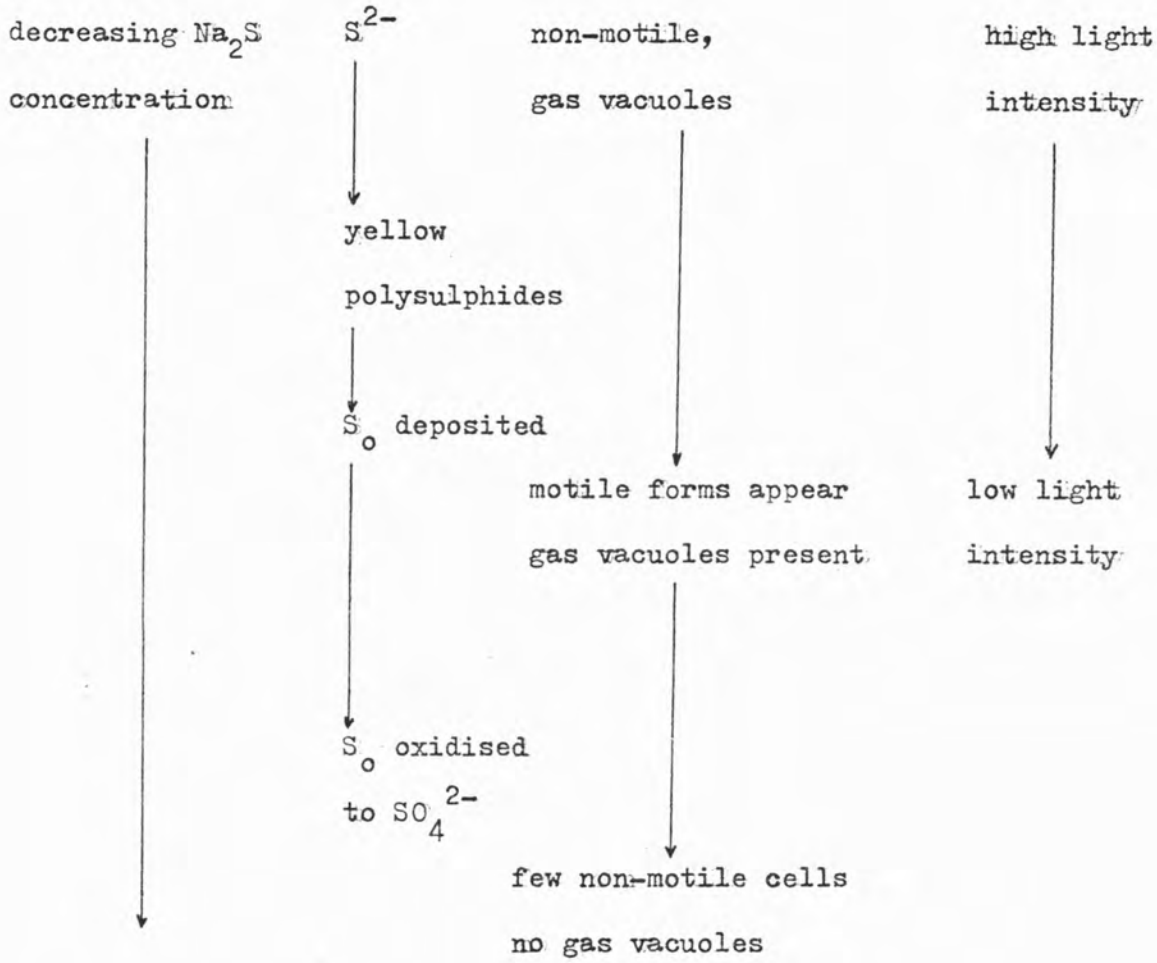
presence of organisms like these was also shown in the coloured bands of the surface trona, and in the absorption spectrum of the saltern liquor (figures 34, 37) at Lake Magadi. Such organisms were also shown to be responsible for the colouration of some of the lake waters in the Wadi Natrun (Imhoff et al., 1978a; 1978b), and may have been responsible for the purple coloured lake water observed by Melack (1978) in Ugandan crater lakes.

The organisms isolated from the Kenyan soda lakes, in this study, resemble members of the genus Ectothiorhodospira in their ability to photooxidise sulphide to sulphate, in depositing sulphur outside the cell as an intermediate, in containing bacteriochlorophyll a and carotenoids of the normal spirilloxanthin series as the major photopigments, and in having a characteristic lamellar type intracytoplasmic membrane system (figures 13.1, 14.1, 54, 55, 56). However, the ability to deposit sulphur outside the cell is not restricted to this particular genus of purple bacteria, since certain species of the so-called non-sulphur Rhodospirillaceae, once thought to be incapable of utilising reduced sulphur compounds as electron donors, have recently been shown to be capable of photooxidising sulphide (Hansen & van Gemerden, 1972; Hansen & Veldkamp, 1973; Keppen & Gorlenko, 1975), in some cases depositing sulphur outside the cell. Furthermore, some members of the Rhodospirillaceae have a lamellar intracytoplasmic membrane system similar, but not identical to those found in members of the genus Ectothiorhodospira (Pfennig & Trüper, 1974; Remsen, 1978; Trüper & Pfennig, 1978). However, in those members of the Rhodospirillaceae capable of utilising sulphide as an electron donor, sulphide is either oxidised to sulphate, without elemental sulphur as an intermediate, or sulphur is deposited extracellularly, without further oxidation to sulphate (Hansen & van Generden, 1972; Hansen & Veldkamp, 1973;

Keppen & Gorlenko, 1975). The ability to utilise sulphide and extracellularly deposited elemental sulphur, therefore, unambiguously distinguishes the strains described here from such members of the Rhodospirillaceae.

The strains described here may be further divided into two groups on the basis of the possession or absence of gas vacuoles (figures 50, 51). The presence of gas vacuoles in the strains, exemplified by strains B3, M2, and M4, appeared to be dependant upon the age of the culture, and the availability of nutrients. In the presence of 0.1% (w/v) $Na_2S.9H_2O$ cells of all the gas vacuolate strains examined were non-motile, a response found in non-gas vacuolate members of the Chromatiaceae (Pfennig & Trüper, 1974). The presence of gas vacuoles in this situation may allow the organism to find the optimal light intensity without the need to be flagellately motile (Walsby, 1978). When the sulphide concentration was lowered, or the gas vacuolate organisms were exposed to oxygen, the cells became motile by polar flagella, retaining their gas vacuoles only if the sulphide concentration was not reduced further (figure 99). These gas vacuolate strains are different from the previously described gas vacuolate members of the Chromatiaceae in the peripheral location of the gas vacuoles (figures 51, 52, 55), and the ability of the cells to float at 26°C as well as at a lower temperature (Pfennig, 1965a; Pfennig & Trüper, 1974; Whittenbury, 1971). The peripheral location of the gas vacuoles may be related to the presence of lamellar type intracytoplasmic membranes extending to the centre of the cell (figure 55), and there may be differences between the structure of these gas vacuoles and those previously described (Blaurock & Wober, 1976; Cohen-Bazire et al., 1969; Walsby, 1972; 1974; 1978). Only one species of gas vacuolate phototrophic bacterium, Lamprocystis roseopercina, has been shown to be capable

Figure 99. The effect of the cultural conditions on strain B3



of flagellate motility, but cells of this species deposit sulphur globules within the cell, and the gas vacuoles are centrally located (Pfennig et al., 1967).

The vacuolate strains described here are, therefore, distinguishable from all previously described members of the Rhodospirillales, in that they resemble members of the genus Ectothiorhodospira in the characteristics of containing bacteriochlorophyll a and carotenoids of the normal spirilloxanthin series, in having a characteristic lamellar intracytoplasmic membrane system, and depositing sulphur outside the cell during the photooxidation of sulphide to sulphate, but they differ in that they contain peripherally located gas vacuoles under certain conditions. The presence or absence of gas vacuoles in members of the Rhodospirillales is of taxonomic significance (Pfennig & Trüper, 1974; Trüper & Pfennig, 1978), and the gas vacuolate strains isolated from the Kenyan soda lakes may, therefore, constitute a separate taxonomic group, which according to present criteria would merit a new taxon. The importance of gas vacuoles in the current taxonomy of the Rhodospirillales may, however, meed to be reviewed in the light of recent findings that plasmids may be involved in the formation of gas vacuoles in cyanobacteria (Walsby, 1977), and non-photosynthetic bacteria of the genus Halobacterium (Simon, 1978; Wiedinger et al., 1979) and Prosthecomicrobium pneumaticum (Das & Singh, 1976). Puchkova et al. (1973) have also questioned the differentiation of the physiologically, metabolically, and morphologically similar genera Chlorobium and Pelodictyon on the basis of the possession of gas vacuoles by the latter genus. Gas vacuolate strains from the Kenyan soda lakes show physiological, metabolic, and morphological similarities to members of the genus Ectothiorhodospira, and the relationship between the genus

Ectothiorhodospira and the gas vacuolate strains may eventually prove to be comparable to that between the genera Chlorobium and Pelodictyon.

All of the non-gas vacuolate strains isolated from the Kenyan soda lakes closely resembled E.shaposnikovii, E.mobilis and the Ectothiorhodospira sp. described by Grant et al. (1979) morphologically and physiologically, and these organisms were largely indistinguishable from one another on the basis of the tests carried out. The gas vacuolate strains were characterised by the presence of gas vacuoles under certain conditions, but in all principle characteristics were indistinguishable from E.mobilis, E.shaposnikovii, and the Ectothiorhodospira sp. described by Grant et al. (1979). In view of these findings it would be unwise to create, at present, a new taxon, at the generic level for this group of gas vacuolate Ectothiorhodospira-like organisms. Their inclusion in the genus Ectothiorhodospira, or in a novel genus must await further study of the taxonomic and possibly phylogenetic relationship between this genus and the gas vacuolate strains.

The organisms which contained gas vacuoles could be divided into at least three groups represented by the strains B3, M2, and M4, whereas the non-gas vacuolate strains could be divided into two groups, represented by the strains E7 and NCL5. The major differences between the various groups within the gas vacuolate and non-gas vacuolate strains were in salinity optima and the range of carbon and sulphur sources utilised for phototrophic growth (tables 8, 11, 12). In view of the present differences recorded for E.shaposnikovii and E.mobilis in this respect, and the lack of any apparent differences in this study, the significance of these parameters in determining the status of separate species is dubious. Trüper (1970) isolated a number of strains of E.mobilis from the

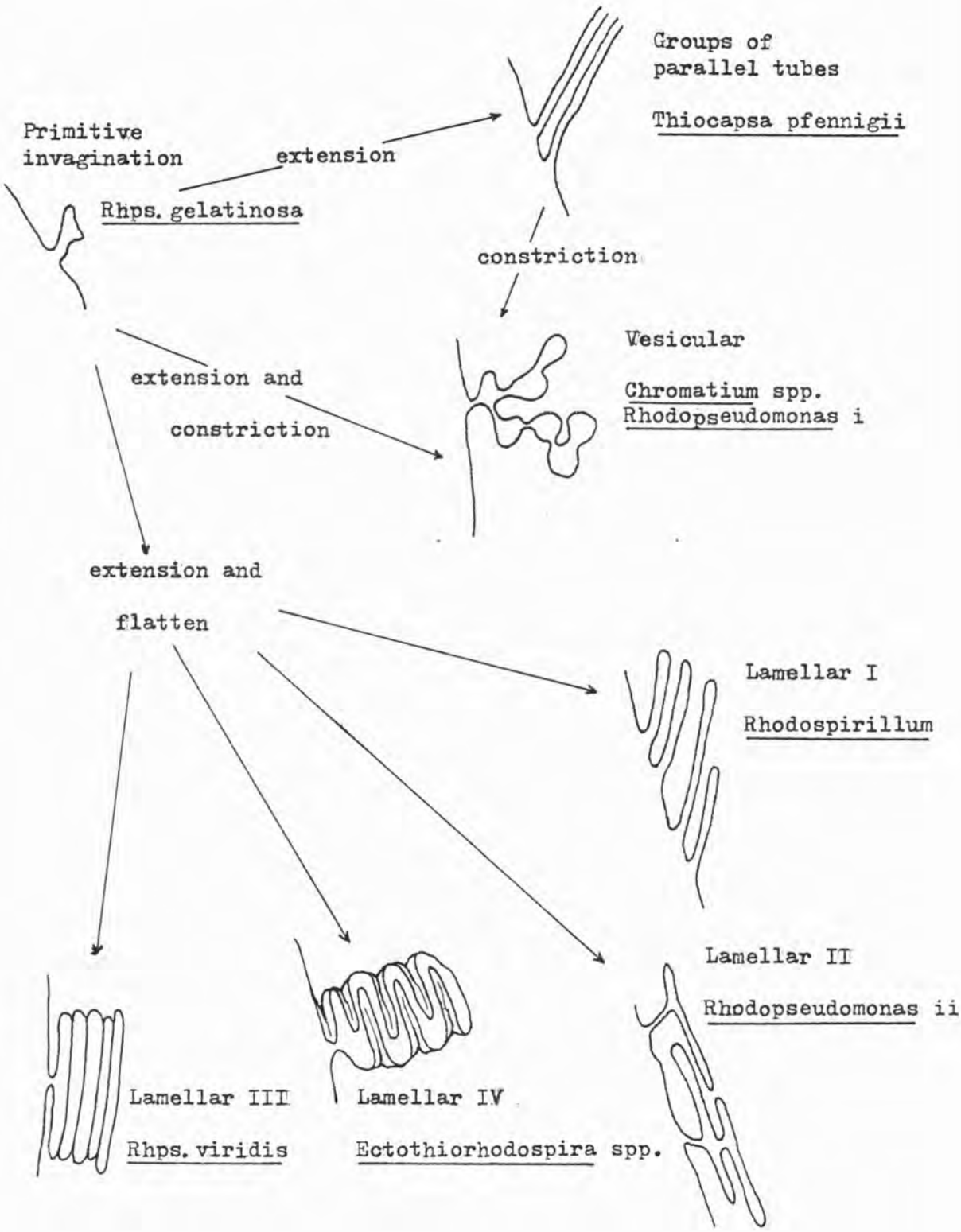
same environment, and all strains showed the 3.0% (w/v) NaCl requirement typical of the type strain of E.mobilis (Trüper, 1968). There would appear to be more grounds for regarding some of the strains isolated from the Kenyan soda lakes as separate species, since in addition to the physiological and biochemical differences there are morphological and GC content differences, which are not apparent in E.mobilis and E.shaposnikovii (Pfennig & Trüper, 1974; Trüper, personal communication; Trüper & Pfennig, 1978). Thus, the physiologically and morphologically distinct strains isolated in the gas vacuolate (strains B3, M2, and M4) and non-gas vacuolate (strains E7 and NCL5) strains may constitute different species within their taxonomic group. Any assignment of these strains to separate species should be supported by a more detailed study of their relationship to one another by features such as DNA homologies.

It is evident that all of the strains isolated here, and members of the genus Ectothiorhodospira isolated previously (Grant et al., 1979; Imhoff & Trüper, 1977; Imhoff et al., 1978a; 1978b; Kondrat'eva, 1956; Matheron & Bauliague, 1972; Raymond & Sistrom, 1967, 1969; Trüper, 1968; 1970; Yang Hui-Fang, 1962) form a well defined physiological group, which is characteristic of particular environments. The pH optima of the strains isolated from the Kenyan soda lakes shows, that at the pH of the lakes, reduced carbon compounds are the most likely source of electron donors for phototrophic growth. However, in other environments, such as the sea, where they have also been found (Herbert & Tanner, 1977; Matheron & Bauliague, 1972; Imhoff & Trüper, 1976; 1978; Trüper, 1968; 1970), these organisms would show better growth in the presence of reduced sulphur compounds (Chesnokov & Shaposnikov, 1936a). The organisms belonging to the Ectothiorhodospira-like group are also distinguishable from other members of the

Rhodospirillineae on the basis of their alkaline pH optimum for growth in the presence of reduced carbon compounds, and the unique ability to utilise polysulphides for photolithotrophic growth.

At present the genus Ectothiorhodospira is included in the family Chromatiaceae (table 5), but the taxonomic position of these organisms is open to dispute (Pfennig, 1977; Pfennig & Trüper, 1974; Trüper, 1968). The similarities between the members of the genus Ectothiorhodospira and the gas vacuolate strains indicate that the two groups are sufficiently closely related to warrant inclusion in the same higher taxon. The members of the Chromatiaceae have, in the past, been recognised as forming a distinct physiological group within the purple phototrophic bacteria, the "Endothiorhodaceae", on account of their ability to deposit sulphur globules within the cell (Baas-Becking, 1925). The members of the genus Ectothiorhodospira, including the gas vacuolate organisms could, therefore, be members of the "Ectothiorhodaceae", on the basis of sulphur deposition outside the cell (Pelsh, 1937; Trüper, 1968). However, the distinction between the purple sulphur and purple non-sulphur bacteria is becoming less distinct as new species of the Rhodospirillaceae are found which show greater similarities to the sulphur metabolism of the Chromatiaceae (Pfennig, 1977). The delineation of the higher taxa of the Rhodospirillales on the basis of more refined structural, biochemical, and physiological tests is not consistent with the present major taxonomic divisions (Pfennig & Trüper, 1974; Trüper, 1976; Trüper & Pfennig, 1978), but such information may eventually assist in the revision of the higher taxa of the Rhodospirillales. In particular, the structure and arrangement of the intracytoplasmic membrane system has suggested novel groupings (figure 100). Thin sections, freeze fracturing, and negative staining have shown that the Chlorobiineae are characterised by the presence of chlorosomes

Figure 100 The possible relationship between intracytoplasmic membrane structures of the Rhodospirillineae in relation to phylogenetic position.



(Schmidt, 1980; Staehelin et al., 1978), whereas the Rhodospirillineae have a diverse system of intracytoplasmic membranes (Oelze & Drews, 1972; Pfennig, 1967; Remsen, 1978). Thin sections and negative staining of cells of gas vacuolate and non-gas vacuolate strains from the Kenyan soda lakes have indicated the presence of intracytoplasmic membranes of the lamellar type (figures 54, 55). Various reviews have considered the structures of the intracytoplasmic membranes in green and purple phototrophic bacteria (Oelze & Drews, 1972, Pfennig, 1967; Remsen, 1978). The intracytoplasmic membrane system of the purple phototrophic bacteria may be divided into several natural groupings. The intracytoplasmic membrane system found in the Chromatiaceae and Rhodospirillaceae is diverse, including vesicular, tubular, and lamellar membranes. The groupings found in the Rhodospirillineae, according to intracytoplasmic membrane structure, are not confined to certain genera (figure 100). The primitive, poorly developed tubular or vesicular membranes are found in Rhps.gelatinosa, Rhc.purpureus, and Rhsp.tenue, whereas the well developed tubular membrane system is characteristic of Thiocapsa pfennigii. The vesicular membrane system is found in most genera of the Chromatiaceae, with the exception of the genus Ectothiorhodospira, and also in several species of the genera Rhodospirillum and Rhodopseudomonas. The lamellar system may be divided into several types, which are characteristic of certain genera (figure 100). The lamellar intracytoplasmic membranes of most members of the genus Rhodospirillum consist of a stack, which lies at an acute angle to the cell membrane, while the lamellar stack of the genus Ectothiorhodospira lie parallel to the cell membrane. In the budding species of the genera Rhodopseudomonas and Rhodomicrobium the lamellae also lie parallel to the cell membrane, but consist of concentric layers, rather than typical stacks.

The characteristic membrane structures are paralleled by certain physiological and morphological properties. All species of the Chromatiaceae, which possess a vesicular membrane system, are capable of depositing sulphur inside the cell, whereas the species of the Rhodospirillaceae, which have a vesicular membrane system, do not, and are only capable of photolithotrophic utilisation of sulphide in trace amounts, depositing sulphur outside the cell, which is not further oxidised. Rhps.sulfidophila is exceptional, in that the organism utilises quite high concentrations of sulphide, up to 0.1% (w/v), has a vesicular membrane system, but oxidises sulphide directly to sulphate, apparently without elemental sulphur as an intermediate (Hansen & Veldkamp, 1973). The members of the genus Rhodospirillum, which contain lamellar membranes of the first type (figures 11.2, 12.2), are incapable of utilising sulphide or other reduced sulphur compounds for photolithotrophic growth (figure 100). Organisms with the third type of lamellar membranes (figures 11.3, 12.3), when capable of photolithotrophic growth, oxidise sulphide to sulphate, without elemental sulphur as an intermediate, while organisms possessing the lamellar membrane system characteristic of the genus Ectothiorhodospira (figures 13.1, 14.1, 55) are capable of photooxidising sulphide to sulphate, with elemental sulphur as an intermediate, which accumulates outside the cell. There may, therefore, be a connection between the subcellular architecture and the sulphur metabolism of the organisms, in particular, the ability to deposit sulphur during the photooxidation of sulphide. Accordingly, apart from members of the genus Ectothiorhodospira, including the gas vacuolate strains, sulphur globules are only deposited extracellularly by members of the purple phototrophic bacteria which have a vesicular membrane system. The members of the green phototrophic bacteria, which also deposit sulphur outside the cell,

are distinguishable from the purple phototrophic bacteria by the possession of bacteriochlorophyll c, d, or e, and carotenoids of the isorenieratene series (table 1), and the presence of chlorosomes as the photosynthetically active membranes. These organisms are capable of photooxidising sulphide to sulphate, via elemental sulphur as an intermediate, which accumulates outside the cell. However, in these organisms the chlorosomes do not appear to be responsible for the formation of the sulphur globules, and a vesicular invagination of the cell membrane appears to allow the formation of the sulphur globules, which are then expelled from the cell (Puchkova et al., 1975). Thus, the presence of vesicular invaginations of the cell membrane appear to be significant in the formation of sulphur globules. The method by which sulphur globules are excreted from the cells of the genus Ectothiorhodospira and the gas vacuolate strains is not known, but may be atypical in the purple phototrophic bacteria.

