Cellular Fatty Acid Composition of *Deleya halophila*: Effect of Growth Temperature and Salt Concentration

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The cellular fatty acid composition of *Deleya halophila*, a moderately halophilic bacterium, grown at different temperatures and NaCl concentrations is reported. When the temperature was lowered the amounts of monounsaturated C_{16:1} and C_{18:1} fatty acids increased with a corresponding decrease in the amounts of saturated C_{16:0} and C_{18:0} fatty acids. Increasing the salt concentration in the medium resulted in an increase of cyclopropane fatty acids, with a concomitant decrease in the monounsaturated fatty acids, suggesting that cyclopropane synthetase is activated or induced by high levels of salt.

INTRODUCTION

Deleya halophila is a moderately halophilic bacterium isolated from hypersaline soils (Quesada *et al.*, 1984). This micro-organism is capable of growing over a wide range of salt concentrations (2.5–25%, w/v, total salts) giving optimal growth at 7.5% when cultured at 32 °C (Ferrer *et al.*, 1987).

The biochemistry of moderately halophilic bacteria has only recently been investigated in detail. However, little is known about the regulatory mechanisms of membrane structure and function in saline environments. It has been suggested that NaCl-induced alterations of membrane lipid composition may be important in controlling the ionic permeability of halotolerant or halophilic bacteria (Hiramatsu *et al.*, 1980; Komaratat & Kates, 1975; Ohno *et al.*, 1979), and it is of interest to determine how the physicochemical properties of the membranes are regulated to adapt to a range of salinities.

Alterations in fatty acid composition occur in some moderately halophilic bacteria with changes in salinity (Hanna *et al.*, 1984; Monteoliva-Sanchez & Ramos-Cormenzana, 1986, 1987a; Ohno *et al.*, 1979), and it has been suggested that perhaps the salt-dependent changes in fatty acid composition are mechanisms for regulating not lipid fluidity but lipid phase (Russell & Kogut, 1985).

Here we report the results of a study undertaken to examine how temperature- and salt-concentration-dependent changes in the fatty acid composition of *D. halophila* could contribute to membrane integrity in this moderately halophilic bacterium.

METHODS

Organism and culture conditions. The bacterium used was *D. halophila* CCM 3662. Cells were grown in the moderately halophilic (MH) medium described by Quesada *et al.* (1983), supplemented with a balanced mixture of sea salt (Subov, 1931) to give a final concentration of 3, 7.5 or 20% (w/v, total salts). The growth temperatures used were 20, 32 and 40 °C. All cultures were incubated in an orbital shaker, harvested in the exponential or stationary phase by centrifugation at 10000 g for 15 min at 4 °C, washed twice with cold saline containing NaCl at a concentration equal to that in the growth medium, and lyophilized.

Fig. 1

Fig. 1. Effect of growth temperature on the ratio of monounsaturated fatty acids ($C_{16:1} + C_{18:1}$, % w/w) and saturated fatty acids ($C_{16:0} + C_{18:0}$) of *D. halophila* grown to stationary phase at different salt concentrations: 3% (●), 7.5% (○) and 20% (▲).

Fig. 2

Fig. 2. Effect of salt concentration on the ratio of monounsaturated fatty acids ($C_{16:1} + C_{18:1}$, % w/w) and cyclopropane fatty acids ($C_{17:0cyc} + C_{19:0cyc}$) of *D. halophila* grown to stationary phase at different temperatures: 20 °C (●), 32 °C (○) and 40 °C (▲).

Fatty acid analysis. Fatty acid methyl esters (FAMES) were prepared from dried cells by saponification and heated with anhydrous methanol acidified with H_2SO_4 (Saito & McElhaney, 1977). FAMES were examined by GLC as previously described by Monteoliva-Sanchez & Ramos-Cormenzana (1987b).

In addition a second 2 m non-polar column (3% methyl silicone, SE-30, on 80/100 mesh Gas-Chrom Q) was used to identify the FAMES. To confirm the identity of unsaturated and cyclopropane fatty acids, hydrogenation and bromination were done as described by Brian & Gardner (1968).

