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Evidence that changes in the growth conditions affect the relative distribution of diether lipids in haloalkaliphilic archaebacteria

(Archaebacteria; extreme halophiles; alkaliphiles; haloalkaliphiles; diether lipids; salinity)

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1. SUMMARY

The polar lipids of members of the family *Halobacteriaceae* are derived from isopranyl glycerol diethers. In many strains only a single, diphytanyl glycerol diether is present. However, certain genera contain strains that produce, in addition to the diphytanyl glycerol diether, a sesterterpanyl-phytanyl glycerol diether. In order to test the hypothesis that the relative amounts of the diethers may alter with changes in the growth conditions, a number of selected strains were grown in media of different salinity. The observed changes in the diether lipid composition indicates that growth conditions affect their distribution, although these studies indicate a complex interaction between genetic, phenetic and physiological parameters in each of the strains.

2. INTRODUCTION

Studies on halotolerant eubacteria have shown that the salinity of the growth medium affects the chemical composition of the cell envelope [1–3]. Investigations of the influence of salinity on the polar lipids indicate a 3-fold effect. Firstly, the relative percentage composition of the different polar lipids may change, which may also be accompanied by changes in the total concentration of the polar lipids. Thirdly, there may also be changes in the fatty acid composition of the polar lipids.

The extremely halophilic archaebacteria represent an unusual group of prokaryotes, which are well adapted to life in the highly saline environment. These organisms are characterised by the presence of polar lipids derived from isopranyl glycerol diethers [4]. While it is possible to study the relative distribution of the polar lipids in relation to salt concentration, the presence of a single diether in many members of the family *Halobacteriaceae* excludes the possibility that there would be changes in the nature of the non-polar, diether core of the polar lipids in response to environmental changes. It has, however, recently been shown that in some *Halobacterium* species, and in members of the genera *Halococcus, Natronococcus,* and *Natronobacterium,* a second diether [5–7], and in some cases a third diether are also present [8,9]. In such organisms it may be possible to detect changes in the diether lipid distribution in response to

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environmental changes. The present paper has sought to investigate this question in relation to changes in the salinity of the growth medium, using members of the alkaliphilic genera *Natronobacterium* and *Natronococcus*.

3. MATERIALS AND METHODS

3.1. Cultures and cultivation

Haloalkaliphilic archaebacterial strains 01YE and 04A were isolated from Owens Lake, California. The isolation and sources of the species *Natronobacterium pharaonis* (DSM2160), a gift from G.S.H. Soliman, University of Bonn, *Nb. gregoryi* (NCMB2189), *Nb. magadii* (NCMB2190), and *Natronococcus occultus* (NCMB2192), a gift from W.D. Grant, University of Leicester, have been described previously [7,10–13]. All strains were grown, with shaking, at 37°C in the medium of Tindall [9], of the following composition (%, w/v): KCl, 0.1; NH_4Cl, 0.1; KH_2PO_4, 0.1; Na_2CO_3, 0.5; sodium glutamate, 0.1; yeast extract (Oxoid), 0.5; casein hydrolysate (Oxoid), 0.5; $MgSO_4$, 0.1 mM; $CaSO_4$, 0.1 mM; 1 ml/l trace element solution of Imhoff and Trüper [14]. NaCl was added to give final concentrations of 3.0, 3.5, 4.0, and 4.5 M, respectively. Cultures were harvested in the late exponential phase by centrifugation, washed with the basal salts growth medium and freeze-dried.

3.2. Acid hydrolysis and chromatography

Freeze-dried cells (100 mg) were hydrolysed in methanol: toluene: conc. H_2SO_4 (5 : 5 : 0.2) at 50°C overnight [15]. Following partition of the diethers into hexane, this phase was subjected to preparative thin-layer chromatography on silica gel thin layers (Macherey Nagel, Art. No. 804 023, cut to 10 × 10 cm), developed in the solvent hexane : diethyl ether : acetic acid (70 : 30 : 1) [16]. The diethers were located with iodine vapour, eluted from the appropriate bands with chloroform, and subjected to gas chromatography without further derivatisation. Gas chromatography was carried out as described previously [9], using a Packard Model 430 gas chromatograph fitted with a 5% SE 30 on Chromosorb (80/100 mesh) 2 m glass column. Separation was carried out isothermally at 310°C.