There are, therefore, clear physiological and structural groupings, which differ from the current taxonomic groupings of the purple phototrophic bacteria. Very recent phylogenetic surveys of certain phototrophic bacteria, based on 16S rRNA and cytochrome c sequencing have supported the view that the nature of the intracytoplasmic membranes, in association with sulphur metabolism may be a more valid guide to the phylogenetic position of a phototrophic bacterium (Ambler et al., 1979a; 1979b; Dickerson, 1980; Gibson et al., 1979; Woese et al., 1980), than is gross morphology. 16S rRNA analyses show that the purple phototrophic bacteria may be divided into groupings which often divide current genera. In particular, the organisms which contain the rather poorly developed tubular or vesicular intracytoplasmic membranes form one closely related group, the remainder of the Rhodospirillaceae form a second group, and the

members of the Chromatiaceae, so far examined, a third (figure 101). The second group appears to divide into at least two sub-groups, which comprise the lamellar membrane containing members of the genera Rhodopseudomonas and Rhodomicrobium, and the vesicular membrane containing members of the genus Rhodopseudomonas. Cytochrome c analyses show a similar pattern (figure 102), which may be related to the structure of the intracytoplasmic membranes. These results also suggest that the purple sulphur and green phototrophic bacteria may occupy groups distinct from members of the Rhodospirillaceae. Within the members of the Rhodospirillaceae there appear to be several groups relating to the tubular, vesicular, and two lamellar intracytoplasmic membranes found in members of this family (Dickerson, 1980). However, members of the genus Ectothiorhodospira have not been included in these studies, and their relationship to the various phylogenetic groupings is unknown.

Polar lipid analyses of certain members of the Rhodopsirillales showed that there were similarities between the organisms isolated from the Kenyan soda lakes, E.mobilis and E.shaposnikovii, and the members of the Chromatiaceae examined (figures 69, 70), suggesting that these members of the genus Ectothiorhodospira, and the gas vacuolate strains, were similar to the Chromatiaceae. The lipids of E.halophila and E.halochloris were, however, significantly different from those of either of the two families examined. These results may suggest a taxonomic difference between the extremely halophilic members of the genus Ectothiorhodospira and other members of this genus. Such differences may reflect the unusual physiological requirements of these species for high salinity. However, cells of E.halophila do not contain lipids characteristic of the halobacteria, and none of the enzymes from E.halophila have been shown to require high concentrations of salt, as do some of the enzymes of the

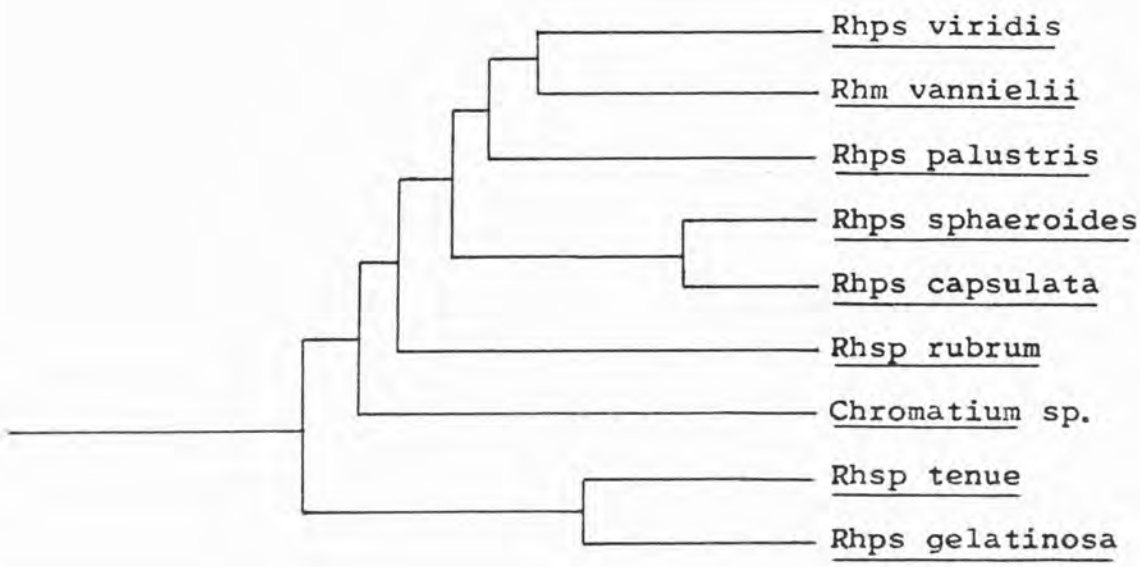


Figure 101 Phylogenetic division of the Rhodospirillineae based upon 16S rRNA analysis

Analysis of the 16S rRNA content of members of the Rhodospirillineae results in the division of the suborder into several distinct groupings (Gibson et al., 1979; Woese et al., 1980), which are not consistent with the present taxonomic groupings (Pfennig & Trüper, 1974; Trüper & Pfennig, 1978). The major divisions are, however, consistent with the groupings produced by membrane type. These results also suggest that the budding species of the genus Rhodopseudomonas may constitute a taxonomic group separate from other members of the genus Rhodopseudomonas.

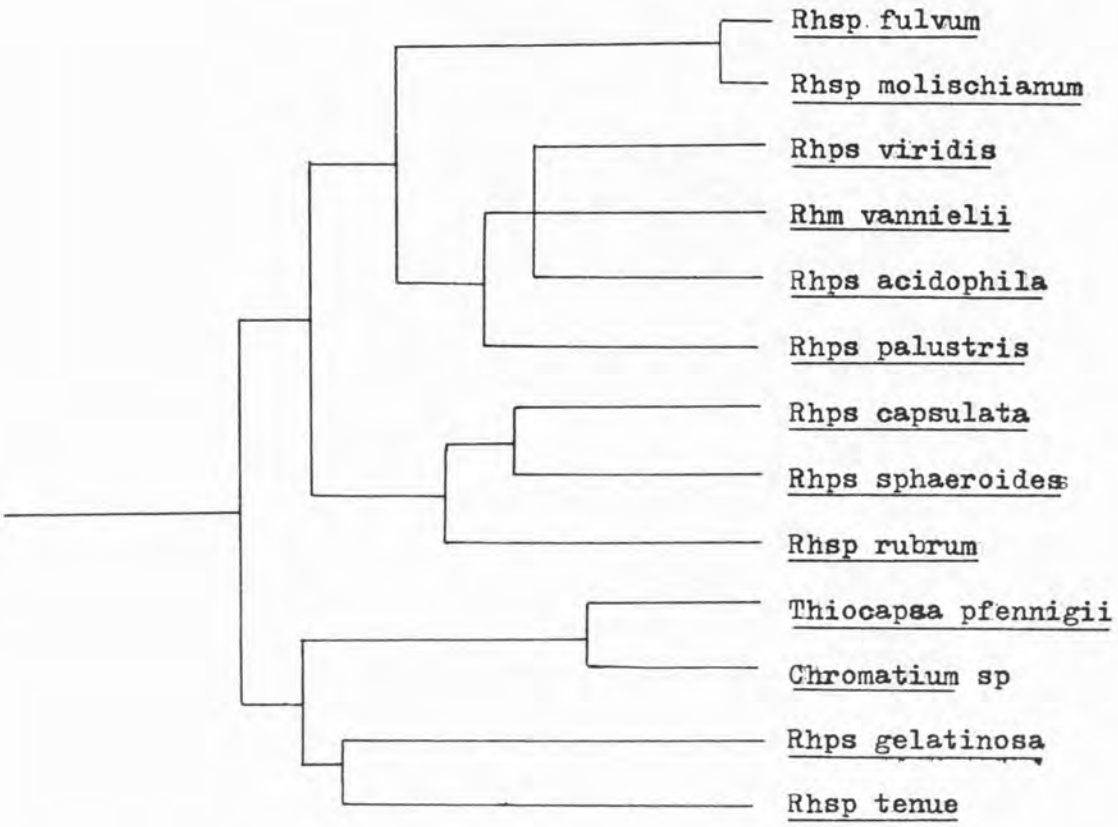


Figure 102 Phylogenetic divisions of the Rhodospirillineae based upon cytochrome c analysis.

The phylogenetic divisions obtained by analysis of the cytochrome c found in members of the Rhodospirillineae results in similar groupings to those obtained from 16S rRNA analysis (figure 101). These results indicate that the current taxonomy, used to characterise these organisms, does not necessarily reflect the possible evolutionary relationships of the organisms (Ambler et al., 1979a; 1979b; Dickerson, 1980).

halobacteria (Tabita & McFadden, 1976; Bayley & Morton, 1978). Accordingly, unlike the halobacteria, the extremely halophilic members of the genus Ectothiorhodospira do not appear to be archaebacteria, and are not, therefore, phylogenetically distinct from other phototrophic bacteria. Apart from their unusual lipid composition the extremely halophilic species, E.halophila, E.halochloris, and E.abd-el-malekii are bipolarly flagellate (Imhoff & Truper, 1977; 1978; personal communication; Raymond & Sistrom, 1967; 1969), a feature restricted to these members of the purple sulphur bacteria, and all three species are larger than cells of this genus which are not extremely halophilic, and are typically spirilla, cells of E.mobilis and E.shaposnikovii being typically vibrios. The differences in cell shape were also reflected in the assignment of an extremely halophilic member of this genus to the genus Rhodospirillum (Baas-Becking, 1928; van Niel, 1933), and the low salinity requiring strains to the genus Rhodopseudomonas (Kondrat'eva, 1956; Onitskaya, 1954; Scardovi, 1950; Yang Hui-Fang, 1962).

There would appear to be some justifiaction for regarding the extremely halophilic members of the genus Ectothiorhodospira as being taxonomically distinct from other members of the genus Ectothiorhodospira, however, these differences may be entirely due to the extreme nature of the environment in which the former organisms evolved rather than the result of separate genetic evolution. The comparison between these members of the present genus Ectothiorhodospira and other members of the phototrophic bacteria is, however, not comprehensive enough to allow firm conclusions to be drawn. The results of a comparison of the DNA of members of this genus (Imhoff, in preparation), and a more extensive study of the cell envelope may provide more definite information on the taxonomic

position of these two physiologically, biochemically, and morphologically distinct groups within the genus Ectothiorhodospira.

Members of the genus Ectothiorhodospira (including the gas vacuolate strains), therefore, differ from members of the Chromatiaceae by virtue of their sulphur metabolism and morphology, and may be considered to have been placed in a family of convenience, in that they are purple sulphur bacteria. It may be argued that these organisms constitute a separate taxonomic group at a higher ranking than the present, which may be distinguished from the Chromatiaceae and Rhodospirillaceae on a number of criteria (tables 1, 17). The criterion of Molisch (1907), as discussed by Pfennig (1977), should not strictly allow the inclusion of the genus Ectothiorhodospira in the family Chromatiaceae, since Molisch's original distinction between the two families "Thiorhodoaceae" (Chromatiaceae) and "Athiorhodoaceae" (Rhodospirillaceae) was based solely on morphology, and the presence of sulphur globules within cells of the Thiorhodaceae. The current order Rhodospirillales maintains the early taxonomic groups, and is subdivided into two suborders, the Rhodospirillineae and Chlorobiineae (table 1), corresponding to the purple and green phototrophic bacteria respectively (Pfennig, 1977; Pfennig & Trüper, 1974; Trüper & Pfennig, 1978). Within the Rhodospirillineae two families are recognised, based on Molisch's criterion, the family Chromatiaceae comprising those species depositing sulphur globules within the cell, and possessing an intracytoplasmic membrane system of the vesicular or tubular type, but also includes the genus Ectothiorhodospira, which deposits sulphur extracellularly and has an intracytoplasmic membrane system of the lamellar type. The family Rhodospirillaceae comprise those species which do not contain sulphur within the cells, and have an intracytoplasmic membrane system of the vesicular, tubular, or lamellar type. Thus, members

of the genus Ectothiorhodospira, including the gas vacuolate strains, resemble members of the Rhodospirillaceae in their fine structure and morphology (Pfennig, 1977), but are characterised by their ability to deposit sulphur extracellularly, which is further oxidised to sulphate, and in this respect may be considered to be similar to members of the Chlorobiaceae. However, since they do not contain chlorosomes, bacteriochlorophyll c, d, or e, and carotenoids of the isorenieratene series, and are flagellate, they cannot properly be considered as belonging within this family.

On morphological grounds it has been argued that this group of purple sulphur bacteria, which deposit sulphur outside the cell, should appropriately be accomodated in the family Rhodospirillaceae under the present taxonomic system (Pfennig, 1977). The GC values are also in agreement with those from the Rhodospirillaceae, which form a tight group (table 4). However, there are certain features which make the genus Ectothiorhodospira difficult to accomodate in the presently defined taxa. Apart from differences in sulphur metabolism, members of the Rhodospirillaceae do not contain gas vacuoles, and the inclusion of the gas vacuolate Ectothiorhodospira-like strains in this family would be anomalous, unless the family was redefined. Lipid analyses also indicate that most members of the genus Ectothiorhodospira and the gas vacuolate strains were more similar to members of the Chromatiaceae (figure 69, 70), although the extremely halophilic species of Ectothiorhodospira are a notable exception. Additionally, examination of the cell wall of members of the Rhodospirillineae for the presence of enterobacterial 'O' antigens has shown that they are present in members of the Rhodospirillaceae and Chromatiaceae, but not in certain members of the genus Ectothiorhodospira (Drews et al., 1978; Trüper, personal communication).

Further investigations of the lipid content may prove useful, since investigations of the neutral lipid fractions have shown that hopanoids are present in members of the Rhodospirillaceae, but not in members of the Chromatiaceae and Chlorobiaceae (Ourisson et al., 1979; Rohmer et al., 1979). The members of the Chromatiaceae and Chlorobiaceae, by virtue of the absence of hopanoids, probably contain squalenes or similar compounds. These results are consistent with the view that the major neutral lipids found in the cells of prokaryotes and eukaryotes may be indicative of phylogenetic groupings. This view is also supported by the finding that isoprenoids, such as squalene, or carotenoids, and hopanoids may functionally replace sterols in the cells membrane (Lanyi et al., 1974; Ourisson et al., 1979; Rohmer et al., 1979). The major groupings, therefore, correspond to those organisms which contain either isoprenoids, hopanoids, or sterols, respectively, as the major neutral lipids in the cell. The groupings created by neutral lipid analysis are not entirely consistent with present taxonomic divisions (Ourisson et al., 1979; Rohmer et al., 1979), however, these results appear to be consistent with the phylogenetic groupings proposed by 16S rRNA and cytochrome c analyses (Ambler et al., 1979a; 1979b; Dickerson, 1980; Gibson et al., 1979).

It would be possible to accomodate the Ectothiorhodospira-like organisms in a separate family, based upon the unique properties of these organisms. Since Pelsh (1937) proposed that the genus Ectothiorhodospira be placed in the "Ectothiorhodaceae", the taxonomy of the phototrophic bacteria has been little altered, and this genus currently occupies an unsatisfactory position in the Chromatiaceae. Pelsh's original family name, "Ectothiorhodaceae", is illegitimate. If the Ectothiorhodospira-like organisms, whether consisting of one or more genera, were to be placed in a new family, then it should

be the Ectothiorhodospiraceae, in which the type species is Ectothiorhodospira mobilis (Trüper, 1968). The Ectothiorhodospiraceae could be defined as containing those species which grow optimally at pH 9.0-10.0, photoorganotrophically, deposit sulphur outside the cell during the photooxidation of sulphide to sulphate, possess bacteriochlorophyll a or b, carotenoids of the normal spirilloxanthin series and glucosides of these carotenoids, and have an intracytoplasmic membrane system of the lamellar type, in which the lamellae of the stack are continuous with one another, rather than discontinuous, as in the lamellar containing members of the Rhodospirillaceae (table 17). The organisms are capable of photolithotrophic growth in the presence of polysulphides, may be flagellately motile, may contain gas vacuoles, and reproduce by binary fission. The resulting three taxa (table 17), the existing Rhodospirillaceae, Chromatiaceae (less the genus Ectothiorhodospira), and the proposed new family, Ectothiorhodospiraceae, would, therefore, constitute clear ecological and physiological groups.

A taxonomically intermediate position for the genus Ectothiorhodospira, including the gas vacuolate strains, between the Chromatiaceae and Rhodospirillaceae (table 17) is strongly suggested by certain data. In particular, some strains utilise sulphide poorly, thus resembling members of the Rhodospirillaceae, although elemental sulphur is deposited outside the cell and is further oxidised to sulphate, like members of the Chromatiaceae. The relationship of the genus Ectothiorhodospira and the gas vacuolate strains to other members of the Rhodospirillineae is central to the discussion of the merits of the current taxonomy of the phototrophic bacteria as compared to the suggestions made by the use of phylogenetic analyses (Ambler et al., 1979a; 1979b; Dickerson, 1980; Gibson et al., 1979; Ourisson et al., 1979; Rohmer et al., 1979;

Table 17. A possible taxonomy of the Rhodospirillineae

Order

Rhodospirillales

Suborder

Rhodospirillineae

Family I	Family II	Family III
Rhodospirillaceae	Ectothiorhodospiraceae	Chromatiaceae
as table 1	cells motile, multiply by binary fission, have pH optima between 8.0 and 10.0, contain lamellar intracytoplasmic membranes of type IV (figure 100). Deposit sulphur globules outside the cell during the photooxidation of sulphide to sulphate.	as table 1
Ectothiorhodospira	(Ectothiohalospira?)	(Ectothiorhodocystis?)
Cells polarly flagellate do not contain gas vacuoles cells short rods to vibrios lipid composition similar to members of the Chromatiaceae e.g. E.mobilis	Cells bipolarly flagellate do not contain gas vacuoles cells distinct spirilla distinctive lipid composition extremely halophilic e.g. E.halophila	Cells polarly flagellate contain peripherally located gas vacuoles cells short rods, may be slightly curved, lipid composition similar to member of the Chromatiaceae e.g. strain B3

Woese et al., 1980). Currently the presence of gas vacuoles is one of the criteria used to distinguish genera of the Rhodospirillales. However, such differences between gas vacuolate and non-gas vacuolate genera are usually supported by other data, although in the case of the genera Chlorobium and Pelodictyon this is not true. Current taxonomic recommendations and the present taxa of the Rhodospirillales suggest that the gas vacuolate Ectothiorhodospira-like organisms are taxonomically distinct from all previously described species, and may justify inclusion in a new genus, for which the name Ectothiorhodocystis would be appropriate. However, such a taxonomic proposal would be unwise, in the light of the present uncertainty about the status of gas vacuoles in the taxonomy of bacteria. These organisms should, therefore, be placed in the same higher taxon as other members of the genus Ectothiorhodospira. Thus, the three type strains, B3, M2, and M4 may also be described as gas vacuolate members of the genus Ectothiorhodospira, and they may constitute new taxa within this genus.

EXTREMELY HALOPHILIC BACTERIA.

Extremely halophilic bacteria, belonging to the family Halobacteriaceae, have been isolated from a number of saline environments, including the sea (Brisou et al., 1974; Colwell et al., 1979; Elazari-Volcani, 1940; Gonzalez et al., 1978; Imhoff et al., 1978b; Mullakhanbhai & Larsen, 1975; Nissenbaum, 1975; Post, 1977; Rodriguez-Valera et al., 1979; Tomlinson & Hochstein, 1972b, 1976). However, most isolates have been obtained from solar salt, salted fish, or salted hides, and the organisms isolated from these sources have, generally shown optimal growth under similar conditions. This is hardly surprising, since solar salt used for salting fish and hides is made by evaporation of sea water, and solar salt produced in many parts of the World has, therefore, a relatively constant chemical composition.

Investigations of more diverse highly saline environments has shown that extremely halophilic bacteria may exist in situations where the pH is less close to neutrality, and the divalent ions are present in either much higher or lower concentrations than in the saltern liquor derived from sea water. In these situations, the optimal conditions for growth of the organisms isolated may reflect the ionic composition of the environment, e.g. H.volcanii, which was isolated from the Dead Sea and grows optimally at pH 6.8 in a medium containing 1.7M Mg^{2+} and 1.7-2.0M NaCl, concentrations approximating to those found in the Dead Sea (Mullakhanbhai & Larsen ,1975). However, "H.marismortui" and H.trapanicum, which were also isolated from the Dead Sea, do not appear to show similar optima (Elazari-Volcani, 1940; 1957). Recently extremely halophilic bacteria have been described in alkaline, highly saline African lakes, where the Mg^{2+} concentrations are very low, and the pH 10.0-11.0 (Imhoff et al., 1978b; Tindall et al., 1980). Organisms isolated from the Great

Salt Lake, Utah, which has a pH of 8.2 and Mg^{2+} and NaCl concentrations of 100mM and 1.5M respectively in the southern arm, may be capable of tolerating the growth conditions found to be optimal for the organisms from the more alkaline African lakes (Litchfield, personal communication; Post, 1977), but precise conditions for optimal growth of these organisms has not been published. Thus, extremely halophilic bacteria may well be found over a wide range of pH, in most highly saline environments, showing specific adaptation to local conditions.

The halobacteria and halococci are distinguished from most other bacteria and all eukaryotes by their characteristic 16S rRNA catalogue, which suggests that they constitute a distinct phylogenetic as well as taxonomic group (Magrum et al., 1978). The 16S rRNA catalogue of the extremely halophilic bacteria shows greatest similarities to the thermoacidophilic bacteria, represented by the genera Thermoplasma and Sulfolobus (Brierley & Brierley, 1973; De Rosa et al., 1975; Magrum et al., 1978; Zillig et al., 1980), and the methanogenic bacteria (Balch et al., 1979; Magrum et al., 1978). These three groups, the extreme halophiles, the thermoacidophilic, and the methanogenic bacteria are known as the urkingdom Archaebacteria (Woese & Fox, 1977; Woese et al., 1978), a name intended to denote antiquity, and characterised by organisms which occupy extreme environments by present standards. The anoxic, highly saline, or hot acid environments, in which archaebacteria are typically found, may have been more common in Archaean times. It is envisaged that life evolved in warm concentrated waters, in an anoxic atmosphere (Deamer, 1978), from a variety of abiotically synthesised hydrocarbon macromolecules (Hargreaves & Deamer, 1978; Oro et al., 1978). From this evolved the two groups of organisms known as the Eukaryotic and Prokaryotic Kingdoms. The prokaryotes are further

divided into the urkingdoms Archaebacteria and Eubacteria (Woese & Fox, 1977; Woese et al., 1978). The relationship of the archaebacteria, eubacteria, and eukaryotes to the early organisms is uncertain, but it has been argued that the archaebacteria may be the closest descendants of these early organisms, since they show adaptation to extreme environments, which may have been common in the Archaean Earth. The division of the prokaryotes into two urkingdoms and their separation from the eukaryotes on the basis of 16S rRNA analyses is further supported by the findings that the archaebacteria show features which allow them to be distinguished from the eubacteria and eukaryotes. The presence of isopranyl ether lipids is also characteristic of members of the archaebacteria (De Rosa et al., 1974b; 1975; 1977a; 1977b; Kates, 1978; Langworthy, 1977a; 1977b; Langworthy et al., 1972; 1974; Magrum et al., 1978; Stoeckenius, 1978; Tornabene, 1978; Tornabene & Langworthy, 1979). Ether lipids are not found in other bacteria, and although ether lipids have been identified in eukaryotes, none of them are of the type found in archaebacteria (Snyder, 1972). The subcellular architecture of archaebacteria is typical of prokaryotes, but the cell envelope is unusual in that muramic acid does not appear to be present, and the usual constituent of the bacterial cell wall, murein, is, therefore, absent (Brown & Cho, 1970; Kandler & Hippe, 1977; Kandler & Konig, 1978; Kushner & Onishi, 1968; Millonig et al., 1975; De Rosa et al., 1975; Weiss, 1974). Halobacteria and halococci thus differ from other bacteria in a fundemantal way, and any fresh examination of this unusual group must take these considerations into account.