RESULTS

Under optimal culture conditions (a salt concentration of 7.5% at 32 °C), the major fatty acid components were $C_{16:0}$ and $C_{18:1}$, together with significant proportions of $C_{16:1}$, $C_{18:0}$ and $C_{17:0cyc}$.

The effect of temperature on the fatty acid composition of *D. halophila* is shown in Table 1 and Fig. 1. When the temperature was lowered, the proportions of both $C_{16:1}$ and $C_{18:1}$ increased, with a corresponding decrease in the proportions of saturated $C_{16:0}$ and $C_{18:0}$ acids. However, the relative proportions of the cyclopropane fatty acid $C_{17:0cyc}$ appeared to be unaffected by temperature; these results were similar for all the salt concentrations investigated.

The fatty acid composition of *D. halophila* was altered when cells were grown in media containing different salt concentrations (Table 1, Fig. 2). For each growth temperature studied, the relative proportion of cyclopropane fatty acids, $C_{17:0cyc}$ and $C_{19:0cyc}$, increased as the salt concentration of the growth medium was raised, with a concomitant decrease of both monounsaturated $C_{16:1}$ and $C_{18:1}$ fatty acids. The relative proportion of saturated $C_{16:0}$ acid was constant with variations in the salt concentration at the three temperatures studied.

To determine whether the phase of growth influenced the fatty acid composition, cells in the exponential and stationary phases grown with 3, 7.5 and 20% salt concentration were analysed (Table 2). No large changes in the total fatty acid composition as a function of growth phase

Table 1. Fatty acid composition of *D. halophila* at the stationary phase of growth as a function of growth temperature and salt concentration

Values are expressed as % (w/w) and are means of three determinations, each using a different batch of cells. In each case, the SD values were not greater than 0.70% of the mean.

Fatty acid	Temp. (°C) ...	Salt concn (% _{w/v}):								
		3			7.5			20		
		20	32	40	20	32	40	20	32	40
<i>Saturated</i>										
C _{10:0}	T	0.37	0.30	ND	ND	T	0.12	1.89	0.90	
C _{11:0}	0.66	0.16	0.74	T	0.31	0.46	0.63	0.37	0.96	
C _{12:0}	2.05	1.77	2.29	1.20	1.91	2.59	2.83	2.42	2.86	
C _{13:0}	1.06	2.72	2.84	1.95	2.49	2.47	1.15	2.71	2.09	
C _{14:0}	2.05	2.16	2.15	1.53	3.86	3.21	3.20	4.30	3.44	
C _{15:0}	0.94	1.08	1.04	0.24	0.12	T	T	T	T	
C _{16:0}	28.89	34.97	43.68	29.02	35.33	41.67	28.86	33.82	40.43	
C _{17:0}	T	ND	ND	ND	T	ND	ND	ND	ND	
C _{18:0}	3.01	4.30	9.88	6.13	8.32	11.81	7.56	10.30	11.41	
C _{19:0}	1.36	T	ND	T	ND	ND	T	1.20	0.75	
C _{20:0}	1.56	1.70	1.91	0.94	1.37	1.50	0.83	0.93	1.05	
<i>Branched</i>										
br-C _{14:0}	0.31	0.96	0.69	1.71	2.52	1.51	1.92	1.28	1.75	
br-C _{15:0}	4.65	4.41	6.75	5.65	5.34	8.70	4.78	5.58	5.79	
br-C _{16:0}	ND	ND	ND	ND	ND	ND	ND	ND	ND	
br-C _{17:0}	0.35	1.13	2.06	0.12	0.54	1.08	T	0.12	0.53	
<i>Unsaturated</i>										
C _{16:1}	13.10	9.53	7.75	10.80	8.82	4.20	6.59	3.99	2.23	
C _{18:1}	35.54	27.35	8.38	30.04	16.88	5.95	18.75	6.22	2.82	
<i>Cyclopropane</i>										
C _{17:0cyc}	4.43	4.88	6.31	8.52	9.01	9.78	11.93	12.52	12.68	
C _{19:0cyc}	1.01	2.48	2.17	2.09	3.12	3.08	10.75	12.27	10.19	

T, Trace content of less than 0.1%. ND, Not detected.

were observed, although cultures in the exponential phase contained more monounsaturated fatty acids and less saturated fatty acids than cultures in the stationary phase. There was a small increase in the proportion of cyclopropane fatty acids in the stationary phase cultures compared with the exponential phase cultures at the three salt concentrations investigated.