4. RESULTS AND DISCUSSIONS

The percentage composition of the diether lipids of the six strains studied are shown in Table 1 and Fig. 1. It has been shown previously [9], that when these strains are grown under the same conditions and harvested in the same growth phase, they have different diether lipid distributions. Comparisons of the strains at any given NaCl concentration confirms these findings (Table 1). However, these results illustrate, in addition, that the relative per-

Table 1

Relative composition of diphytanyl glycerol diethers, expressed as a percentage of the total diether (diphytanyl and sesterterpanyl-phytanyl = 100%) composition, in haloalkaliphilic archaebacterial strains in relation to the NaCl concentration of the growth medium

Strain	Molar NaCl concentration in growth medium			
	3.0	3.5	4.0	4.5
Natronobacterium pharaonis (DSM2160)	62.2	57.5	51.5	45.6
Nb. gregoryi (NCMB2189)	73.8	47.8	43.4	42.9
Nb. magadii (NCMB2190)	73.8	59.6	54.2	42.5
Natronococcus occultus (NCMB2192)	79.3	75.2	73.2	67.2
Strain 01YE	57.4	52.8	63.4	63.8
Strain 04A	80.4	67.9	70.1	66.4

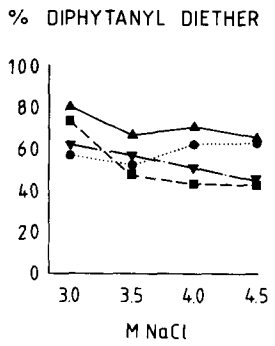

Fig. 1. Graphical representation of the influence of NaCl concentration of the growth medium on the relative composition of the diphytanyl glycerol diether, expressed as a percentage of the total diether (100%) composition. Cells were grown to the late exponential phase, harvested, and the diethers obtained by acid hydrolysis. The relative percentage composition of the constituent diphytanyl and sesterterpanyl-phytanyl glycerol diethers were determined by gas chromatography. Experimental conditions are given in MATERIALS AND METHODS. Strain 01YE (●------●); Strain 04A (▲———▲); *Nb. gregoryi* (NCMB2189) (■ — — — ■); *Nb. pharaonis* (DSM2160) (▼·–·–·▼).

centage of the diether lipids are not constant, and change in response to changes in the growth conditions.

The exact response to the changes in salinity of the growth medium are, however, not entirely clear. In five of the strains examined (Table 1) there appears to be a tendancy for the relative amounts of the diphytanyl glycerol diether to decrease with increasing salinity. In strain 01YE there appears to be an increase in the amount of diphytanyl glycerol diether with increasing salinity (Fig. 1). One possible reason for these differences could be related to the NaCl optima of the strains studied. In the case of *Nb. gregoryi* and *Nc. occultus* their NaCl optima are nearer 3.0–3.5 M, while in the other strains the optima are nearer 3.5–4.0 M NaCl. However, *Nb. gregoryi* and *Nc. occultus* show the same response to salinity as do *Nb. magadii* and *Nb. pharaonis*. Thus, it cannot be concluded that the diether lipid composition is directly determined by a simple combination of the NaCl optimum of the strain and the actual salinity of the growth medium. The results from strain 01YE indicate the possibility that the selection of the direction of the change of the relative diether composition (i.e. increase or decrease) may be controlled genetically, rather than by biophysical limitations. This would suggest that there is no way of predicting if an organism increases or decreases the relative proportion of the diphytanyl glycerol diether in response to increasing salinity. The implications of this require further study.

While it was the purpose of this work to use different salinities to test the hypothesis that the diether lipid composition of the strains may change in response to changes in the environment, the results presented here indicate that all of the organisms tested are capable of altering the relative amounts of the diethers present in the polar lipids. These results also indicate that the actual extent of the changes in any given strain are probably genetically fixed, rather than determined solely by external parameters. Whether this is also reflected in different rates of biosynthesis of the diethers under different conditions also requires to be investigated, although the data presented here strongly suggests that at some point changes in the external environment may also affect the biosynthesis of the different diethers.

A second question, which this work does not answer, is whether the salinity of the medium has a direct or indirect effect on the diether lipid distribution. While all organisms were harvested in the same growth phase, due to the different NaCl optima of the strains, each showed different specific growth rates. Further experiments must be designed to test if variations in the growth rates at the same salinity affect the diether lipid composition. It is important to distinguish between the direct influence of salinity, which determines the diether lipid composition irrespective of the growth rate, and growth rate-related effects, which are brought about indirectly by altering the salinity.

These preliminary results indicate, that with the discovery of other diethers [5–9] in the extremely halophilic archaebacteria it is now possible to study the influence of environmental parameters on the non-polar, diether core of the polar lipids of these organisms. This system is unique among halotolerant and halophilic prokaryotes, and provides a simple system, comprising a maximum of three components. This simplifies the interpretation of

the results when compared with studies on eubacteria, where the degree of saturation, branching, or cyclisisation, as well as changes in the chain length of the fatty acids must be taken into consideration.

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