Organisms isolated, in this study, from Lake Magadi, exemplified by the type strains SP1, SP2, and MS3, resemble halobacteria by the usual criteria (Gibbons, 1974; Tindall et al., 1980), but grow

optimally at pH 9.0-10.0, in Mg^{2+} concentrations of 0.1-2.0mM, values similar to the pH and ionic composition of Lake Magadi and the saltern liquor. The ability of these organisms to grow at very low Mg^{2+} concentrations and high pH seems to be unique, and sets them apart from other recorded examples of halobacteria, with the possible exception of those observed by Imhoff et al. (1978b) in some of the lakes of the Wadi Natrun. The known species H.halobium, H.cutirubrum, and H.salinarium may be adapted to grow at pH 7.5 in Mg^{2+} concentrations as low as 10mM, but under these conditions cells undergo an irreversible change from rods to spheres (Brown & Gibbons, 1959). Growth was not recorded at Mg^{2+} concentrations less than 10mM. Colwell et al. (1979) have also shown that some strains of Halobacterium are capable of growth when inoculated into a standard medium at pH 9.0, but the method by which the pH was adjusted was not recorded, nor was the final pH, after inoculation and incubation, since alkalinity is eventually lost due to the associated precipitation of Mg^{2+} as $MgCO_3$, caused by the formation of CO_3^{2-} during the inevitable solution of CO_2 from the atmosphere under alkaline conditions. Thus, there are no accounts of growth of any of the known species of Halobacterium at high pH in the presence of very low concentrations of Mg^{2+}. These properties appear to be characteristic of organisms isolated from alkaline, highly saline environments. In the strains SP1, SP2, and MS3 the morphology of the cells is affected by lowering of the pH or increasing the Mg^{2+} concentration to 10.0mM or above, suggesting that these strains differ from known species in certain aspects of the structure of the cell envelope. Studies on the cell envelope of H.cutirubrum have shown that pH and Mg^{2+} have an important function in maintaining the integrity of the cell envelope (Kushner & Bayley, 1963; Kushner & Onishi, 1966; Kushner et al., 1964; Onishi & Kushner, 1966). Cells

of this species were able to maintain their rod shaped morphology in 4.5M NaCl, over the pH range 4.9 to 11.0 (Kushner & Bayley, 1963), whereas cells of strains SP1, SP2, and MS3 lost their rod shaped morphology, becoming spherical or distorted when the pH of the suspending medium was adjusted to pH 7.0, but adjusting the pH of the suspending medium to pH 11.0 had no effect on the morphology of the cells. These changes may also have been affected by changes in Mg^{2+} concentration associated with changes in pH. In H.cutirubrum the cells maintained their rod shaped morphology only while the cell wall maintained the regular hexangonal array, indicating that the cellular morphology was only maintained while the cell wall maintained its integrity (Kushner & Bayley, 1963; Kushner et al., 1964). The results from the strains SP1, SP2, and MS3 suggest that the cell wall of these strains may be structurally different in some way, which would account for the morphological transformation of the cells at pH 7.0. The morphology of cells of strains SP1, SP2, and MS3 were also affected by the Mg^{2+} concentration in the medium. In media containing more than 5.0mM Mg^{2+} at pH 9.5 or pH 8.5 the cells of these strains were distorted, in that at pH 9.5 the cells became spherical, whereas at pH 8.5 the cells became elongated. This is in contrast to the effects observed with known species (Brown & Gibbons, 1955), cells of such species requiring at least 0.1-0.2M Mg^{2+}, and becoming spherical in concentrations below 100mM. Mg^{2+} has been shown to be involved in the maintainance of the structure of the cell envelope, as well as being important in stabilising the ribosomes (Bayley & Morton, 1978; Kushner & Bayley, 1963; Kushner & Onishi, 1966; Lanyi et al., 1974; Stoeckenius & Rowen, 1964). In particular, the cell wall of H.cutirubrum requires greater concentrations of Mg^{2+} for stability when lipids are removed from the cell envelope. This suggests that there may be

ionic interactions between the cell envelope proteins and the positively charged groups within the lipid fraction. The low requirement of the strains SP1, SP2, and MS3 for Mg^{2+} suggests that the interactions within the cell envelope of these strains may be modified to allow the organism to cope with a more alkaline environment and the associated low levels of Mg^{2+}. In Gram-positive eubacteria grown under conditions of low Mg^{2+} concentration, teichoic acids are produced in larger quantities in both the cell wall and cytoplasm to increase capacity for Mg^{2+} binding (Ellwood & Tempest, 1972; Reaveley & Burge, 1972), but these compounds have not been described in the cell walls of members of the Halobacteriaceae (Bayley & Morton, 1978; Brown & Cho, 1970; Mohr & Larsen, 1963; Smithies et al., 1955; Steber & Schleifer, 1975; 1979; Steensland & Larsen ,1969), or any other archaebacteria (Balch et al., 1979; Brock et al., 1972; De Rosa et al., 1975; Kandler & Hippe, 1977; Kandler & Konig, 1978; Jones J et al., 1977; Millonig et al., 1975; Weiss, 1974).

The cell walls of H.salinarium, H.cutirubrum, and H.halobium have been shown to contain a glycoprotein of molecular weight 200,000d, as the main structural component (Konewicz, 1972; Mescher, personal communication, Mescher & Strominger, 1976a; 1976b; 1978; Mescher et al., 1974). Cells of strains SP1 and MS3 appear to contain a component which resembles this glycoprotein, in its electrophoretic mobility and staining characteristics (figure 95). Cells of strain SP2 did not contain such a glycoprotein (figure 95), but did possess a major protein staining band with a molecular weight of 50,000d, a weight comparable to the value found for the large molecular weight glycopeptide from H.salinarium (Mescher & Strominger, 1976a; 1978). The glycoprotein from the cell wall of H.cutirubrum, H.halobium, and H.salinarium is thought to be

responsible for the regular hexagonal array visible in electron micrographs of the outer region of the cell wall (Cho et al., 1967; D'Aoust & Kushner, 1972; Kirk & Ginzburg, 1972; Kushner & Bayley, 1963; Kushner et al., 1964; Steensland & Larsen, 1969). A similar subunit hexagonal, tetragonal, or pentagonal array has also been observed as a feature of the outer regions of the cell walls of the archaebacterial Sulfolobus acidocaldarius and certain methanogens (Balch et al., 1979; Jones J et al., 1977; Romesser et al., 1979; Weiss, 1974), and in the outer layer of cell walls of several Gram-positive and Gram-negative eubacteria (Drews et al., 1978; Sletyr, 1976; Thornley, 1976). The cell wall of the archaebacteria which possess this type of patterned array does not, however, contain a peptidoglycan layer below these subunit arrays, and it has been shown that in these organisms these subunits constitute the major structural layer of the cell wall (Jones J et al., 1977; Kandler & Konig, 1978; Mescher & Strominger, 1976b; 1978; Romesser et al., 1979; Steensland & Larsen, 1969; Stoeckenius & Rowen, 1964; Weiss, 1974). The strains SP1, and MS3 resemble extremely halophilic bacteria, in that they appear to contain a glycoprotein as the major structural component of the cell wall, but the occurrence of a cell wall devoid of glycoprotein is unusual in strain SP2, and is the first reported absence of a glycoprotein from the cell wall of an extremely halophilic bacterium belonging to the archaebacteria. Some other members of the archaebacteria have, however, been shown to contain an entirely proteinaceous cell wall (Balch et al., 1979; Millonig et al., 1975; Weiss, 1974), and a proteinaceous, glycoprotein free, cell wall may occur in other extremely halophilic archaebacteria, unexamined in this respect, such as H.volcanii, H.saccharovorum, H.vallismortis, and "H.marismortui" (Ginzburg, 1978; Kirk & Ginzburg, 1972; Mullakhanbhai & Larsen, 1975; Tomlinson &

Hochstein, 1976; Gonzalez et al., 1978). The major protein in the cell wall of strain SP2, of molecular weight 50,000d, may be similar in some respects to the large molecular weight glycopeptide found in H.salinarium (Mescher & Strominger, 1976a; 1978).

A characteristic feature of the organisms, which have a peptidoglycan in the cell wall, is their sensitivity to the antibiotic bacitracin, which is thought to inhibit glycosylation of the peptidoglycan backbone, and organisms containing murein,or the archaebacterial pseudomurein are sensitive to this antibiotic (Hammes et al., 1979; Siewert & Strominger, 1967; Storm & Strominger, 1973). However, H.salinarium, H,halobium, H.cutirubrum, and Methanococcus vannielii have also been shown to be sensitive to this antibiotic, although they contain neither murein, pseudomurein, nor any other comparable polymer (Jones J et al., 1977; Mescher & Strominger, 1975; 1976a; 1978). It has been postulated that the sensitivity of these species to bacitracin is due to inhibition of glycosylation of the structural glycoprotein, which may proceed via a lipid carrier as in glycosylation of peptidoglycan (Hammes et al., 1979; Mescher & Strominger, 1975; 1978). It has recently been proposed that bacitracin may also inhibit glycosylation of membrane lipids in H.cutirubrum (Basinger & Oliver, 1979), but the methanogenic archaebacteria, Methanosarcina barkerii, which has a heteropolysaccharide cell wall, and Methanospirillum hungatii, which has a proteinaceous, but not hexagonally patterned cell wall, are not sensitive to bacitracin (Hammes et al., 1979; Kandler & Hippe, 1977; Kandler & Konig, 1978), although the lipids found in the cell membrane of these two species are similar to those found in Methanococcus spp. and Halobacterium spp., which have glycoprotein in the cell wall (Balch et al., 1979; Jones J et al., 1977; Kates & Kushwaha, 1978; Mescher & Strominger, 1978). Within the halobacteria,

H.saccharovorum has been shown to be insensitive to 30µg of bacitracin (Tomlinson & Hochstein, 1976), a concentration which inhibits H.cutirubrum, H.halobium, and H.salinarium (Basinger & Oliver, 1979; Mescher & Strominger, 1975), and this may imply the absence of a glycoprotein. These results suggest that bacitracin may be useful in conjunction with other antibiotics in distinguishing the cell wall types of the archaebacteria as either proteinaceous/ heteroploysaccharide or glycoprotein/peptidoglycan (Hammes et al., 1979). At present only the glycoprotein and heteropolysaccharide cell wall types have been conclusively shown to occur in the genera Halobacterium and Halococcus respectively, among the extremely halophilic archaebacteria (Mescher & Strominger, 1976a; 1978; Mescher et al., 1974; Steber & Schliefer, 1975; 1979).

Similarities may be found between strain SP2, H.saccharovorum, H.vallismortis, H.volcanii, and "H.marismortui" in their physiology, biochemistry, morphology, and possible cell wall structure (table 18). In particular, all these organisms are capable of utilising carbohydrates for growth, and none of them has yet been shown to be capable of purple membrane synthesis (Ginzburg, 1978; Gonzalez et al., 1978; Larsen, personal communication; Stoeckenius, personal communication; Stoeckenius et al., 1979; Tomlinson & Hochstein, 1976). H.saccharovorum is insensitive to 30µg of bacitracin, which normally inhibits those species of halobacteria with a glycoprotein containing cell wall, but the recently isolated H.vallismortis is sensitive to bacitracin, implying that a glycoprotein was present in the cell wall, but this organism was tested in the presence of 150µg of bacitracin, and may result in the inhibition of other cellular functions (Basinger & Oliver, 1979; Gonzalez et al., 1978; Tomlinson & Hochstein, 1976). Cells of H.vallismortis also resemble those of strain SP2, when grown at low temperature. The

non-motile species "H.marismortui" also appears to resemble strain SP2 in its cell shape, and the formation of coccoid cells on solid media (Elazari-Volcani, 1957). There are also differences in the properties of the cell envelope of "H.marismortui" and H.halobium, seen in the permeability of the cell to macromolecules and appearance in electron micrographs (Ginzburg, 1978; Kirk & Ginzburg, 1972), which imply differences in the cell wall structure. Similarly, the low concentrations of sugar amines in the cell wall of strain SP2 and H.volcanii may be indicative of the absence of a glycoprotein (Mullakhanbhai & Larsen, 1975).

Cells of strains SP1 and MS3 were similar to H.halobium, H.cutirubrum, and H.salinarium, in that they contain a glycoprotein of similar molecular weight in the cell envelope (figure 95), and have comparable levels of sugar amines in the cell wall (table 18). All these organisms were similar in being able to produce a purple membrane (or similar membrane), and their inability to utilise carbohydrates for growth.

The amino acid composition of the bulk protein of cells of strains SP1, SP2, MS3, and H.halobium (table 16) were similar to those found previously in halobacteria and halococci (Brown, 1963; Mullakhanbhai & Larsen, 1975; Onishi & Kushner, 1966; Reistad, 1970; Steensland & Larsen, 1969). The results from the bulk protein analyses show an excess in the acidic amino acid content over the basic amino acid content of 15.7-19.7 mol%. The value of 20.9mol% acidic amino acid excess in the archaebacterium Methanospirillum hungatii (Kandler & Konig, 1978), and Acinetobacter strain MJT/F5/199A (Thornley, 1976) are comparable to those found in the bulk protein of the extremely halophilic archaebacteria, but in these two species the analyses apply to the cell wall, and the patterned, proteinaceous, external layer, respectively. The cell wall of

Table 18. Taxonomic features of the extremely halophilic archaebacteria.

	purple membrane produced	sugars[a] utilised	gas from sugars	nitrate reduction	gas from nitrate	gelatin hydrolysed	starch hydrolysed	motile	cell wall[b]
H.cutirubrum	+	-	-	-	-	+	-	+	G
H.halobium	+	-	-	+[c]	-	+	-[d]	+	G
H.salinarium	?	-	-	-	-	+	-	+	G
H.volcanii	?	+/O	-	-	-	-	-	-	P?
H.saccharovorum	-	+/F	+	+	-	-	-	+	P?
H.vallismortis	?	+/F	+	+	+	-	+	+	P?
H.trapanicum	-	-	-	+	+	-	-	-	?
Gt-1*	?	+/F	+	-	-	n.d.	n.d.	+	?
M_7*	?	+/F	+	+	+	n.d.	n.d.	+	?
SP1	+	-	-	-	-	-	-	+	G
SP2	-	+/?	-?	-	-	+	-	-	P
MS3	+	-	-	-	-	+	-	+	G
Hc.morrhuae	?	-	-	-	-	+	+/-	-	HP

a: sugars utilised by either a fermantative (F) or oxidative (O) pathway.

b: cell wall types, glycoprotein (G), proteinaceous (P), and heteropolysaccharide (HP).

c: results vary according to source

d: salt sensitive amylase detected

* strains isolated by Tomlinson & Hochstein (1972)

H.salinarium gives an excess of acidic amino acids over basic amino acids of 30 mol% (Mescher & Strominger, 1978), and the cell wall of the thermoacidophilic archaebacterium, S.acidocaldarius, has an excess in acidic amino acids over basic amino acids of only 7.2 mol% (Weiss, 1974). Thus, although the amino acid composition of the bulk protein of the extremely halophilic archaebacteria may give values similar to those for specific parts of the cell envelope of other archaebacteria and eubacteria, comparisons with the cell envelope of the halobacteria show that the highly acidic cell envelope may be characteristic of the halobacteria. The highly acidic nature of the cell wall in these organisms may account for the extremely halophilic nature of the cells, although it is known that other proteins of these extremely halophilic organisms are highly acidic (Werber et al., 1978). The strains SP1, SP2, and MS3 are distinguishable from H.salinarium in the greater excess of acidic amino acids (table 16), which may reflect the alkaliphilic nature of these extreme halophiles, which otherwise resemble members of the halobacteria.

The polar lipid composition of the strains SP1, SP2, and MS3 is different to that of members of the Halobacteriaceae so far examined (figures 97, 98) (Kates, 1978; Kates et al., 1966). The absence of glycolipids in these three strains suggest that the alkaline environment may influence the polar lipid composition. In the thermoacidophilic archaebacteria Thermoplasma and Sulfolobus (including the MT strains) the major polar lipids present are glycolipids (De Rosa et al., 1977a; 1977b; Langworthy, 1977b; Langworthy et al., 1972; 1974). The polar lipid composition of the halobacteria and halococci comprises both phospholipids and glycolipids, although the phospholipids constitute the major part of the polar lipid content (Kates et al., 1966; Kates, 1978). Like the lipids found in other archaebacteria they are glycerol ethers.

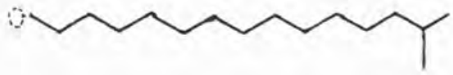
However, the Halobacteriaceae contain only di-O-phytanyl glycerol ethers, and the dibiphytanyl tetraethers present in many other genera of the archaebacteria are not present (Balch et al., 1979; De Rosa et al., 1974b; Kates, 1978). The presence of phospholipids as the predominant polar lipids, and the apparent absence of glycolipids in cells of strains SP1, SP2, and MS3 may account, in part, for the low Mg^{2+} requirement, since the increased phosphate content of the membrane may have a similar effect to the presence of teichoic acids in the cell wall of Gram-positive bacteria, grown under conditions of Mg^{2+} deficiency (Ellwood & Tempest, 1972; Reavely & Burge, 1972). However, this would only account for the stability of the membrane, and not the cell wall's integrity, or the function of the ribosomes (Bayley & Morton, 1978). The predominance of acidic phospholipids and acidic amino acids in the cell wall may serve some effect in screening the interior of the cell, and promote ionic interactions within the cell wall and membrane, resulting in greater envelope stability. The significance of these findings may be elucidated by a comparison of these organisms with extremely halophilic archaebacteria and possible alkaliphilic archaebacteria from Boulder Springs (Brock et al., 1971).

The virtual absence of fatty acids in extracts of cells treated by acid methanolysis, the absence of muramic acid, and the presence ether linked lipids in cells of strains SP1, SP2, and MS3 confirm that these organisms are members of the archaebacteria (Oro et al., 1978; Woese et al., 1978). The polar lipids from these three strains appear to be derived from an ether linked lipid, which is similar in its chromatographic properties to the 2,3-di-O-phytanyl glycerol ether found in methanogenic and extremely halophilic archaebacteria (Balch et al., 1979; Kates, 1978; Kates et al., 1966; Tornabene & Langworthy, 1979). The minor differences between the

chromatographic mobilities of the 2,3-di-O-phyatnyl glycerol ether and the equivalent ether from strains SP1, SP2, and MS3 may be indictaive of differences between the two lipids. A number of ether lipids have been found in the archaebacteria (figures 17, 18), but so far they all appear to be derivatives of a mono-phyatnyl ether, diphytanyl ether, or dibiphytanyl ether (figures 17, 18) found in various members of the archaebacteria (Balch et al., 1979; De Rosa et al., 1977a; 1977b; Hancock & Kates, 1977; Kates, 1978; Tornabene & Langworthy, 1979). Recently Chappe et al. (1979) isolated two novel ether lipids from the kerogen fraction of the Messel oil shale, which may represent two further ether lipids from either ancestors of the present day archaebacteria, or from organisms extant today, but as yet undiscovered. The lipids are a mono-pentadecyl ether, and a bipentadecyl ether (figure 103), and the geological history of the Messel oil shales suggests that they may have been present in organisms similar to the methanogenic archaebacteria, but not the extremely halophilic or thermoacidophilic archaebacteria (Chappe et al., 1979; Ourisson, personal communication).

Geochemical investigations for the presence of carbanaceous compounds have shown that numerous compounds are present with a great variation in chain length. However, a number of workers (Chappe et al., 1979; Hare & Hoering, 1972; Michaelis & Albrecht, 1979; Moldowan & Seifert, 1979) have shown that the biphytanyl residue from the dibiphytanyl ether may be isolated from oils, and that the intact glycerol ethers may be found in present day and fossil sediments (Anderson et al., 1977; Chappe et al., 1979). The degradation of lipids is largely dependant on the prevailing conditions (Didyk et al., 1978; Maxwell et al., 1973), and the ether lipids of the archaebacteria may be particularly resistant to biological degradation (Kushwaha & Kates, 1978), so they may remain

a.



b.

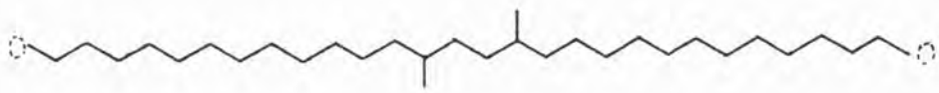


Figure 103. Structure of isopranyl side chains from ether lipids found in the Messel Oil Shales.

The isopranyl side chains isolated from ether lipids in the kerogen fraction of the Messel Oil Shales include the phytanyl and biphytanyl side chains similar to those found in the archaebacteria (figures 18a, b), and the novel pentadecyl (a) and bipentadecyl (b) isopranyl side chains.

unaltered for long periods of time.

The phytanyl, diphytanyl, and dibiphytanyl ethers found in the archaebacteria form a typical lipid bilayer in the cell membrane. The measured dimensions of the cell membrane are similar, whether the major lipids present are diphytanyl or dibiphytanyl ethers. The only major differences being that membranes consisting of dibiphytanyl ethers have no natural cleavage plane in the hydrophobic region (Langworthy, 1977a; Weiss, 1974), suggesting that the dibiphytanyl ether spans the cell membrane (figure 104). The similar dimensions of the diphytanyl ether cell membrane, and the presence of a natural cleavage plane suggests that in this type of membrane the lipid bilayer consists of two identical subunits (figure 104) (Cho et al., 1969; Kirk & Ginzburg, 1972; Steensland & Larsen, 1969). Examination of the possible spacial configurations of the pentadecyl and bipentadecyl ethers, isolated by Chappe et al., (1979) from the Messel oil shale, show a similar relationship to the phytanyl and biphytanyl ethers (figure 105), in that the bipentadecyl side chain is a dimer of the pentadecyl isopranyl side chain. Membranes composed of these two lipids would, theoretically, show little difference from the dimensions of the membranes formed from the diphytanyl and dibiphytanyl ethers. There is no evidence that the lipids of Chappe et al. (1979) are present in archaebacteria, although there is no apparent limitations on their occurence in archaebacterial type membranes. It is unlikely that the lipids found in strains SP1, SP2, and MS3 are similar to these ether lipids, since significant differences in the number of carbon atoms present may be expected to show up as significant differences in the mobility of the 2,3-di-O-phytanyl glycerol ether and possible pentadecyl or bipentadecyl ethers. However, since these two lipids were unavailable for comparison, these structures can not be

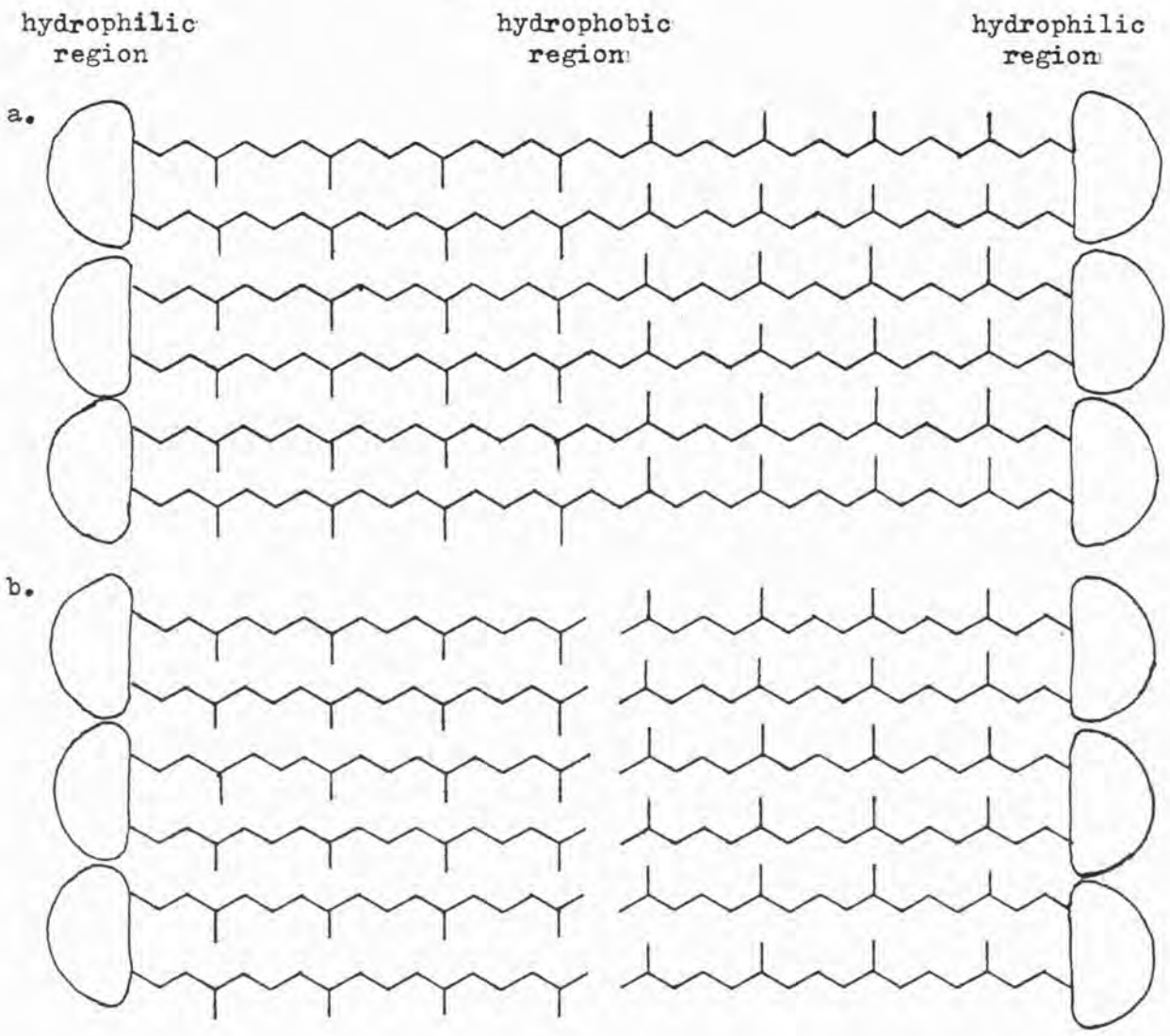


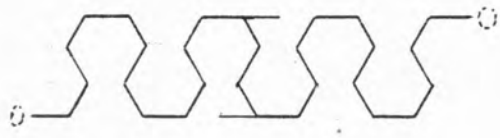
Figure 104. Ether lipids in the cell membrane of archaebacteria.

The ether lipids found in members of the archaebacteria form a typical lipid bilayer. Examination of the cell membrane of the thermoacidophiles suggests that the dibiphytanyl tetraethers span the membrane, creating a membrane in which there is no natural cleavage plane in the hydrophobic region (a). The lipid bilayer in the extreme halophiles has the same dimensions as that found in the thermoacidophiles, and is presumably composed of two similar lipids spanning the membrane, since the membrane has a natural cleavage plane in the hydrophobic region (b).

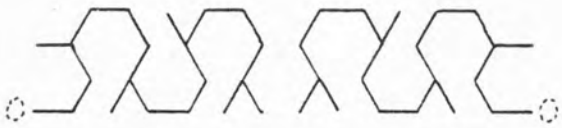
Figure 105. The possible spacial configuration of isopranyl side chains found in ether linked lipids.

The spacial configuration of the various isopranyl ether lipids are represented in two dimensions, and further illustrates their relationship to membrane structure. Measurements of the dimensions of the dibiphytanyl tetraether lipids from thermoacidophiles suggests that the isopranyl side chains are folded. Assuming regular folding of the isopranyl chain, the biphytanyl and bipentadecyl chains may be seen to have similar configurations. If the cell membrane is spanned by the biphytanyl side chain, then the bipentadecyl chain may have a similar role, if found in archaebacterial membranes. If, as suggested by study of the cell membrane of the halobacteria, two phytanyl side chains are required to span the cell membrane, then the two phytanyl side chains may also take up a configuration similar to that of the biphytanyl side chain. The pentadecyl side chain may also adopt a similar configuration to the phytanyl side chains, also suggesting that they may have a similar function if found in archaebacterial membranes.

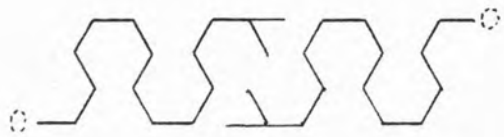
biphytanyl isopranyl side chain



bipentadecyl isopranyl side chain



phytanyl isopranyl side chain



pentadecyl isopranyl side chain

discounted until the nature of the isopranyl side group is determined by chromatography or mass spectroscopy.

Certain ether lipids form a semi-cyclic configuration, as seen in the occurrence of five membered rings (figure 18) in the lipids of the thermoacidophilic Sulfolobus-like MT strains of De Rosa and co-workers (De Rosa et al., 1977a; 1977b). The ring structures are probably present to stabilise the hydrophobic region against the effects of high temperature, since lipids extracted from cells of the MT strains show a greater degree of cyclisisation at higher temperatures (De Rosa et al., 1977a). It is unlikely that the lipids of strains SP1, SP2, and MS3 are derived from the w',w-biphytanyl containing ethers, whether straight chain or cyclic, on the basis of Rf values. However, the straight chain and cyclic forms of the ether lipids found in the MT strains, in certain solvent systems, are indistinguishable from one another (De Rosa et al., 1977a; 1977b). This does not preclude that the lipids of strains SP1, SP2, and MS3 may be cyclisised forms of the 2,3-di-O-phytanyl glycerol ether found in the halobacteria. It seems that the ether lipids of the strains SP1, SP2, and MS3 are similar to those found in the halobacteria, since the presence of the phytanyl ethers is a constant feature of this group of extremely halophilic organisms. The data (figures 97, 98) also suggests that there may be differences in the functional side groups of some of the lipids of H.salinarium, and strains SP1, SP2, and MS3. Thus, the lipids of the organisms isolated from the salterns, and Lake Magadi, may show some differences to those found in the Halobacteriaceae, but their nature is likely to conform to the restrictions on lipid structure found in biological membranes.

The neutral lipids of strains SP1, SP2, and MS3 were predominantly squalenes and isoprenoid carotenoids, indicating

similarities to the recognised members of the Halobacteriaceae, and taken together with the polar lipid analysis further supports their inclusion as members of the archaebacteria. The involvement of squalenes in the stabilisation of polar lipid membranes in the halobacteria has been demonstrated by Lanyi et al. (1974), and illustrates that these compounds may functionally replace sterols in the cell membrane.

The polar lipids found in prokaryotes and eukaryotes form the basis of the membrane structures proposed by Ourisson and co-workers (Ourisson et al., 1979; Rohmer et al., 1979). The polar lipids are usually spacial arranged within the cell membrane in association with neutral lipids, which may be divided into three classes, isoprenoids, hopanoids, and sterols (Rohmer et al., 1979; Ourisson et al., 1979). Examination of their structure shows that squalenes, hopanoids, and sterols are of similar dimensions, and would occupy the hydrophobic region of the membrane. It has been speculated that hopanoids are phylogenetic precursors of sterol. Bloch (1976) has also speculated that squalenes would be the end product of sterol biosynthesis in an anoxic environment, which suggests that such compounds may be phylogenetic precursors of hopanoids and sterols. The division of prokaryotes and eukaryotes into phylogenetic groupings according to neutral lipid analyses may be seen to produce results similar to 16S and 18S rRNA analyses (Gibson et al., 1980; Ourisson et al., 1979), in that the archaebacteria contain only squalenes or its derivatives as the major neutral lipids (table 19), adding weight to the speculation that such organisms evolved in an anoxic environment. However, squalenes have also been found in a number of Gram-positive and Gram-negative eubacteria (Amdour et al., 1976; Novitsky & Kushner, 1978), indicating that these organisms could also be considered to have evolved in an anoxic environment. These

Table 19. The major phylogenetic divisions of life.

Kingdom	Urkingdom	nature of polar lipids	nature of major neutral lipids	type of cell wall
Prokaryota	Archaebacteria	glycerol linked to isopranyl side chains via ether bonds	isoprenoids	a. no cell wall b. proteinaceous c. glycoprotein d. peptidoglycan e. polysaccharide
	Eubacteria	glycerol linked to fatty acid side chains via ester bonds	isoprenoids hopanoids	a. no cell wall b. peptidoglycan
Eukaryota		glycerol linked to side chains via ether or ester bonds	hopanoids sterols	a. no cell wall b. proteinaceous c. glycoprotein d. polysaccharide

two groups are, however, distinguishable from one another in 16S rRNA analyses. Organisms containing hopanoids may be considered as forming another phylogenetic group, although this includes both eubacteria and eukaryotes (table 19), which are distinguishable in rRNA analyses. The third group consists of eukaryotes, which are dependant on oxygen for the synthesis of sterols (table 19).

The discovery of a heavy membrane fraction in strains SP1 and MS3, with an absorption spectrum not too dissimilar to that of the purple membrane from H.halobium and H.cutirubrum, indicates that a membrane with a similar function may exist in these strains. Although this membrane has not been isolated in pure form a number of points indicate that this membrane may not be identical to the purple membrane of H.halobium and H.cutirubrum. The purple membrane consists of lipids and bacteriorhodopsin as discrete patches in the cell membrane. SDS polyacrylamide gel electrophoresis (figure 95) indicates that in the strains SP1 and MS3 a bacterio-opsin like protein may be present, but is apparently absent from strain SP2, which does not contain a purple membrane-like fraction. The inability to detect retinal in cells and membrane fractions from strains SP1 and MS3 may suggest that the extraction proceedures used for the purple membrane were inappropriate for this type of membrane, but it may also be noted that a number of other isoprenoids may substitute for retinal in artificially prepared membranes (Stoeckenius et al., 1979), although all-trans and 13-cis retinal are the only naturally occuring isoprenoids found in bacteriorhodopsin. However, cyanopsin, containing 11-cis-3-dehydro-retinal, has an absorption maximum at 620nm, similar to the maximum of 625nm for membranes from strains SP1 and MS3, which may account for the absence of retinal in extracts of the heavy membranes from strains SP1 and MS3 (Hubbard et al., 1971). Investigations of the

lipid content of red and purple membrane fractions of H.halobium and H.cutirubrum (Kushwaha et al., 1975a; 1976) have shown that the ether derivatives of phosphatidyl glycerosulphate, the triglycosyl, and glycolipid sulphate present in H.halobium and H.cutirubrum are almost exclusively confined to the purple membrane, and may serve an important function in ion transport, particularly proton translocation across the purple membrane. Significantly, these lipids were not detected in extracts of strains SP1 and MS3, suggesting that the lipids of the heavy membrane fraction from strains SP1 and MS3 may contribute to some of the differences observed. That the different absorption maximum of the heavy membrane from strains SP1 and MS3 is not due to the effect of pH on a typical halobacterium purple membrane is illustrated by the blue shift in the absorption maximum of purple membrane at acid pH, and a red shift at alkaline pH (Oesterhelt & Stoeckenius, 1971; Stoeckenius et al., 1979), an effect converse to that required to account for the blue shift in the alkaliphilic halobacterial membrane (figure 92). The speculated changes in structure and physical properties may be the result of adaptation of the membrane to alkaline conditions to allow for its continued function at high pH. In purple membrane isolated from H.halobium and H.cutirubrum the rate of dark adaption is affected by alterations in the pH (Lozier et al., 1974; Ohno et al., 1977). At pHs of 9.0 and above the rate of dark adaption becomes very slow, which affects the efficiency of the purple membrane. Any membrane with a similar function, from an organism living at a pH greater than 9.0 may be expected to function at an optimal rate close to the pH of the environment, which may result in changes in the maximal rate of dark adaption to pH 9.0 or greater. Furthermore, it has been shown that the highest cytoplasmic pH tolerated by even alkaliphiles may

be pH 9.0-9.5, the cytoplasm remaining at this pH, and becoming acidic, with respect to the medium, when the external pH was raised above pH 9.5 (Guffanti et al., 1977). This also raises the question of any potential proton gradient across the membrane of these alkaliphilic halobacteria. If a specialised photosensitive membrane exists for proton translocation, as in purple membrane containing halobacteria, then is the direction of proton translocation reversed above pH 9.0-9.5?

The present taxonomy of the family Halobacteriaceae is based on the examination of a restricted group of isolates, mainly H.cutirubrum, H.halobium, H.salinarium, and Hc.morrhuae (Colwell et al., 1979; Gibbons, 1974; Kocur & Hodgkiss, 1973). There is little dispute about the taxonomic position of the halococci, but despite the plethora of work on the purple membrane of the halobacteria the taxonomy of this group is still ill-defined. Bergey's eighth edition (1974) recognises only two species, H.halobium and H.salinarium, H.cutirubrum being regarded as a synonym of H.salinarium. The organisms H.trapanicum, "H.marismortui", and "Amoebobacter morrhuae" are regarded as species incertae sedis, due to the lack of sufficient data on these organisms. The taxonomy of the Halobacteriaceae is apparently based on the unpublished data of Colwell and Gibbons (Gibbons, 1974). Recently, Colwell et al. (1979) have published a taxonomic study of the red halophilic bacteria, which apparently includes the unpublished data of Colwell and Gibbons. The taxonomy of the Halobacteriaceae is further confused in this study, in which the species H.cutirubrum and H.salinarium are recognised, but H.halobium is regarded as being similar to H.cutirubrum, and may be synonymous with this species (Colwell et al., 1979). The present taxonomy of the Halobacteriaceae requires revision in view of the isolation of the new species H.volcanii (Mullakhanbhai &

Larsen, 1975), H.saccharovorum (Tomlinson & Hochstein, 1976), and H.vallismortis (Gonzalez et al., 1978), and the reisolation of "H.marismortui" (Ginzburg, 1978). Other halobacteria have been isolated (Tomlinson & Hochstein, 1972a), which are different to presently described species, and suggest that the reduction of nitrate or nitrite, and the utilisation of carbohydrates may be significant taxonomic features (Dundas, 1978; Elazari-Volcani, 1957; Tomlinson & Hochstein, 1972a).

The members of the archaebacteria have recently been subject to a revision of their taxonomy, which may serve to provide criteria for the revision of the taxonomy of the halobacteria (Balch et al., 1979). Analysis of the 16S rRNA sequence of the methanogenic bacteria in the family Methanobacteriaceae (Bergey's Manual, 1974) has shown that there are at least three major groupings present, which have been described by the three taxa, at order level, Methanobacteriales, Methanomicrobiales, and Methanococcales (Balch et al., 1979). The three orders are divided into four families, which are characterised by certain features, and are consistent with the groupings proposed by 16S rRNA analysis. Analysis of the DNA-depandant RNA polymerase, also found in other archaebacteria, from the Sulfolobus-"Caldariella" group has also led to a revision of the taxonomic divisions of these thermoacidophiles (Zillig et al., 1980). A similar analysis of the 16S rRNA sequence and DNA-dependant RNA polymerase of the extremely halophilic bacteria would be invaluable in determining the phylogenetic and perhaps the taxonomic relationship of this group. In the absence of such information there are, never the less, certain indications of groupings, which may prove useful in further studies.

The extremely halophilic bacteria may be divided into two distinct morphological groups, the rod shaped halobacteria, and the coccoid

halococci. It should, however, be noted that one of the so called halobacteria species, H.volcanii, is not a rod, and has a plate like morphology (Mullakhanbhai & Larsen, 1976). Scanning electron micrographs of cells illustrate clearly the dished or cup-shaped morphology of the cells (Larsen, unpublished micrograph). The halococci presently constitute a separate genus, Halococcus, within the family Halobacteriaceae (Gibbons, 1974), separated from the genus Halobacterium on the basis of its Gram-positive cell wall sacculus, the absence of motility, and its coccoid morphology. The division of the halococci from the halobacteria is supported by previous studies (Colwell et al., 1979; Kocur & Hodgkiss, 1973). The halobacteria are a more heterogeneous group than the halococci, and fall into several natural groupings. The organisms which do not utilise carbohydrates, exemplified by H.cutirubrum, H.salinarium, and H.halobium, constitute one group. The strains SP1 and MS3 are similar to these organisms, in that they all appear to contain a glycoprotein in the cell wall, have a satellite DNA band, are capable of synthesising a purple or similar membrane, and are incapable of utilising carbohydrates for growth. The organisms are typically motile rods, and there is evidence that these organisms are sensitive to bacitracin (30μg), and the ferredoxin found in these organisms (perhaps excluding strains SP1 and MS3) may be characteristic (Ginzburg, 1978; Kerscher et al., 1976; Mescher & Strominger, 1975; Susman et al., 1979; Werber & Mevarech, 1979b; Werber et al., 1978). The ability to grow anaerobically in the presence of nitrate has been observed in "H.marismortui" (Werber & Mevarech, 1979a), which may be associated with the two different functions of the structurally different, plant-like, 2Fe-ferredoxins found in H.halobium and "H.marismortui" (Ginzburg, 1978; Kerscher et al., 1976; Sussman et al., 1979). The inability to utilise

carbohydrates for growth may be connected with the synthesis of purple or equivalent membranes, although it has been shown that H.halobium contains a salt sensitive amylase. It has been suggested that the satellite DNA band is responsible for the formation of gas vacuoles and purple membrane (Simon, 1979; Weidinger et al., 1979). The possibility that purple membrane synthesis is associated with the satellite DNA band needs to be clarified, particularly since it may contain one or more plasmids, and the investigation of the presence of satellite DNA bands in the carbohydrate utilising species may ellucidate the relationship.

Organisms which use carbohydrates fermentatively are represented by "H.marismortui", H.saccharovorum, H.vallismortis, and possibly strain SP2 (table 18), and do not appear to be capable of synthesising purple membrane (Ginzburg, 1978; Stoeckenius, personal communication; Stoeckenius et al., 1979; Tomlinson & Hochstein, 1976). Strain SP2 does not possess a satellite DNA band, and a glycoprotein was not evident in the cell wall. The species "H.marismortui", H.saccharovorum, H.vallismortis, and H.trapanicum have not been examined with regard to these features, although the insensitivity of H.saccharovorum to 30µg bacitracin suggests that a glycoprotein may be absent (Hammes et al., 1979; Mescher & Strominger, 1975; Tomlinson & Hochstein, 1976). H.saccharovorum and H.vallismortis are apparently motile, although flagella have not been observed, while "H.marismortui", H.trapanicum, and strain SP2 are non-motile (Elazari-Volcani, 1957; Gonzalez et al., 1978; Tomlinson & Hochstein, 1976).

The plate-like organism, H.volcanii, is the only isolate of this type of organism (Mullakhanbhai & Larsen, 1975), but Walsby (1980) has observed a morphologically similar organism in highly saline pools in the Sinai Penninsula. H.volcanii is a non-motile

fragile, slightly dished flat cell, which utilises carbohydrates oxidatively. Some similarity to the cell wall of strain SP2 is suggested by the low values of sugar amines found in these two organisms (table 14) (Mullakhanbhai & Larsen, 1975).

The members of the halobacteria described to date may be divided in to at least two groups on the basis of the data which is presently available. The organisms exemplified by H.cutirubrum, H.halobium, H.salinarium, and strains SP1 and MS3 are similar to one another in their inability to utilise carbohydrates, the possession of a satellite DNA band, the presence of a glycoprotein in the cell wall, and, with the possible exception of H.salinarium, are usually capable of synthesising a purple or similar membrane. There is less data on the other species of halobacteria, but the evidence suggests that H.saccharovorum, H.vallismortis, H.trapanicum, "H.marismortui", H.volcanii, and strain SP2 are similar to one another. Although all these organisms have not been tested with regard to all the following criteria, the characteristics of this group appears to be the ability to utilise carbohydrates for growth, the inability to produce purple or similar membranes, the absence of a satellite DNA band, and the absence of a glycoprotein in the cell wall (table 18).

The organisms which produce purple membrane are able to grow under low oxygen tension in the presence of light, whereas other organisms would fermantatively utilise carbohydrates under similar conditions. Although salterns have often been described as aerobic environments, the presence of such high concentrations of salt, together with the elevated temperatures associated with efficient solar salt production, would limit the solubility of oxygen, even if it were produced abundantly by organisms such as the alga Dunaliella. The ability to produce energy under conditions of low

oxygen tension would, therefore, have a selective advantage, and some of the carbohydrate utilising strains have been shown to be capable of facultative anaerobic growth (Gonzalez et al., 1978; Werber & Mevarech, 1979a).

Analysis of the cell envelope of the methanogenic bacteria has shown that cell wall structure and nature of the ether lipids in the cell wall are indicative of the taxonomic divisions proposed by comparison of the 16S rRNA sequences, and are, therefore, also indicative of the phylogenetic divisions of these organisms (Balch et al., 1979). The separation of the Gram-positive methanogenic bacteria at the family level suggests that the Gram-positive genus Halococcus may be deserving of higher taxonomic status, at the family level (Balch et al., 1979; Brown & Cho, 1970) (table 20). The cell wall is not atypical among the archaebacteria, in that it resembles the heteroploysaccharide found in Methanosarcina (Steber & Schliefer, 1975; Kandler & Hippe, 1978), although it has recently been proposed that it may be a novel peptidoglycan (Steber & Schliefer, 1979), perhaps similar to that found in the genera Methanobacterium and Methanobrevibacter (Balch et al., 1979; Kandler, 1979; Kandler & Konig, 1977). The insensitivity of the extremely halophilic cocci of the genus Halococcus to penicillin (Colwell et al., 1979) does not prove that the cell wall is not a peptidoglycan, although it is not murein (Hammes et al., 1979). The Gram-negative halobacteria may belong to the same family, but separate from the halococci. Differences in the nature of the cell wall (table 18), particularly in the presence of glycoproteins, may indicate generic differences as in the methanogenic bacteria (table 20) (Balch et al., 1979), and the evidence suggests that there may, therefore, be more than one major group within the halobacteria.

The lipid analyses show that most members of the halobacteria

are essentially similar in their polar lipid content (Kates, 1978; Kates et al., 1966), but the strains isolated in this study are a notable exception, in that they do not contain glycolipids, contain novel phospholipids, and may be derived from a novel ether lipid (figures 96, 97, 98). These strains also show physiological differences, which suggest that, although the strains SP1, SP2, and MS3 are halobacteria, in requiring 3.0-4.5M NaCl for growth (figures 83, 84, 85), they show peculiarities, in being alkaliphilic halobacteria, and may, therefore, represent a phylogenetic and taxonomically distinct branch of the halobacteria, deserving of generic and possibly family status (table 20).

The strains SP1, SP2, and MS3 may be differentiated from one another on a number of physiological differences. Strain SP2 is evidently different from strains SP1 and MS3, in that it does not possess a satellite DNA band, or heavy membrane fraction, and is capable of utilising carbohydrates . The absence of a glycoprotein in the cell wall of strain SP2 also clearly distinguishes this strain from strains SP1 and MS3, These differences appear to justify its classification in a separate taxon at the specific, and possibly generic level. It may be argued that such differences are due to the absence of the satellite DNA band in strain SP2, which is a large plasmid. However, evidence from the methanogenic archaebacteria suggests that differences in the cell wall are stable genetic features, and there is no evidence to suggest that the satellite band carries information for the suppression of carbohydrate utilisation, or that it is ever lost from those species which contain the satellite DNA. Investigations of the plasmid composition of the satellite suggest that it may not be entirely composed of plasmids, although they are present. The differences between strains SP1 and MS3 are less significant, and the differences in NaCl optima (figures 83, 85) of

Table 20 Reappraisal of the taxonomy of the extremely halophilic archaebacteria (Halobacteriales, ordo nov.)

Group I: Halococci (family Halococcaceae fam. nov.)

genus, Halococcus, Schoop, 1934

species, Halococcus morrhuae (Farlow, 1880) Kocur & Hodgkiss, 1973

Gram-positive, non-motile cocci, with a heteropolysaccharide in the cell wall. Have a satellite DNA band.

Group II: Halobacteria (family Halobacteriaceae, Gibbons, 1974)

genus I, Halobacterium, Elazari-Volcani, 1957

species, Halobacterium cutirubrum, (Lochhead, 1934) Elazari-Volcani, 1957

Halobacterium halobium, (Petter, 1931) Elazari-Volcani, 1957

Halobacterium salinarium, (Harrison & Kennedy, 1922) Elazari-Volcani, 1957

Gram-negative, motile rods, with a glycoprotein in the cell wall. May produce purple membrane, and do not utilise carbohydrates for growth. Have a satellite DNA band.

genus II, (Halomonas, gen. nov.)

species, Halobacterium saccharovorum, Tomlinson & Hochstein, 1976

Halobacterium vallismortis, Gonzalez et al., 1978

Halobacterium trapanicum, (Petter, 1931) Elazari-Volcani, 1957

Halobacterium marismortui, Elazari-Volcani, 1957

Gram-negative rods, with a proteinaceous cell wall. Do not produce purple membrane, and are capable of utilising carbohydrates fermentatively. May not have a satellite DNA band. Species may be motile or non-motile.

Table 20 (cont.)

Group II:

genus III, (Halopedia, gen. nov.)

species, Halobacterium volcanii, Mullakhanbhai & Larsen, 1975

Gram-negative, non-motile plates. May not have a satellite DNA band, and may not produce purple membrane, may utilise carbohydrates oxidatively, and may not have a glycoprotein in the cell wall.

Group III: Alkaliphilic halobacteria (Natralobacteriaceae, fam. nov.)

genus I, (Natralobacterium, gen. nov.)

species, strain SP1 (MS3)

Gram-negative, motile rods, with a glycoprotein in the cell wall. May produce a purple-like membrane, and do not utilise carbohydrates for growth. Have a satellite DNA band. Are obligately alkaliphilic, growing media almost devoid of Mg^{2+} at pH 9.5-10.0, and have a lipid composition distinctive from Group II.

genus II, (Natralomonas, gen. nov.)

species, strain SP2

Gram-negative, non-motile rods, without a glycoprotein in the cell wall. Do not produce a purple membrane, and are capable of utilising carbohydrates for growth. Do not have a satellite DNA band. Are obligately alkaliphilic, growing media almost devoid of Mg^{2+} at pH 9.5-10.0, and have a lipid composition distinctive from Group II

The generic name Halomonas has recently been used to describe a new genus of salt tolerant bacteria (Vreeland et al., 1980). This genus is not, as the generic name seems to imply, a member of the extremely halophilic archaebacteria.

these two strains may be indicative of natural variation between strains, as may be the differences in the GC content. The sensitivity of strain MS3 to O/129 indicates a similarity to strain SP2, as does the ability of strain MS3 to rapidly hydrolyse gelatin. In most respects strain MS3 is more similar to strain SP1, in its possession of a satellite DNA band, and a heavy membrane fraction, motility, inability to utilise carbohydrates, and in the occurrence of a glycoprotein in the cell wall. However, the differences between strains SP1 and MS3 may be indicative of two different species within the same genus, although their relationship remains to be determined by other means. The further study of these strains may prove useful in determining the taxonomic position of the various extremely halophilic archaebacteria extant today.

WATER CHEMISTRY OF THE KENYAN SODA LAKES AND ITS INFLUENCE ON THE ECOLOGY OF THE PHOTOTROPHIC BACTERIA.

The microorganisms living in the alkaline saline lakes of the Rift Valley, Kenya have been little studied in relation to their ecology and physiology. The algae constitute a significant part of the primary productivity of these type of lakes (Hecky & Kilham, 1973; Talling et al., 1973), but the presence of members of the Rhodospirillaceae has not been thoroughly investigated. The occurrence of microorganisms in Lake Magadi and in the salterns has not been properly investigated until now. Previous work suggested that the colour of the saltern liquor was due to either carotenoids from flamingo feathers, or to the presence of red algae (Baker, 1958; Walther, 1922). The results of this investigation show that large numbers of extremely halophilic bacteria, together with unidentified members of the Rhodospirillales impart the characteristic colour to the saltern liquor. The nature of the organisms present, their physiological adaptations, and their evolution in the alkaline saline lakes may contribute to our understanding of the limits of life on Earth, or indeed other planets (Mills, personal communication).

It has been shown that macromolecules found in living cells, such as lipids and proteins may be synthesised abiotically under conditions present in the Early Pre-Cambrian Earth (Deamer, 1978). These molecules may form into large organised aggregates when present in the correct concentrations. The concentration of organic compounds probably increased by evaporative concentration, which would have been possible due to the elevated temperatures of the primitive Earth (Hargreaves & Deamer, 1978; Oro et al., 1978). There is good reason to believe, therefore, that life probably evolved in a hot aqueous environment, where the concentrations of organic macromolecules and inorganic solutes were raised by evaporative

concentration. In certain cases isolated areas of water may have concentrated to salinities similar to those found in saline lakes. In the more dilute regions, including the sea, the pH was probably slightly alkaline, due to the presence of bicarbonate (Hargreaves & Deamer, 1978), but it is possible that in very concentrated brines the alkalinity was further increased by the concentration of bicarbonate, giving rise to the presence of large amounts of carbonate, forming an alkaline, saline lake (Eugster & Hardie, 1978; Hardie & Eugster, 1970), similar to those found today in the Rift Valley, Kenya. In extreme cases this would result in the formation of saline lakes with saturated brines, such as those at Lake Magadi and those of the Wadi Natrun (Eugster, 1970; Eugster & Hardie, 1978; Hardie & Eugster, 1970; Imhoff et al., 1978b; Jones B et al., 1977).

It has been possible to show that both ester and ether lipids may be synthesised abiotically. In particular, diether glycerol lipids, which were indistinguishable from those found in H.cutirubrum, have been synthesised abiotically under primitive Earth conditions (Hargreaves & Deamer, 1978). If ester lipids and these archaebacterial ether lipids were synthesised under alkaline, saline conditions, then alkaliphilic halobacteria and other alkaliphiles may have evolved in this type of environment. There is some evidence to suggest that the formation of early membranes from abiotically synthesised macromolecules may have been enhanced by the low concentrations of divalent ions typical of alkaline, saline lakes (Eugster & Hardie, 1978; Hargreaves & Deamer, 1978; Hardie & Eugster, 1970), and that, although a wide range of pH did not interfer with membrane formation, ether lipids were more stable in the alkaline environment (Hargreaves & Deamer, 1978). However, the significance of large numbers of alkaliphilic halobacteria at Lake Magadi does not directly support any speculation that such an

environment may have given rise to the earliest organisms of this type from abiotically synthesised macromolecules, since none of the Kenyan soda lakes are older than the Cretaceous (Baker, 1958; Baker et al., 1972; McCall, 1967; Thompson & Dodson, 1963). The geological record of the Rift Valley shows that a number of lakes have occupied the floor of the rift before the present day lakes (Baker, 1958; McCall, 1967; Thompson & Dodson, 1963). Many of the early lakes were more extensive and were probably more dilute. During the course of geological time the lake levels in the rift have fluctuated due to a combination of geological and climatic changes. Although all the lakes were formed by water sheds within the rift draining into the lakes, those lakes in central and southern Kenya, which lay south of the highest point of the Kenyan dome would have no outflow from the Rift Valley. In the wettest periods the lakes of the central region may have overflowed into the lowest point in the south of the rift, at the present day lakes Magadi and Natron. Evidence that the fluctuating water levels affects the biota of the lakes by changes in the solute concentrations have been observed at Lake Naivasha (Richardson & Richardson, 1972). During the wettest periods of the Rift Valley's history many of the present day lake basins were part of more extensive lakes, such as the Gamblian Lake(s) covering lakes Naivasha, Nakuru, and Elmentieta (Thompson & Dodson, 1963), the lake once joining the basins of Lake Bogoria and Lake Baringo (McCall, 1967), and the Olorgasaille Lake covering the Magadi basin (Baker, 1958). The interconnection of the present day lakes at that time by more extensive bodies of water, and outflow to other lakes (Butzer et al., 1972) would have led to the transfer of biological matter between the present day lake basins, so that when these basins were eventually isolated by climatic and geological changes, the

organisms found in the lakes would probably have been similar. This would account for the similarities between the lakes in the organisms examined, but not for their introduction. Possible sources for the organisms found in the soda lakes include, the sea, inflowing waters, and other alkaline, saline lakes. Birds, such as the flamingos, which migrate vast distances between the soda lakes of the African Rift Valley, would be capable of transfering microorganisms from one lake to another. Organisms similar to those found in the Kenyan soda lakes have been isolated from a number of environments. Ectothiorhodospira-like organisms have been isolated from a wide range of environments, including freshwater, the sea, and alkaline, saline lakes (Baas-Backing, 1928; Herbert & Tanner, 1977; Imhoff & Trüper, 1976; Imhoff et al., 1978a; 1978b; Kondrat'eva, 1956; Matheron & Bauliague, 1972; Pelsh, 1937; Yang Hui-Fang, 1962), although they occur most abundantly in alkaline environments over a wide range of salinity (Grant et al., 1979; Imhoff et al., 1978b; 1978a). These organisms are not the only phototrophic bacteria found in the Kenyan Rift Valley, and under suitable conditions organisms resembling members of the purple non-sulphur and green gliding phototrophic bacteria may be found. The extremely halophilic bacteria are more restricted in their ecology, occupying only extremely saline environments, although it has recently been shown that halococci may survive in the sea for some time (Rodriguez-Valera et al., 1979). The alkaliphilic halobacteria seem to be characteristic of the alkaline, extremely saline environment (Tindall et al., 1980; Imhoff et al., 1978b). The closest extremely saline environments to the Wadi Natrun and Lake Magadi, which have been studied, are in the Middle East and East Africa. Studies of the Dead Sea have shown that halobacteria, represented by the species H.volcanii, H.trapanicum, and H.marismortui

are the commonest extreme halophiles (Elazari-Volcani, 1957; Ginzburg, 1978; Mullakhanbhai & Larsen, 1975). An organism morphologically resembling H.volcanii has been described from saline pools in the Sinai Penninsula (Walsby, 1980), and an investigation of the hypersaline Lake Assal, in French Somaliland, which has a chemical composition similar to that of the Dead Sea (Brisou et al., 1974) has shown that H.trapanicum is also present. These saline lakes, which lie fairly close to the Wadi Natrun and Lake Magadi, have a distinctive population of extremely halophilic bacteria, while the alkaline, saline lakes of the Wadi Natrun and Lake Magadi harbour physiologically similar organisms. Whether these organisms from the alkaline, saline lakes, those of the Dead Sea, and those from sea water salterns, such as H.halobium and H.saccharovorum, all arose independantly in these environments, or arose by adaptation of a common ancestor is a moot point. In contrast, the members of the Rhodospirillales isolated from the soda lakes show a physiological adaptation to the alkaline conditions, which is manifest in similar organisms isolated from less alkaline waters. These organisms appear to constitute a separate ecological and physiological group within the Rhodospirillales.

Both groups of organisms examined show adaptation to the saline conditions, which appear to influence the ecology of the lakes. In the more dilute lakes the extremely halophilic archaebacteria are absent, and the prevalent phototrophs grow optimally in salinities of 1.0-5.0% (w/v) NaCl, which represents typical salinity fluctuations within these lakes. In the more saline lakes, such as Lake Magadi, the extremely halophilic archaebacteria are evident, and the phototrophs present are capable of optimal growth at the salinity of the lake, or of tolerating such high salinities (Imhoff & Trüper, 1977; in preparation; Imhoff et al., 1978a; 1978b). The presence

of significant amounts of CO_3^{2-} ions in these saline lakes results in an alkaline pH between 9.0 and 11.0 (Imhoff et al., 1978b; Talling and Talling, 1965). The optimal pH for growth of the alkaliphilic extremely halophilic archaebacteria is pH 9.0-10.0, and is similar to that found for the phototrophs grown under photoorganotrophic conditions. It is evident from this and other studies that the upper limit of pH for growth is within the region pH 11.0-12.0 (Brock & Darland, 1970; Gee et al., 1980; Guffanti et al., 1978; Souza & Deal, 1977; Souza et al., 1974). The optimal pH for growth of many alkaliphilic organisms is often close to pH 9.5, and this may be connected to the finding that the internal pH of some alakliphiles does not become acidic with respect to the external environment until the external pH reaches pH 9.0-9.5 (Guffanti et al., 1978). It also appears that in the organisms studied by Guffanti et al. (1978), the transmembrane potential, essential for growth, was dissipated as the pH increased above pH 9.5, and approached 11.0. A further consequence of growth in an alkaline, CO_3^{2-} rich environment is the ability to tolerate the low concentrations of divalent cations. This was particularly noticable in the alkaliphilic, extremely halophilic archaebacteria from the Wadi Natrun (Imhoff et al., 1978b) and Lake Magadi (Tindall et al., 1980). In the organisms isolated from Lake Magadi the optimal concentration of Mg^{2+} for growth was 0.1-2.0mM, although concentrations as low as 0.02mM Mg^{2+} did not significantly affect growth. Although the optimum Mg^{2+} and Ca^{2+} concentrations for growth of the phototrophs was not determined, the removal of Mg^{2+} and Ca^{2+} by the precipitation of $MgCO_3$ and $CaCO_3$, and their subsequent removal from the medium by filtration did not affect the growth of the organisms significantly, suggesting that the concentrations of 0.5mM Mg^{2+} and 0.3mM Ca^{2+}, found by flame spectroscopy, were sufficient to allow optimal growth

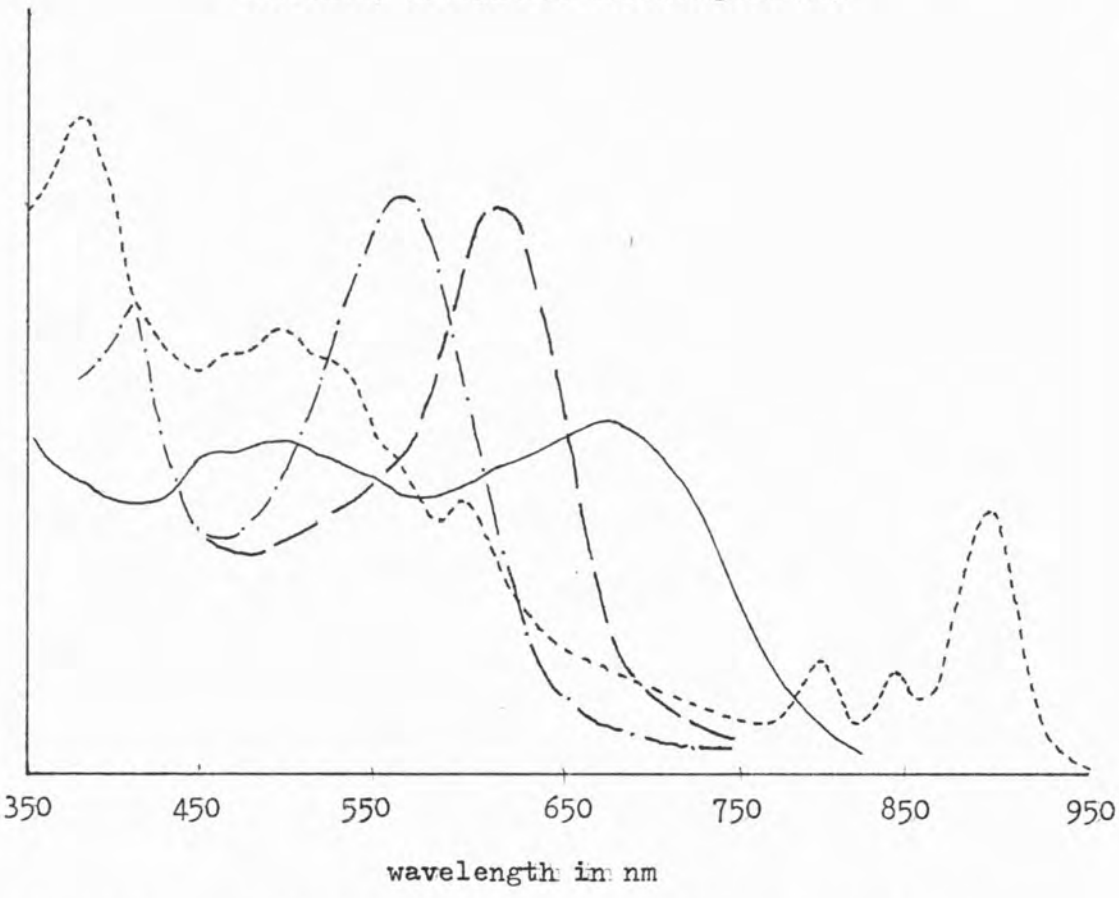
of the organisms. The optimal concentrations for these ions required by other members of the purple phototrophic bacteria is about 4.0mM Mg^{2+} and 9.0mM Ca^{2+} (van Niel, 1931; Pfennig, 1965a). The organisms isolated from the Kenyan soda lakes, therefore, appear to be adapted to the environment in which they live, irrespective of the major taxonomic and phylogenetic differences between the two groups examined.

The examination of recent and ancient organic sediments has shown that some of the molecules found in them are similar to many of those found in bacteria (Chappe et al., 1979; Didyk et al., 1978), and may be useful indicators of the type of organisms which were present in the sediments during their deposition. The environment in which organic macromolecules were deposited, and their resistance to degradation must influence the interpretation of the analysis of organic sediments (Didyk et al., 1978). The interpretation of phytol degradation to pristane, and the steric alteration of phytol to other steroisomers has proven useful in determining the conditions of deposition (Didyk et al., 1978), and the extent of biological and geochemical alterations likely to have taken place. In particular, it has recently been observed that many organic sediments contain lipids which are similar to those found in the archaebacteria (Anderson et al., 1977; Chappe et al., 1979; Hare & Hoering, 1972; Michaelis & Albrecht, 1979; Moldowan & Seifert, 1979). Due to the rather unusual nature of the ether lipids found in the archaebacteria they are relatively resistant to biological alteration (Bayley & Morton, 1978; Kushwaha & Kates, 1978), and may prove useful in tracing archaebacteria in the fossil record (Chappe et al., 1979). Applying this technique to organic sediment analysis to particular environments, with known geological history, such as Lake Magadi, may prove useful in attempting to trace the ancestors of the extremely

halophilic archaebacteria found there today by comparison with fossil lipids and those found in the extremely halophilic bacteria.

Organisms capable of synthesising energy from light include the extremely halophilic archaebacteria, green and purple phototrophic bacteria, cyanobacteria, and eukaryotic algae and plants. In a highly saline environment prokaryotic and eukaryotic organisms capable of energy production using light are present, represented by the archaebacterial extreme halophiles, members of the genus Ectothiorhodospira, and eukaryotic algae of the genus Dunaliella. In such a situation competition for light may be expected to be a major factor in the ecology of highly saline waters. However, each of the three groups of organisms have different absorption maxima for their primary light harvesting pigments, bacteriorhodopsin, bacteriochlorophyll a or b, and chlorophyll a and b (figure 106), and none significantly overlap. Even the presence of a novel type of purple membrane with an absorption maximum at 625nm, in strains SP1 and MS3 would not be coincident with any of the previously described maxima. At first sight such apparent inter-relationship between three phylogenetically dissimilar lines utilising light for energy production by three distinguishable processes may seem peculiar, but it must be remembered that any overlap of the spectral requirements would lead to competition and perhaps, also the exclusion of those organisms at a selective disadvantage. These observations suggest that the phototrophic organisms may have arisen either at the same time, or in consecutive stages, which were physiologically limited. Either of these two possiblities would support the recent hypothesis (Gibson et al., 1979) that phototrophic mechanisms may have arisen several times during evolution in one or more of the major evolutionary lines.

Figure 106. Absorption maxima of the photopigments from extremely halophilic microorganisms.



Ectothiorhodospira halophila, ·····; Halobacterium purple membrane, – · — · –; strain MS3 purple membrane, —— —— ——

Dunaliella sp., ————

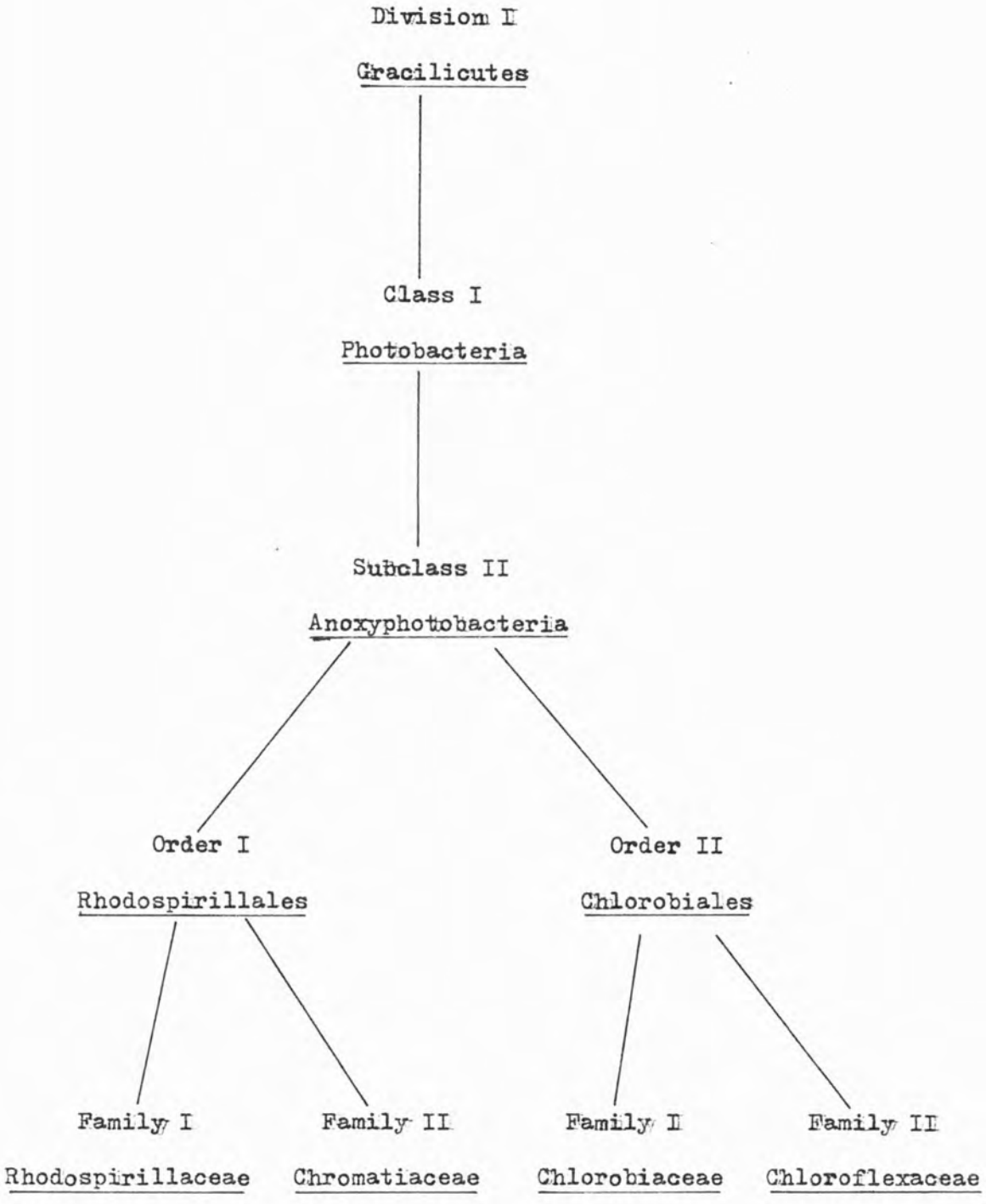
POST SCRIPT

Phototrophic bacteria.

In a recent revision of the higher taxa of the bacteria, Gibbons and Murray (1978) proposed that the Gram-negative prokaryotes be placed in a new division, the Gracilicutes, while the Gram-positive prokaryotes be placed in the division of the Firmacutes. The slime-moulds were placed in the Mollicutes, and the so-called archaebacteria in the division Mendocutes. The Gracilicutes were further divided in to two classes, the phototrophic Photobacteria, and the non-phototrophic Scotobacteria. The Photobacteria include the cyanobacteria, the prochlorophytes, and the purple and green phototrophic bacteria. The purple and green phototrophic bacteria were distinguished from the cyanobacteria and prochlorophytes by their anoxygenic phototrophic metabolism, and were placed in the new subclass, the Anoxyphotobacteria (table 21). The Anoxyphotobacteria are further divided into two orders, the Rhodospirillales, containing the purple phototrophic bacteria, and the Chlorobiales, containing the green phototrophic bacteria (Gibbons and Murray, 1978; Trüper and Pfennig, in press). Thus, the new order Chlorobiales corresponds to the old suborder Chlorobiineae (Pfennig and Trüper, 1974).

Working independantly, on the lakes of the Wadi Natrun, Egypt, Dr.J.F.Imhoff has isolated an organism similar to the gas vacuolate strain B3. The organism is similar to strain B3, in its ability to deposit sulphur outside the cell during the photooxidation of sulphide to sulphate, in its salinity optimum, GC base content, carbon and sulphur source utilisation, and in its pH optima. In particular the presence of two distinct optima have been noted in the presence of carbon compounds or sulphur compounds, which is consistent with the findings of this study and those of Chesnokov

Table 21. Higher taxa of the purple and green phototrophic bacteria.



and Shaposnikov (1936a). In discussions with Dr.Imhoff, Dr.W.D.Grant, Dr.A.Walsby, Prof.Dr.H.G.Trüper, and Dr.M.D.Collins the taxonomic position of these gas vacuolate organisms has been discussed. It is current practice to assign gas vacuolate organisms within the Anoxyphotobacteria to a separate genus, and recently Gorlenko has described a motile, gas vacuolate organism resembling a Chromatium sp., which has been assigned to a new genus Lamprobacter (Imhoff, personal communication). Following such examples it would be appropriate to consider placing gas vacuolate Ectothiorhodospira-like organisms in a new genus, Ectothiorhodocystis. However, in view of the present uncertainty concerning the involvement of plasmids in the formation of gas vacuoles, this must be questioned, since plasmid loss or gain would result in the creation of new taxa, which are otherwise indistinguishable from one another. The gas vacuolate strains may, therefore, be placed in the genus Ectothiorhodospira, as a genus of convenience until the taxonomic position of these organisms in studied in more detail. In the case of strains M2 and M4, they may be clearly distinguished from all existing species of this genus, and their assignment as gas vacuolate members of this genus seems satisfactory, however, the same cannot be said for strain B3. Strain B3 cannot easily be distinguished from E.mobilis and E.shaposnikovii, apart from the presence of gas vacuoles and its more pronounced rod shape. Leaving these features aside it would be difficult to distinguish strain B3 from these two species of Ectothiorhodospira, and it may, more properly be described as a gas vacuolate form of E.mobilis or E.shaposnikovii. The taxonomic position of the gas vacuolate organisms of the 'Ectothiorhodospiraceae' presents a problem to the taxonomist at present, but the study of these organisms may help to resolve the status of gas vacuoles in the differentiation of the

taxa of the Anoxyphotobacteria.

Extremely halophilic bacteria.

The revisions of the higher taxa of the prokaryotes by Gibbons and Murray (1978) include recommendations for assigning the so-called archaebacteria to a new division, the Mendocutes. However, these proposals imply that the Mendocutes are more closely related to the Gracilicutes, Firmacutes, and Mollicutes than to the eukaryotes, when phylogenetic studies suggest that the Mendocutes are no more closely related to these prokaryotes than to the eukaryotes.

Alkaliphilic halobacteria are not confined to the soda lakes of the Rift Valley, Kenya, and an organism similar to strain SP2 has been isolated by Soliman and Trüper (unpublished) from the Wadi Natrun, Egypt. The organism apparently resembles strain SP2 in its ability to utilise carbohydrates, in not containing a satellite DNA band, and its NaCl, pH, and Mg^{2+} requirements. Soliman and Trüper have proposed that their organism be assigned to a new species of Halobacterium, however, in view of the more detailed information on strain SP2 it has been suggested that this strain be designated the type strain of the new species. The assignment of this strain to a new species of Halobacterium also supports the proposal that the alkaliphilic halobacterium, strain SP1 be placed in a new species on account of its physiological and biochemical properties, which differ from other halobacteria, including strain SP2.

Further work on the taxonomy of the halobacteria, including strains SP1, SP2, and MS3 has confirmed that these strains are unusual among the halobacteria in not containing glycolipids (H.N.M.Ross, Ph.D. thesis, in preparation). Investigations of the products of acid methanolysis in the solvent hexane:diethyl ether (85:15) gave results essentially similar to those obtained

in this study. However, the suggestion by Dr.M.D.Collins that the acid methanolysis products would be better differentiated by redevelopment in a second solvent led to the findings that the alkaliphilic halobacteria (strains SP1, SP2, and MS3) contain two ether linked moieties, one of which is similar to the 2,3-di-O-phytanyl-sn-glycerol found in other halobacteria (Ross et al., in press). However, a number of little used strains of halobacteria also contain two ether linked moieties, although they are apparently not alkaliphilic (for details see H.N.M.Ross, Ph.D. thesis, in preparation), and does not clarify the taxonomic position of the alkaliphilic halobacteria. These findings show that the nature of the ether linked lipid derivative does not necessarily indicate taxonomic differences, although the absence of glycolipids from the alkaliphilic halobacteria does seem to be taxonomically significant. It is hoped that further studies of the taxonomy of the extremely halophilic archaebacteria will resolve the status of present species, and the taxonomic proposals suggested in this thesis (H.N.M.Ross, Ph.D. thesis, in preparation).

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The Isolation of Alkalophilic Bacteria

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Over the last few decades, many reports have appeared of organisms growing at extreme pH values. Most of the reports however, concern acidic environments, and little information exists about the alkaline limits of life, testified to by the paucity of detail in review articles concerned with the effects of pH (Vallentyne 1963; Skinner 1968; Kushner 1971; Langworthy 1978).

There are a number of man-made highly alkaline environments, e.g. food processing waste, textile wastes, but naturally occurring high alkaline (>pH 10) environments are relatively uncommon. There are certain desert soils where pH values of 10 and above have been recorded (Rupela & Tauro 1973) but the most alkaline, naturally occurring environments on earth are the soda lakes and soda deserts, now restricted to a few areas (for a list see Te-Pang 1890) where pH values of 10 and above are common. The areas where such lakes and deserts are to be found are mostly closed basins where evaporative concentration takes place. The genesis of the alkalinity is open to some dispute, but the most widely accepted explanation is that a weak solution of metal bicarbonates, largely sodium, leached from rocks by CO_2-charged surface waters, concentrates in the absence of significant amounts of calcium (which removes alkalinity by calcite precipitation) to produce a $NaHCO_3/Na_2CO_3$ brine (Hardie & Eugster 1970). At pH values above 10, Na_2CO_3 preponderates (Golterman & Clymo 1971), and most of the soda lakes have from 2–10% (w/v) Na_2CO_3 at pH values of 10–11, with varying amounts of other salts, often including NaCl as a major component. Extreme examples such as the Wadi Natrun in Egypt and Lake Magadi in Kenya have crystalline deposits reflecting total dissolved solids of up to 40% (w/v). Dilute alkaline springs are also found where the calcium content may be high (Souza *et al.* 1974)—in these cases the genesis of the alkalinity is not clear, and obviously different from that in the soda lakes.

No definition of the term alkalophile seems to exist (more correctly alkaliphile from the Arabic root, but the term alkalophile is now in common usage and will continue to be used in this article). However, the assumptions generally implicit in its use appear to be that those organisms incapable of growing at neutrality, capable of growth at pH 10 and above, and with pH optima at pH 9 or above are designated alkalophiles. The much larger group of organisms capable of growing over a wider range of pH from the acid side of neutrality to pH values in excess of 10, and with pH optima closer to neutrality should therefore be designated alkalotolerant.

Eukaryotic micro-organisms have been described in alkaline habitats (Souza *et al.* 1974; Siegel & Giumarro 1965; Langworthy 1978) but pure culture studies have seldom been made, so it is not clear if these organisms are alkalophiles or merely alkalotolerant. A considerable number of alkalotolerant species of bacteria are known from a wide variety of habitats, including members of the genera *Streptococcus* (Chesbro & Evans 1959), *Agrobacterium* (Allen & Allen 1950) and *Bacillus* (Bornside & Kallio 1956; Chislett & Kushner 1961). Alkalophilic species of bacteria have been isolated from a variety of habitats, some not particularly alkaline in nature. Most of the strains have been soil isolates of Gram positive sporing rods assigned to the genus *Bacillus*. Probably the best known alkalophile is *B. pasteurii*, a common soil isolate, which requires both high pH and NH_3 (Wiley & Stokes 1962). Other soil isolates include *B. alcalophilus* (Boyer & Ingle 1972; Vedder 1934) and the large number of incompletely characterized *Bacillus* spp. described by Horikoshi and his coworkers (Horikoshi 1971*a*,*b*; 1972; Yamamoto *et al.* 1972; Nakamura & Horikoshi 1976). Horikoshi has also isolated a *Bacillus* sp. from rayon waste (Ikura & Horikoshi 1977), and Ohta and his colleagues have isolated a *Bacillus* sp. from fermenting indigo leaves (Ohta *et al.* 1975). The extraordinary organism *Kakabekia barghoorniana* described by Siegel (Siegel & Siegel 1970; Siegel 1977) has been isolated from several soil samples. This particular organism grows in very high concentrations of NH_4OH, but has never been grown in the absence of soil particles. Alkaline springs have yielded several alkalophilic bacteria, including a coryneform organism (Souza & Deal 1977), a *Flavobacterium* sp., and an unidentified anaerobe (Souza *et al.* 1974; Deal *et al.* 1975).

Potentially the best source of alkalophilic micro-organisms, the soda lakes and soda deserts, are virtually unexplored microbiologically. No consideration of these environments would be complete without some mention of the massive blooms of blue green algae which occur in soda lakes periodically at pH values considerably in excess of 10. The most frequently described species are members of the genera *Spirulina* and

Anabaenopsis (Talling & Talling 1965; Melack & Kilham 1974). These algae have seldom if ever been isolated from these environments and studied in pure culture, so it is not clear if these organisms are true alkalophiles. However, in general there is evidence that blue green algae are more abundant in alkaline waters (Fogg 1956) and in some cases may be true alkalophiles. Bacteriological studies on soda lakes are scarce. Some years ago Isachenko (1951) observed several morphological types of bacteria in Crimean soda lakes, some of which were photosynthetic bacteria. Reports of pure culture studies on isolates from such lakes have appeared only recently and so far are restricted to photosynthetic bacteria. Imhoff and Trüper (1977), and Grant *et al.* (1979) have described alkalophilic *Ectothiorhodospira* spp. from different soda lakes. It is to be expected that these lakes will yield a wide variety of different types of alkalophilic bacteria in the near future.

The biochemical basis of alkalophily remains to be elucidated. Ohta *et al.* (1975) and Koyama *et al.* (1976) have shown that membrane exclusion mechanisms may be important in allowing the transport of charged compounds. Recently the problems posed by alkalophily in relation to ATP synthesis have been explored in *B. alcalophilus* (Guffanti *et al.* 1978) and alkalophiles may prove to be useful tools in furthering our understanding of the mechanics involved in energy generation.

Enrichment and Isolation Procedures

In principle, there is no reason why a variety of physiological groups of bacteria should not be isolated from an appropriate environment. In most cases the pH of the initial enrichment is achieved and maintained by adding an appropriate quantity of Na_2CO_3 (or $Na_2CO_3.10H_2O$). Occasionally NaOH has been used (Souza *et al.* 1974). Na_2CO_3 is the additive of choice for many enrichments in view of the high concentrations of the compound to be found in most naturally occurring alkaline environments, and there is some evidence that growth is enhanced in its presence (Nakamura & Horikoshi, 1976; Imhoff & Trüper 1977; Grant *et al.* 1979). pH values of 10–11 can be achieved in most media by the addition of 1–5% (w/v) Na_2CO_3 or the equivalent amount of $Na_2CO_3.10H_2O$. The pH of the enrichments should be checked and adjusted if incubation periods longer than 48 h are to be used. High pH media quickly absorb CO_2 from the atmosphere and drop markedly in pH after a relatively short period. For this reason growth after some time in media with a starting pH in excess of 11 should be checked very carefully, and claims of growth at pH 12–13 (Allen & Allen 1950; Kingsbury 1954) are to be viewed with suspicion unless careful pH measurements

have been made. At this present time the highest pH recorded for growth in pure culture may be as low as 11·5.

It is not possible to maintain pH values in excess of 11·0 with the Na_2CO_3 system due to the buffering of atmospheric CO_2, but if a constant pH monitoring system is available then automatic addition of NaOH can be used to retain very high pH values (Souza *et al.* 1974; Souza & Deal 1977). However, in our experience enrichments at pH 10–11 yield most alkalophiles, and enrichments which have been kept in excess of pH 11·0 seldom yield organisms.

Enrichment of the following three groups is outlined as an example of the system. Our experience with highly alkaline environments is restricted to African soda lakes which yield a variety of types of bacteria including alkalophilic aerobic heterotrophs, alkalophilic photosynthetic bacteria, and alkalophilic halophiles. We have applied enrichments for alkalophilic heterotrophic bacteria to a variety of ordinary soil types with success.

Heterotrophic bacteria

Several different types of semi-defined media are possible, including nutrient broth, tryptic soy broth (Souza & Deal 1977) adjusted to the appropriate pH value. The most useful general medium is a modification of that described by Horikoshi (1971*a*).

Composition (gl^{-1}): glucose, 10·0; peptone (Difco), 5·0; yeast extract (Difco), 5·0; K_2HPO_4, 1·0; $MgSO_4.7H_2O$, 0·2; distilled water to 900 ml. The medium is sterilized by autoclaving. Agar at 1·5 % (w/v) may be included if desired. 100 ml 20 % (w/v) $Na_2CO_3.10H_2O$ (sterilized separately by autoclaving) is added to give a final concentration of $Na_2CO_3.10H_2O$ in the medium of 2 % and a final pH of 10·5. Agar containing media should be mixed with $Na_2CO_3.10H_2O$ at 60°C and when mixed poured immediately. A variety of modifications of this medium have been used by Horikoshi and his coworkers to isolate bacteria capable of degrading different carbon sources (Horikoshi 1972; Nakamura & Horikoshi 1976; Ikura & Horikoshi 1977).

A small amount of inoculum is added to the media and incubation is carried out at 25°C or 37°C with shaking for 24–48 hours. Material is then streaked on agar media of the same composition. Both alkaline muds and ordinary soils yield a variety of alkalophilic aerobic heterotrophs by this procedure.

Photosynthetic bacteria

A modification of the medium described by Grant *et al.* (1979) is suitable

for the enrichment and purification of several morphological types of photosynthetic bacteria. As yet these have only been isolated from soda lakes, and enrichments using ordinary river and pond muds have not yielded organisms at high pH.

Composition (gl^{-1}): NH_4Cl, 1·0; NaCl, 0·4; $MgSO_4.7H_2O$, 0·4; $CaCl_2.2H_2O$, 0·5; KH_2PO_4, 1·0; sodium acetate, 1·0; yeast extract (Difco) 1·0. Distilled water to 900 ml. A solution of trace elements containing (gl^{-1}): $ZnSO_4.7H_2O$, 5·5; $FeSO_4.7H_2O$, 5·0; $Na_2MoO_4.2H_2O$, 1·0; $MnCl_2.4H_2O$, 5·5; $CuSO_4$, 1·5; $CoCl_2$, 1·5 is added to the medium at 1 ml l^{-1}.

The medium is sterilized by autoclaving. Agar at 1·5 % (w/v) may be included. 100 ml 20 % (w/v) $Na_2CO_3.10H_2O$ (sterilized separately by autoclaving) is added to give a final concentration of $Na_2CO_3.10H_2O$ of 2 % (w/v) and pH 10·3. Na_2S at 0·1 % may be included in the medium (sterilized separately by autoclaving) if desired, and the NaCl content may be increased depending on the salinity of the environment. Agar containing media should be mixed with $Na_2CO_3.10H_2O$ at 60°C and poured immediately.

Imhoff & Trüper (1977) have used a high salt medium for the isolation of an alkalophilic halophilic *Ectothiorhodospira* sp. from an Egyptian soda lake.

This medium contains (gl^{-1}): $CaCl_2.2H_2O$, 0·05; KH_2PO_4, 0·5; NH_4Cl, 0·8; $MgCl_2.6H_2O$, 0·1; NaCl, 180·0; Na_2SO_4, 20·0; Na_2CO_3, 20·0; yeast extract, 0·5; sodium succinate, 1·0; Na_2S, 1·0.

A solution of trace elements at 1 ml l^{-1} of the following composition (gl^{-1}): $FeCl_2.4H_2O$, 1·8; $CoCl_2.6H_2O$, 0·25; $NiCl_2$, 0·01; $CuCl_2$, 0·01; $MnCl_2.4H_2O$, 0·07; $ZnCl_2$, 0·10; H_3BO_3, 0·5; $Na_2MoO_4.2H_2O$, 0·03; $Na_2SeO_3.5H_2O$, 0·01 is added to the medium, followed by 1 ml l^{-1} of vitamin solution of the following composition (gl^{-1}): biotin, 0·1; nicotinamide, 0·35; thiamine, 0·3; *p*-aminobenzoic acid, 0·2; pyridoxal chloride, 0·1; Ca-pantothenate, 0·1; Vit B_{12}, 0·05.

The final pH of the enrichment is 9·6.

The initial inoculum is 25 % (v/v) mud and incubation is at 30°C at 5000 lx in bottles completely filled. Subculture may be carried out in media of the same composition, incorporating 0·05 % (w/v) ascorbate if organic acids alone are to be used as hydrogen donors. Purification can be achieved by repeated agar shake dilution, or alternatively by streaking on agar media in 25 mm plastic Petri dishes, and incubating these in transparent polycarbonate jars (GasPak Anaerobic Systems: Becton, Dickinson and Company, Cockerysville, MD 21030, USA) in the anaerobic environment produced by the GasPak system. The Petri dishes may be held upright round the walls of the jar by an inner glass

sleeve, thus affording much better illumination of the plates (Fig. 1). The transparent anaerobic glove box system described by Aranki *et al.* (1969) with an atmosphere of 85 % O_2-free N_2/5 % CO_2/10 % H_2 is particularly useful if large numbers of tests are to be made, and liquid media in divided dishes can be used in this system. Engelhard D catalyst is used in such a system to maintain an O_2 free environment (Engelhard Ltd., Valley Road, Cinderford, Gloucestershire, GL14 2PB) (Fig. 2).

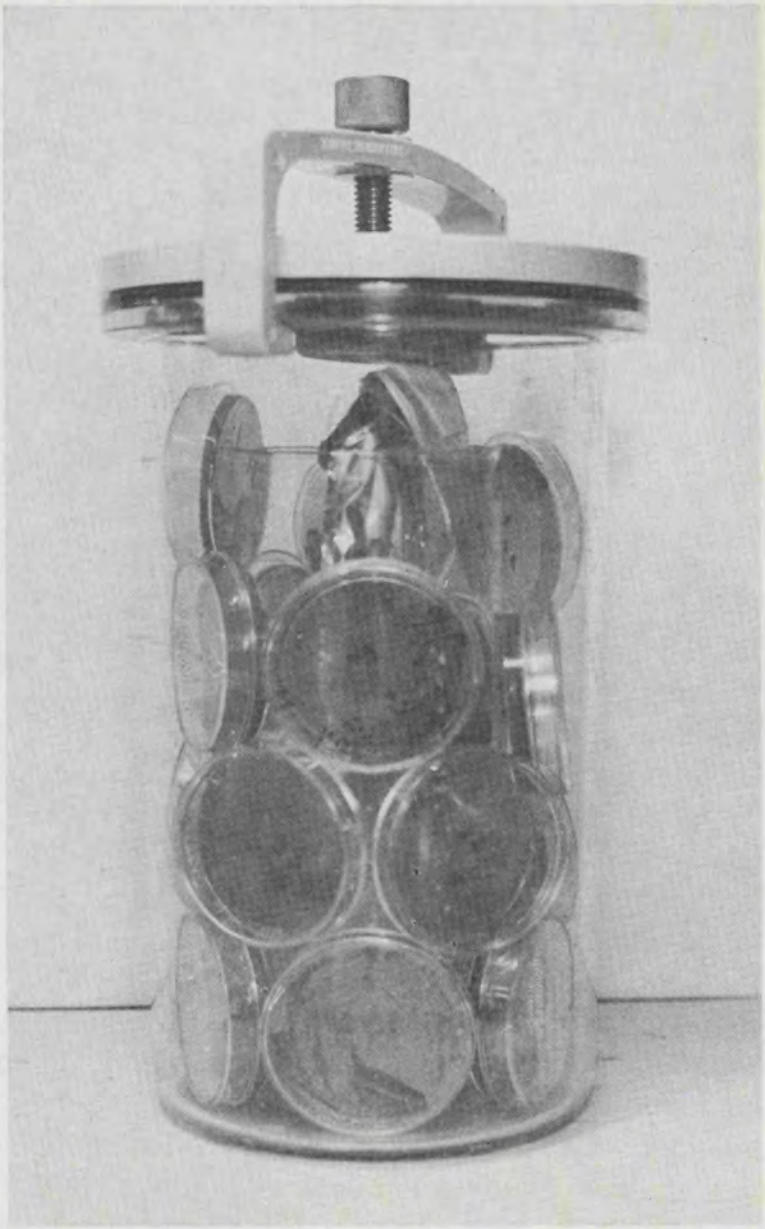

Fig. 1. GasPak polycarbonate jar with glass liner.

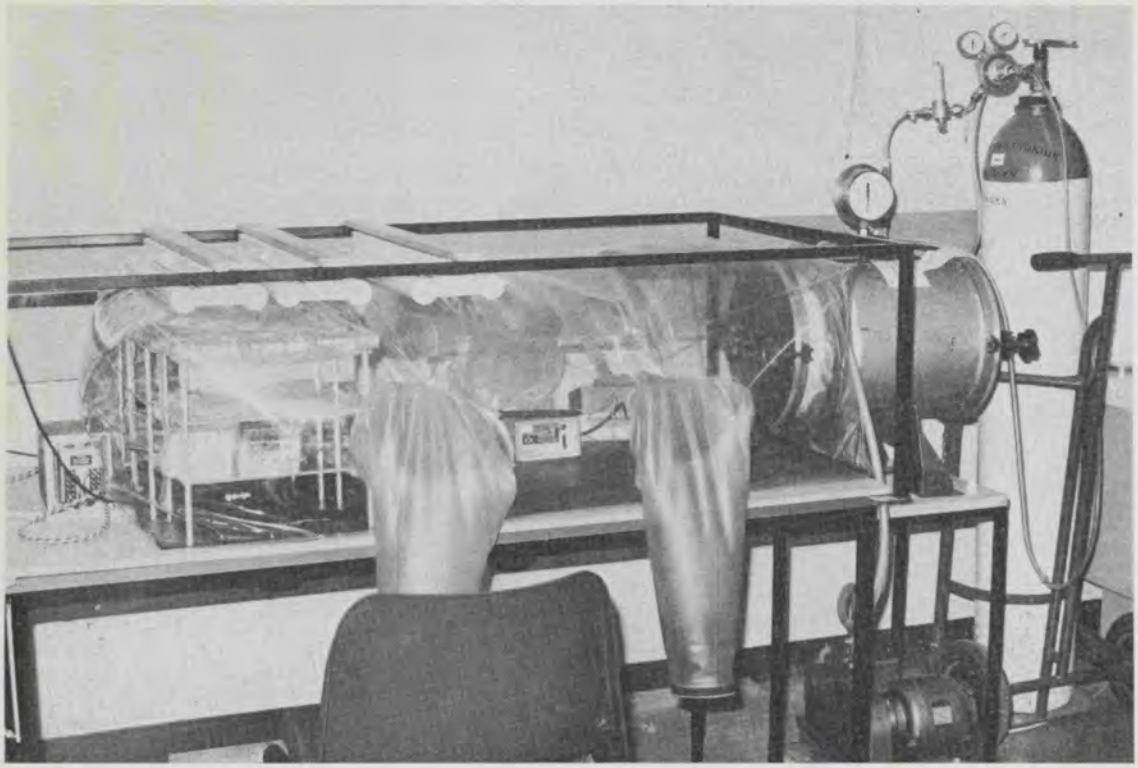

Fig. 2. Clear plastic anaerobic glove box after Aranki *et al.* (1969). Atmosphere 85% O_2 free N_2/5% CO_2/10% H_2. Catalyst Engelhard D. Access by flushable port.

Halophilic bacteria

Extreme examples of soda lakes such as the Wadi Natrun and Lake Magadi yield alkalophiles which are additionally extreme halophiles. One of these is the photosynthetic *Ectothiorhodospira* sp. described by Imhoff & Trüper (1977), the enrichment medium for which has already been described. The solar evaporation ponds at Lake Magadi which contain 16% (w/v) Na_2CO_3, and 16% (w/v) NaCl yield a red alkalophilic halophile which does not contain bacteriochlorophyll. This organism which is probably related to the genus *Halobacterium* (B. J. Tindall and W. D. Grant, unpublished results) can be enriched for in a medium of the following composition (gl^{-1}): $Na_2CO_3.10H_2O$, 200; NaCl, 200; yeast extract (Difco), 1·0; KCl, 1·0; $MgCl_2$, 1·0; NH_4Cl, 1·0. A solution containing 200 g NaCl and 200 g $Na_2CO_3.10H_2O$ is sterilized by autoclaving in a total volume of 500 ml. The other components (including 15 g agar if desired) are also sterilized in a total volume of 500 ml, and the two solutions mixed. Agar containing media should be mixed at 60°C and poured immediately. The pH reading obtained for the medium using sodium corrected probes is 10·6–10·8.

The organism can also be grown in a standard halophile medium adjusted to the appropriate pH value (Brown 1963).
Composition: (gl^{-1}): $MgSO_4$, 20·0; sodium citrate, 3·0; KCl, 2·0; peptone (Difco), 1·0. Make up to 500 ml with distilled water. Sterilization is by autoclaving. A solution containing 250 g NaCl and 50 g $Na_2CO_3.10H_2O$ in 500 ml distilled water is sterilized by autoclaving, and added to the other salts. The final pH of the medium is 10·0. Agar may be incorporated at 1·5 % (w/v) and sterilized with the latter solution.

Enrichment is in shallow static layers of medium at 37°C for 3–4 weeks at 2000–5000 lx. Purification is carried out by streaking on agar media in Petri dishes which are incubated in the light at 37°C. It is necessary to seal the edges of the Petri dishes with transparent adhesive tape to prevent drying out and crystallization of the salt.

Determination of pH Optima

The use of pH gradient plates

The pH range of growth of certain isolates may be rapidly determined using pH gradient plates. Square Petri dishes are suitable for the purpose, and media of the appropriate composition but without $Na_2CO_3.10H_2O$ is poured to a depth of at least 1 cm and allowed to set. A uniform trough 1 cm in width is removed from one end and agar containing 20 % $Na_2CO_3.10H_2O$ and 0·2 M NaOH is poured into the trough. The trough agar is prepared by mixing together equal volumes of sterile 0·4 M NaOH/40 % (w/v) $Na_2CO_3.10H_2O$ and 4 % (w/v) agar at 60°C. Plates left at 37°C overnight develop a uniform gradient from pH 12–pH 7·0 which is stable for 2 days. Organisms are streaked across the plate at right angles to the trough. After 48 h the pH at the alkaline end drops due to CO_2 absorption, and therefore if prolonged incubation is necessary the technique is not as useful for determining the upper limits of pH, although useful information on the lower limits can still be obtained. Plates incubated in a CO_2 charged atmosphere, e.g. GasPak system, show a more rapid equilibration of the gradient, and the technique only yields information about the lower limits of growth. The surface pH of such plates can be determined using a suitable membrane electrode such as the EIL 1070-2 flat head electrode. The technique is particularly useful for fast growing heterotrophs, and often the pH optima can be predicted from the appearance of the growth. Figure 3 shows isolates from an ordinary soil and an alkaline mud, and clearly shows that one strain is alkalotolerant, whereas the others are alkalophilic.

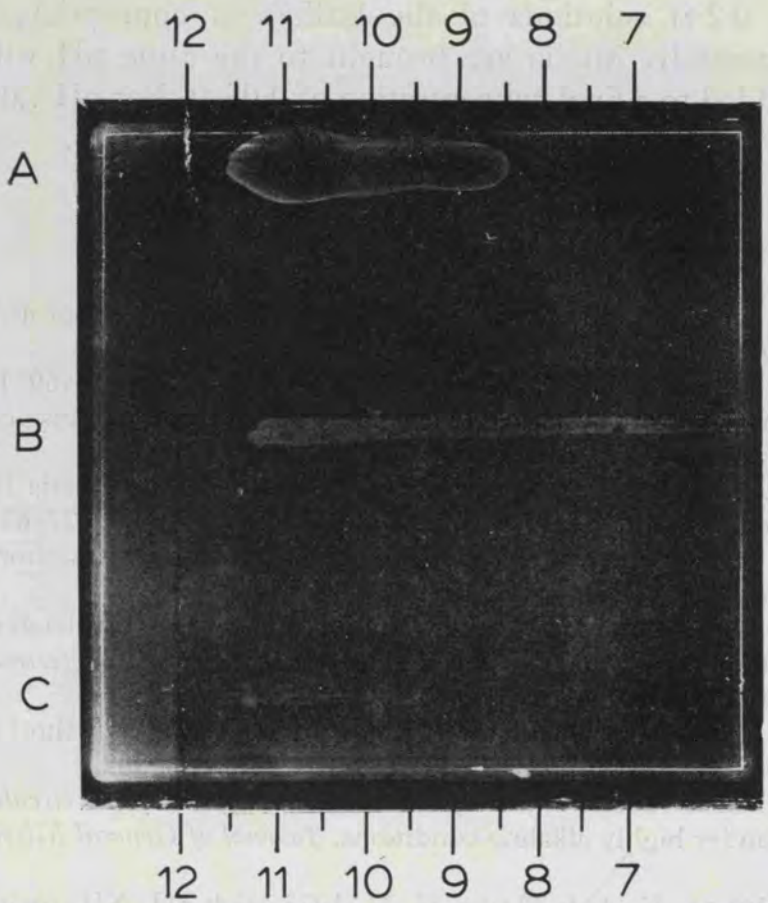

Fig. 3. pH gradient plate showing alkalotolerant and alkalophilic heterotrophs. Strain B is an alkalotolerant isolate from a British garden soil, strain A an alkalophilic isolate from an alkaline mud sample, and strain C an alkalophilic isolate from a British garden soil. The medium is the modified Horikoshi medium (1971*a*) described in the text.

Buffer systems for high pH

The naturally occurring buffer system in most alkaline environments is the $NaHCO_3/Na_2CO_3$ system. However the system is unsatisfactory for use in determination of pH optima due to the rapid absorption of CO_2 from the atmosphere, which tends to bring high pH media down to between pH 9 and 10 even if relatively high concentrations of Na_2CO_3 have been used. It may be no accident that most of the alkalophiles isolated from naturally occurring environments have pH optima between 9 and 10. The alkaline soda lakes presumably maintain pH values above 10 by constant evaporative concentration and generation of alkalinity by photosynthesis. The most useful buffers over the range 9–12 in a variety of media are borax/NaOH and Na_2HPO_4/NaOH. These systems are less effected by atmospheric CO_2 and are non-toxic to a wide range of

alkalophiles. 0·2 M solutions of the buffers at appropriate pH's are sterilized separately. Media are brought to the same pH with NaOH and buffer added to a final concentration of 0·05 M. For pH values below 9, NaH_2PO_4/Na_2HPO_4 and borax/boric acid may be used.

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SHORT COMMUNICATION

An Alkalophilic Red Halophilic Bacterium with a Low Magnesium Requirement from a Kenyan Soda Lake

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A *Halobacterium* species isolated from solar evaporation ponds and sodium sesquicarbonate deposits at Lake Magadi, Kenya, differs from known species of *Halobacterium* in its GC content, in being obligately alkalophilic with a pH optimum between 9·0 and 10·0, and in having a Mg^{2+} requirement of between 0·1 and 2·0 mM for optimum growth.

INTRODUCTION

Extremely halophilic bacteria of the genus *Halobacterium* characteristically occur in highly saline environments, such as solar evaporation ponds, salt lakes, and on various salted food products, turning them from orange/red to pink or even purple (Dundas, 1977; Gibbons, 1974; Larsen, 1967). The currently described species of *Halobacterium* are characterized by the presence of carotenoids and by a minimum requirement of NaCl for growth of 2 to 3 M. All the recorded isolates have an additional requirement for relatively large amounts of Mg^{2+} (Brown & Gibbons, 1955) which might be related to the stability of the cell wall and/or of the protein-synthesizing apparatus (Kushner, 1978). Mg^{2+} above 2 M inhibits most species of *Halobacterium*, although the recently described *H. volcanii* tolerates these concentrations, reflecting its natural habitat, the Dead Sea (Mullakhanbhai & Larsen, 1975). The habitats described so far for *Halobacterium* species have been mainly neutral to acidic, highly saline environments, where divalent cations such as Mg^{2+} are readily soluble, none of the recorded isolates growing in semi-defined media in the absence of added Mg^{2+} (Brown & Gibbons, 1955).

The African Continent contains a number of alkaline soda lakes where the main ions are Na^+, CO_3^{2-}, HCO_3^- and Cl^-, with varying amounts of SO_4^{2-} and K^+ (Talling & Talling, 1965). The more dilute lakes have a characteristic population of phototrophic bacteria (Grant *et al.*, 1979). Concentrated soda lakes exist, such as the Wadi Natrun, Egypt, and Lake Magadi, Kenya, where the total dissolved salts may exceed 35% (w/v) at a pH of 10·5 to 11·0 (Imhoff *et al.*, 1978). Under these conditions divalent cations are present in only trace amounts, since precipitation occurs as carbonates during formation of the lake waters (Hardie & Eugster, 1970). Parts of one such lake, Lake Magadi, are solid sodium sesquicarbonate (trona, $Na_2CO_3.NaHCO_3.2H_2O$) for most of the year. The crystalline trona, which is mined by the Magadi Soda Company as a source of soda ash, has an orange/pink crust which is similar in colour to the local solar evaporation ponds used to produce common salt. The solar evaporation ponds are unusual in that Na_2CO_3 and NaCl are present in equally large amounts in the liquor (16%, w/v) at a pH of 10·8. Microscopic examination of the liquor reveals large numbers of bacterial rods. The Mg^{2+} concentration is less than 0·5 mM both in the pan liquor and in Lake Magadi surface waters (Talling & Talling, 1965).

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Recently Imhoff *et al.* (1978), as part of an ecological and microbiological survey of the lakes in the Wadi Natrun, a similar environment to Lake Magadi, reported red halophilic bacteria growing in the highly saline, alkaline waters where the concentration of Mg^{2+} was below the level of detection. Isolates obtained from this environment were by implication alkalophilic or alkalotolerant and grew at Mg^{2+} concentrations of less than 10 mM.

We report here the isolation of a red obligately halophilic and alkalophilic bacterium from both the trona crust and the solar evaporation ponds at Lake Magadi. We believe that this organism and others like it are responsible for the orange/pink coloration. The organism differs from previously described members of the *Halobacteriaceae* in its GC content, alkalophily and ability to grow in media containing less than 0·5 mM-Mg^{2+}.

METHODS

Halobacterium cutirubrum (NCMB 763), *H. volcanii* (NCMB 2012), *H. halobium* (NCMB 736), *H. salinarium* (NCMB 764) and *H. trapanicum* (NCMB 767) were obtained from the National Collection of Marine Bacteria and maintained on the medium of Payne *et al.* (1960). The NaCl content was lowered to 2 M for *H. volcanii*.

Enrichments were originally performed in a modification of the medium of Brown (1963) of the following composition (%, w/v): bacteriological peptone (Oxoid), 1·0; trisodium citrate, 0·3; $MgSO_4.7H_2O$, 2·0; KCl, 0·2; $Na_2CO_3.10H_2O$, 5·0; NaCl, 25; pH 9·5. Samples from the trona crust and the solar evaporation ponds were inoculated into this medium and incubated at 37 °C under fluorescent lighting (Philips daylight). Isolates were purified by streaking on to media solidified with 2·0% (w/v) agar. The isolates were subsequently maintained in the medium of Payne *et al.* (1960) modified by adding 5% (w/v) $Na_2CO_3.10H_2O$ and decreasing the $MgSO_4.7H_2O$ content to 0·1% (w/v); the final pH was 9·5.

The related effects of pH and Mg^{2+} concentration were studied in media prepared either without $MgSO_4.7H_2O$ or with 0·1 or 2·0% (w/v) $MgSO_4.7H_2O$. Varying amounts of sterile 20% (w/v) $Na_2CO_3.10H_2O$ or $NaHCO_3$ were added to produce pH values of 7·5, 8·5 and 9·5.

To determine the optimal concentration of Mg^{2+} for growth in media at pH 9·5, media of different $MgSO_4.7H_2O$ contents were prepared and the concentration of Mg^{2+} in solution was then determined with a Varian Tectron atomic absorption spectrophotometer, using media passed through a 0·45 μm Millipore filter (Golterman *et al.*, 1978).

The GC content of the cells was determined by the buoyant density (Mandel *et al.*, 1968) and the melting point (Marmur & Doty, 1962) methods using DNA prepared by the method of Marmur (1960).

The amino acid analysis of the bulk protein prepared by the method of Reistad (1970) was performed on a Locarte Mark IV Floor Model automatic amino acid analyser.

Growth tests were done as previously described (Colwell *et al.*, 1979; Gibbons, 1957; Gonzalez *et al.*, 1978; Sehgal & Gibbons, 1960; Tomlinson & Hochstein, 1976).

RESULTS AND DISCUSSION

A motile Gram-negative rod, designated strain SP-1, was isolated from a sample of solar evaporation pond liquor after enrichment in the modified medium of Brown (1963) for 2 to 3 weeks. A similar organism was isolated from the trona crust of the lake.

The cells were 0·7 μm by 1·5 to 3·0 μm in medium containing 4·5 M-NaCl and 2 mM soluble Mg^{2+} at pH 9·5. Orange–red colonies were obtained after 2 weeks incubation at 37 °C on the modified medium of Payne *et al.* (1960); these were convex with an entire edge, 3 to 4 mm in diameter, translucent and mucoid.

Cells became spherical in medium containing 1 M-NaCl and lysed as the salt content was lowered further; cells suspended in distilled water lysed rapidly. Growth occurred between 3·0 and 5·5 M (saturation) NaCl, with an optimum at 4·0 M at 37 °C, pH 9·5.

Absorption spectra of whole cells showed maxima at 475, 505 and 545 nm, characteristic of the bacterioruberins found in halobacteria. The characteristics of the isolate, therefore, indicated a close relationship to the genus *Halobacterium*.

Growth of strain SP-1 and known *Halobacterium* species was tested at pH 7·5, 8·5 and

9·5 in media containing 2·0 or 0·1 % (w/v) $MgSO_4.7H_2O$ or without added $MgSO_4.7H_2O$. Strain SP-1 grew well at pH 9·5 with 0·1 % (w/v) $MgSO_4.7H_2O$ (0·1 mM soluble Mg^{2+}). It also grew at pH 9·5 in media without any added $MgSO_4.7H_2O$, the concentration of soluble Mg^{2+} in the medium under these conditions being less than 0·05 mM. The Mg^{2+} optimum for strain SP-1 at pH 9·5 was between 0·1 and 2 mM (more precise determinations of the optimum were not possible due to the difficulty of constructing media with a precise range of soluble Mg^{2+} at pH 9·5). With 2% (w/v) $MgSO_4.7H_2O$ (10 mM soluble Mg^{2+}) at pH 9·5, some inhibition of growth of strain SP-1 occurred and the cells became coccoid in morphology. All the recognized species of *Halobacterium* showed good growth in media containing 2% (w/v) $MgSO_4.7H_2O$ at pH 7·5 but grew poorly with 0·1 % (w/v) $MgSO_4.7H_2O$ and not at all without added $MgSO_4.7H_2O$. Strain SP-1 grew well at pH values between 9·0 and 10·0, but not below pH 8·5. Cells were elongated at pH 8·5. It survived inoculation into medium of pH 11·0, although the pH dropped to 9·5 to 10·0 as growth occurred. It did not grow at pH 7·5 irrespective of the Mg^{2+} concentration, indicating that the requirement for high pH was not a consequence of the concentration of soluble Mg^{2+}. Growth of the known species of *Halobacterium* was good in 2% (w/v) $MgSO_4.7H_2O$ at pH 7·5 but poor and variable at pH 8·5; no growth occurred at pH 9·5, agreeing with other reports (Colwell *et al.*, 1979). It was not possible to separate the effects of pH and Mg^{2+} in this case, since high pH and high Mg^{2+} concentrations are mutually incompatible.

Strain SP-1 resembled the known species of *Halobacterium* in showing positive reactions for gelatinase, catalase, oxidase, S^{2-} from $S_2O_3^{2-}$, and negative reactions for amylase, reduction of NO_3^-, reduction of NO_2^-, production of gas from NO_3^-, and production of gas from NO_2^-. The antibiotic sensitivity of strain SP-1 also resembled that of known species of *Halobacterium* in that inhibition of growth was recorded in the presence of novobiocin, but not in the presence of penicillin G, polymixin B, ampicillin, tetracycline or dihydrostreptomycin. Strain SP-1 did, however, differ from known species of *Halobacterium* in being sensitive to vibriostat O/129.

Further similarities between *Halobacterium* spp. and strain SP-1 were indicated by amino acid analysis of the bulk protein. Comparison of *H. halobium* with strain SP-1 indicated a similar pattern of amino acids, the total acidic amino acid content being greater than the basic amino acid content by 14·4 mol % and 13·9 mol %, respectively (not corrected for amide). These values are characteristic of the genus *Halobacterium* (Brown, 1964; Kushner & Onishi, 1966; Mullakhanbhai & Larsen, 1975; Steensland & Larsen, 1969).

Buoyant density centrifugation indicated no satellite bands, in contrast to most species of *Halobacterium* (Moore & McCarthy, 1969). The GC content was 59·0 ± 1·0 mol % (buoyant density) and 59·5 ± 1 mol % (T_m). These values are considerably lower than those found for the major DNA bands of recognized *Halobacterium* species (Gibbons, 1974).

Strain SP-1 appears to differ significantly from the recognized species of *Halobacterium* by virtue of its alkalophily and low Mg^{2+} requirement. Highly saline, alkaline environments are relatively rare in the world compared with highly saline, neutral environments and, possibly, such environments harbour a unique microbial population. Strain SP-1 may be similar to the *Halobacterium* sp. described by Imhoff *et al.* (1978) from the Wadi Natrun.

Further work is in progress on a comprehensive comparison of strain SP-1 with other isolates from highly saline, alkaline environments and type strains of *Halobacterium*. Whether strain SP-1 is also a member of the Archaebacteria remains to be determined (Woese *et al.*, 1978).

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A Rapid Procedure for the Detection of Archaebacterial Lipids in Halophilic Bacteria

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Whole-organism acid methanolysates of strains of halophilic bacteria were examined for glycerol diether moieties and other long-chain constituents by thin-layer chromatography. Glycerol diether moieties were detected only in acid methanolysates of archaebacterial halophiles. Eubacterial halophiles contained only fatty acid methyl esters. Spots on thin-layer plates attributed to diether moieties were further identified by infrared spectroscopy. Thin-layer chromatographic analysis of whole-organism methanolysates provides a simple and rapid method of distinguishing archaebacterial halophiles from eubacterial halophiles. The majority of archaebacterial halophiles produced only one chromatographically distinct glycerol diether moiety, but certain strains, including the recently described alkalophilic halophiles, produced two distinct moieties.

INTRODUCTION

Molecular genealogical analysis based upon transfer and ribosomal RNA sequence homologies has revealed that the bacteria do not constitute a phylogenetically monolithic grouping (see Woese & Fox, 1977). The kingdom *Procaryotae* actually comprises two phylogenetically distinct groups – the 'archaebacteria', and the 'eubacteria' including the cyanobacteria. The first organisms recognized to be archaebacteria were the methanogens (Woese & Fox, 1977) and subsequent studies indicated that certain thermoacidophiles (*Caldariella*, *Sulfolobus* and *Thermoplasma*) and extreme halophiles (*Halobacterium* and *Halococcus*) should also be included in this group (Woese *et al.*, 1978). Amongst other distinctive features of the archaebacteria are the absence of cell wall peptidoglycan (Brown & Cho, 1970; Kandler & Hippe, 1977) and the presence of ether-linked lipids (deRosa *et al.*, 1974, 1980; Kates, 1978; Tornabene & Langworthy, 1978; Balch *et al.*, 1979).

At present, archaebacteria have only been isolated from a few restricted environments and there is considerable interest in establishing whether they occur elsewhere. The assignment of a particular isolate to the archaebacteria is currently a laborious procedure. During recent investigations into the taxonomic status of alkalophilic halophiles from Kenyan soda lakes (Tindall *et al.*, 1980; W. D. Grant, unpublished results) we have developed a rapid screening procedure for detecting archaebacteria, based on the presence of the ether-linked lipids. The ether lipids of halophilic bacteria have been the subject of extensive investigation by Kates and associates (Sehgal *et al.*, 1962; Kates *et al.*, 1966; Joo *et al.*, 1968; Kates, 1978), but no simple procedure existed for the detection of these lipids. This paper demonstrates that the pattern of spots corresponding to glycerol diether moieties obtained by thin-layer chromatographic (t.l.c.) analysis of whole-organism methanolysates (Minnikin *et al.*, 1975) provides a means of distinguishing archaebacterial halophilic taxa from eubacterial halophilic

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taxa. The method described is rapid, simple, requires no expensive equipment and should be applicable in routine laboratories.

METHODS

Cultures and cultivation. Details of the test strains and their sources are shown in Table 1. *Actinopolyspora halophila* and members of the genera *Halobacterium* and *Halococcus* were grown at 37 °C in liquid shake culture on the medium of Payne *et al.* (1960) with the exception of *Halobacterium volcanii* which was grown in the

Table 1. *Grouping of test strains based on chromatographic analysis of whole-organism methanolysates*

(*a*) *Halophilic bacteria lacking fatty acid methyl esters but containing glycerol diether moieties*

Strain	Source*
Halobacterium cutirubrum	NCMB 763
Halobacterium halobium	NCMB 736
Halobacterium halobium	CCM 2090
Halobacterium halobium	NCMB 777
Halobacterium halobium	NCMB 2080
Halobacterium saccharovorum	NCMB 2081
Halobacterium salinarium	NCMB 764
Halobacterium salinarium subsp. *proteolyticum*	NCMB 786
Halobacterium salinarium	CCM 2148
Halobacterium simoncinii subsp. *neapolitanum*	NCMB 773
Halobacterium sp. (Cagliari)	NCMB 768
Halobacterium trapanicum	NCMB 784
Halobacterium volcanii	NCMB 2012
Halococcus morrhuae	NCMB 787
Halococcus morrhuae	NCMB 749
Halococcus morrhuae	CCM 2526
Sarcina morrhuae†	NCMB 761
Sarcina litoralis†	NCMB 757
Sarcina sreenivasani†	NCMB 776
Alkalophilic halophiles: SP1	W. D. Grant
SP2	W. D. Grant
MS3	W. D. Grant

(*b*) *Halophilic bacteria containing fatty acid methyl esters but lacking glycerol diether moieties*

Strain	Source*
Actinopolyspora halophila	ATCC 27976
Brevibacterium halotolerans	ATCC 25096
Ectothiorhodospira halophila	DSM 244
Ectothiorhodospira sp. (S1)	W. D. Grant
Flavobacterium halmephilum	NCMB 1971
Micrococcus halobius	CCM 2526
Micromonospora halophytica subsp. *halophytica*	NRRL 2998
Micromonospora halophytica subsp. *nigra*	NRRL 3097
Paracoccus halodenitrificans	NCMB 700
Planococcus halophilus	ATCC 27964
Pseudomonas beijerinckii	NCIB 9041
Vibrio costicola	NCMB 701

* ATCC, American Type Culture Collection, Rockville, U.S.A.; CCM, Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia; DSM, Deutsche Sammlung von Mikroorganismen, Munich, W. Germany; NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland; NCMB, National Collection of Marine Bacteria, Aberdeen, Scotland; NRRL, Northern Utilization Research and Development Division, U.S. Department of Agriculture, Illinois, U.S.A.; W. D. Grant, Department of Microbiology, University of Leicester, Leicester.

† Now classified as *Halococcus morrhuae* by Kocur & Hodgkiss (1973).

medium of Mullakhanbhai & Larsen (1975). *Flavobacterium halmephilum*, *Micrococcus halobius*, *Micromonospora halophytica*, *Paracoccus halodenitrificans*, *Pseudomonas beijerinckii* and *Vibrio costicola* were grown in liquid shake culture in nutrient broth no. 2 (Oxoid) supplemented with 10% (w/v) NaCl at 30 °C. *Brevibacterium halotolerans* was grown in nutrient broth no. 2 (Oxoid) supplemented with 5% (w/v) NaCl at 30 °C. The alkalophilic halophiles (SP1, SP2, MS3) and *Ectothiorhodospira* strains (*E. halophila*, S1) were grown in the media of Tindall *et al.* (1980) and Imhoff & Trüper (1977), respectively, at 37 °C. *Planococcus halophilus* was grown at 30 °C in liquid medium containing tryptone (5 g l^{-1}), proteose peptone (5 g l^{-1}) and NaCl (58·4 g l^{-1}). Cultures were checked for purity at maximum growth, harvested by centrifugation (10000 *g*) and freeze-dried.

Whole-organism acid methanolysis. Dry cells (100 mg) were mixed with methanol (3 ml), toluene (3 ml) and conc. H_2SO_4 (0·1 ml) and heated at 50 °C for 15 to 18 h as described by Minnikin *et al.* (1975). The long-chain components were extracted from this mixture by adding 1·5 ml hexane (Minnikin *et al.*, 1975). Hexane extracts were chromatographed on Merck silica gel $60F_{254}$ aluminium-backed thin-layer plates (10 × 10 cm) using petroleum ether (b.p. 60 to 80 °C)/diethyl ether (85 : 15, v/v) as developing solvent (Minnikin *et al.*, 1975). Further resolution of glycerol diether moieties was achieved by developing the t.l.c. plate in one dimension using petroleum ether (b.p. 60 to 80 °C)/acetone (95 : 5, v/v) followed by redevelopment in the same direction using toluene/acetone (97 : 3, v/v). Confirmation of the results obtained from the whole-organism methanolysis method of Minnikin *et al.* (1975) was achieved by using the anhydrous methanolic HCl procedure of Kates *et al.* (1966) on the total lipid fraction of the test strains. Lipids were revealed by spraying the t.l.c. plates with 10% dodecamolybdophosphoric acid in absolute ethanol and heating for 15 min at 150 °C. All lipids appeared as dark blue spots on a yellow background.

RESULTS AND DISCUSSION

The patterns obtained by chromatographic analysis [using petroleum ether (b.p. 60 to 80 °C)/diethyl ether; 85 : 15, v/v] of methanolysates of halophilic bacteria are shown in Fig. 1.

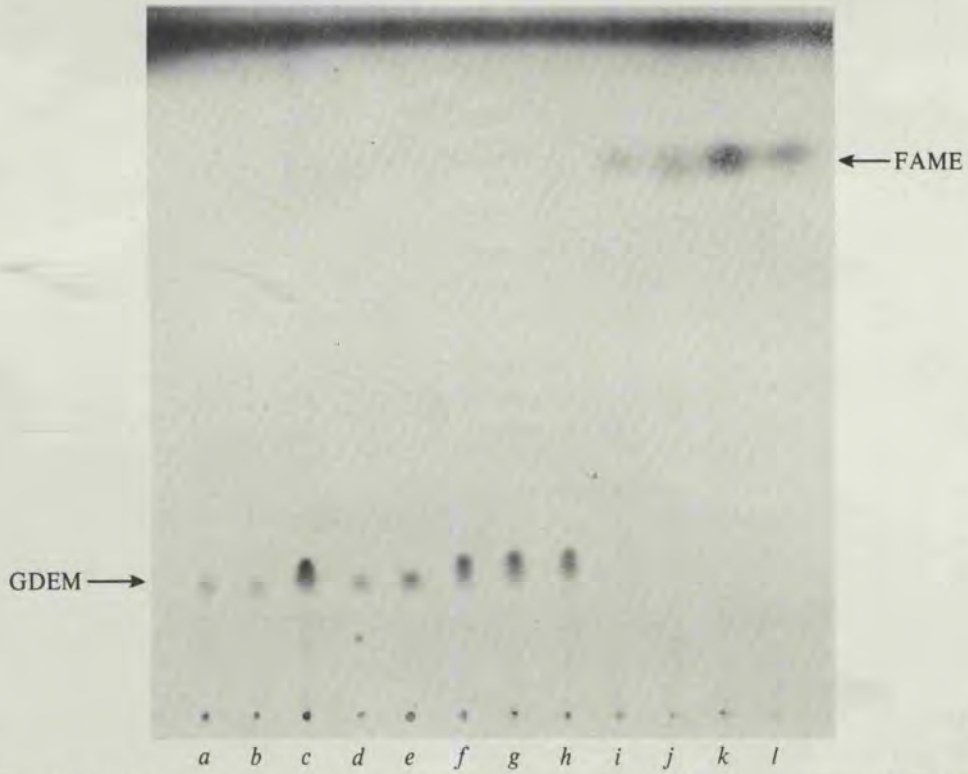

Fig. 1. Thin-layer chromatographic analysis of whole-organism methanolysates of (*a*) *Halococcus morrhuae* NCMB 787, (*b*) *Halococcus morrhuae* NCMB 749, (*c*) *Halobacterium halobium* NCMB 777, (*d*) *Halobacterium salinarium* NCMB 764, (*e*) *Halobacterium volcanii* NCMB 2012, (*f*) SP1, (*g*) SP2, (*h*) MS3, (*i*) *Planococcus halophilus* ATCC 27964, (*j*) *Actinopolyspora halophila* ATCC 27976, (*k*) *Vibrio costicola* NCMB 701 and (*l*) *Pseudomonas beijerinckii* NCIB 9041. Plates were developed in petroleum ether (b.p. 60 to 80 °C)/diethyl ether (85 : 15, v/v). FAME, fatty acid methyl esters; GDEM, glycerol diether moieties.

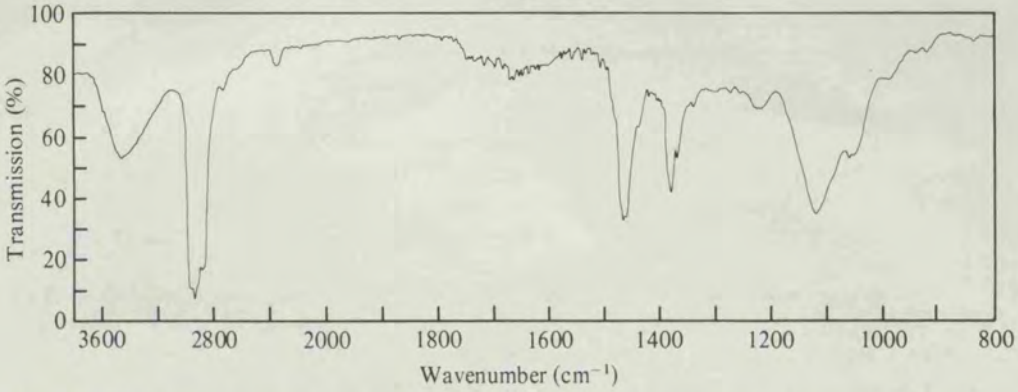

Fig. 2. Infrared spectrum of the purified glycerol diether moiety from *Halobacterium cutirubrum* NCMB 763.

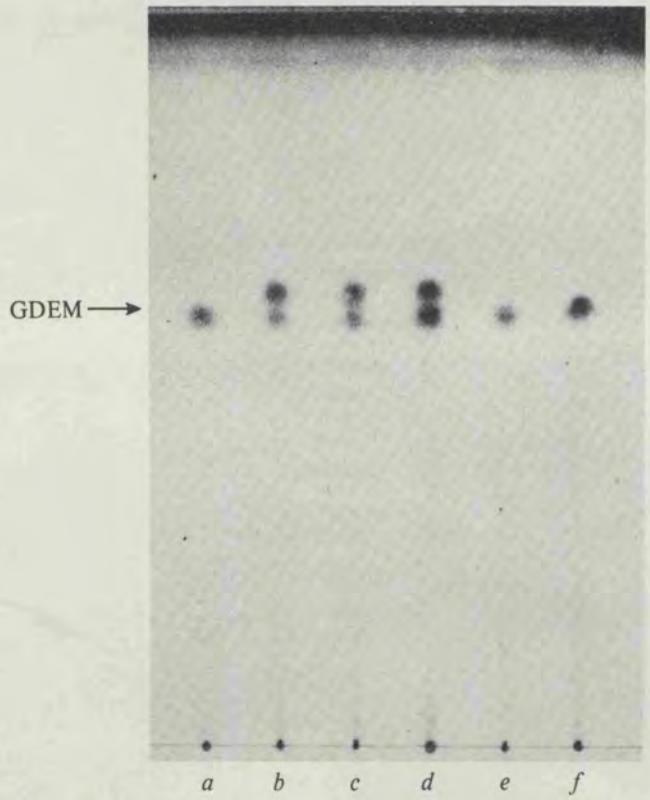

Fig. 3. Thin-layer chromatographic analysis of whole-organism methanolysates of (*a*) *Halobacterium cutirubrum* NCMB 763, (*b*) SP1, (*c*) SP2, (*d*) MS3, (*e*) *Halobacterium salinarium* NCMB 764 and (*f*) *Halococcus morrhuae* NCMB 749. Plates were first developed in petroleum ether (b.p. 60 to 80 °C)/acetone (95 : 5, v/v), then dried and redeveloped in the same direction in toluene/acetone (97 : 3, v/v). GDEM, glycerol diether moieties.

Spots with R_F values of about 0·2 were attributable to glycerol diether moeities (GDEM) whereas components having R_F values greater than 0·6 corresponded to methyl esters of non-hydroxylated fatty acids (FAME) (Minnikin *et al.*, 1975, 1978). On the basis of their chromatographic patterns, the test strains were divided into two broad groups: taxa containing GDEM and lacking FAME (Table 1 *a*), and taxa containing FAME only (Table 1 *b*). The absence of ester-containing lipids in the archaebacterial halophiles (Table 1 *a*) was demonstrated by a negative reaction with the hydroxamate/$FeCl_3$ ester spray reagent (Skidmore & Entenman, 1962; Kates, 1975). This reagent unequivocally distinguishes

glycerol diether moieties from ester-containing components such as hydroxylated fatty acid methyl esters, which have similar R_F values (about 0·2). The presumptive diether moieties, purified by preparative t.l.c., were further confirmed by infrared spectroscopy. Notable was the complete absence of ester absorption in the range 1730 to 1750 cm^{-1} (Fig. 2). However, the spectra did show strong absorption bands indicative of long-chain groups (2960, 2940, 2880, and 1460 cm^{-1}), OH groups (3400 cm^{-1}, broad) and ether C–O–C groups (1120 cm^{-1}) (Fig. 2). Doublets at 1365 to 1380 cm^{-1} were indicative of isopropyl units.

It was noted that the glycerol diether spots of certain archaebacterial strains (spots *c*, *f*, *g* and *h*, Fig. 1) were elongated. An attempt to resolve these components further was made by developing the t.l.c. plate in one dimension using petroleum ether (b.p. 60 to 80 °C)/acetone (95:5, v/v) followed by redevelopment in the same direction in toluene/acetone (97:3, v/v). Using this procedure, two separate spots were observed, suggesting two distinct glycerol diether moieties (Fig. 3). Further confirmation that these strains contain two chromatographically distinct glycerol diether moieties was obtained by applying the alternative acid methanolysis procedure of Kates *et al.* (1966). Chromatographic analysis of these extracts yielded identical results. This is the first report of two distinct glycerol diether moieties within the extreme halophiles. The extensive investigations of Kates and associates were confined to strains (notably *Halobacterium cutirubrum*) which possess only one major glycerol diether moiety (Fig. 3). Work is in progress to elucidate the structural relationship between these two chromatographically distinct diether moieties.

We believe that thin-layer chromatographic analysis of whole-organism methanolysates provides a simple and effective way of distinguishing archaebacterial halophiles from eubacterial halophiles. In addition, the methanolysis procedure of Minnikin *et al.* (1975) can be scaled down to about 15 to 20 bacterial colonies and thus provides a useful screening procedure for archaebacterial halophiles.

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PHOTOTROPHIC BACTERIA FROM KENYAN SODA LAKES.

Ph.D. thesis

by

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University of Leicester, 1980.

The soda lakes of the Rift Valley, Kenya, are a group of closed-basin, alkaline, saline lakes. The most extreme example in the Rift Valley is Lake Magadi, where semi-solid trona deposits fill the lake, and are mined commercially. At present the lakes are isolated from one another, although this may not have always been the case.

The microbiological investigations of these lakes have shown that characteristic phototrophic bacteria and extremely halophilic archaebacteria occur abundantly under certain conditions. The phototrophic bacteria observed at the lakes include Chloroflexus-like organisms of the hot springs, and organisms resembling members of the genus Ectothiorhodospira. Ectothiorhodospira-like organisms isolated from the lakes were similar to Ectothiorhodospira mobilis and Ectothiorhodospira shaposhnikovii, in their physiology and biochemistry, but could be divided into two groups on the basis of the occurrence of gas vacuoles in one group. These organisms, which deposit sulphur outside the cell during the photooxidation of sulphide to sulphate, constitute an ecological and physiological group adapted to life in the soda lakes. The taxonomic relationships of the organisms isolated from the lakes is discussed, including the taxonomic status of the gas vacuolate and extremely halophilic organisms of the Ectothiorhodospira group.

The extremely halophilic archaebacteria, isolated from Lake Magadi were similar to known archaebacterial extreme halophiles, in their possession of ether linked lipids, NaCl requirement, and the lack of muramic acid in the cell wall, but were different in other respects, including adaptation to low Mg^{2+} concentrations and pH 9.0-10.0. The organisms constitute an ecological and physiological group within the extremely halophilic archaebacteria, and their taxonomic relationship is discussed.