DISCUSSION

Investigation of the changes in fatty acid composition of cells grown under extreme conditions of temperature and salt concentration should be useful for studying the relationship between the fatty acid composition of lipids and their physical properties relevant to growth in halophilic environments. This study is the first report on the fatty acid composition of *D. halophila* CCM 3662, and has shown that it was significantly affected by the incubation temperature and salt content of the growth medium.

The predominant alteration in fatty acid composition detected in response to a decrease in growth temperature was an increase in unsaturation (Fig. 1). These results are in accord with earlier findings for other bacteria (Melchior, 1982; Russell, 1984). The increase in unsaturation may be due to an increase in the amount of the dehydrase/desaturase enzyme (Cronan, 1975) or the altered activity of enzymes at lower temperatures (Sinensky, 1971). The exact influence of temperature on physical properties of the membrane of *D. halophila* cannot be deduced from the present results since compositional effects may partly counteract one another (Barran & Miller,

Table 2. *Effect of phase of growth on the fatty acid composition of D. halophila grown at 32 °C and at different salt concentrations*

Values are expressed as % (w/w) and are means of three determinations, each using a different batch of cells. In each case, the SD values were not greater than 0.70% of the mean.

Fatty acid	Salt concn (% w/v)					
	3		7.5		20	
	Exp.	Stat.	Exp.	Stat.	Exp.	Stat.
<i>Saturated</i>						
C _{10:0}	0.28	0.37	0.12	ND	0.84	1.89
C _{11:0}	T	0.16	0.20	0.31	0.26	0.37
C _{12:0}	0.40	1.77	0.46	1.91	2.61	2.42
C _{13:0}	2.45	2.72	2.98	2.49	2.87	2.71
C _{14:0}	1.80	2.16	2.64	3.86	4.83	4.30
C _{15:0}	1.12	1.08	0.15	0.12	0.21	T
C _{16:0}	32.64	34.97	33.84	35.33	32.62	33.82
C _{17:0}	ND	ND	ND	T	T	ND
C _{18:0}	3.86	4.30	7.37	8.32	8.86	10.30
C _{19:0}	ND	T	ND	ND	T	1.20
C _{20:0}	0.38	1.70	T	1.37	T	0.93
<i>Branched</i>						
br-C _{14:0}	0.84	0.96	2.13	2.52	1.93	1.28
br-C _{15:0}	4.66	4.41	4.42	5.34	4.02	5.58
br-C _{16:0}	ND	ND	ND	ND	ND	ND
br-C _{17:0}	1.40	1.13	0.94	0.54	T	0.12
<i>Unsaturated</i>						
C _{16:1}	12.95	9.53	11.46	8.82	7.19	3.99
C _{18:1}	31.08	27.35	21.69	16.88	11.10	6.22
<i>Cyclopropane</i>						
C _{17:0cyc}	3.95	4.88	8.47	9.01	11.19	12.52
C _{19:0cyc}	2.06	2.48	3.09	3.12	11.38	12.27
<i>Growth rate</i>						
Generation time (h)	1.38	9.62	1.18	6.30	1.95	10.66
Incubation time (h)	12.00	26.00	12.00	26.00	16.00	30.00

Exp, exponential cultures. Stat, stationary cultures. T, Trace content of less than 0.1%. ND, Not detected.

1976), and also the change in growth rate with change in temperature may contribute to changes in lipid composition (Brown & Rose, 1969). The compositional adaptation may serve to maintain a constant membrane fluidity in response to temperature changes (McElhaney, 1976).

Increasing the salt concentration resulted in a decrease in the proportion of monounsaturated fatty acids with a concomitant increase in cyclopropane fatty acids (Fig. 2). In the non-halophile *Escherichia coli* growth in the presence of 0.3 and 0.6 M-NaCl resulted in an increase of cyclopropane fatty acids, detected only at the stationary growth phase (McGarrity & Armstrong, 1975, 1981). However, for the moderately halophilic bacterium *Pseudomonas halosaccharolytica*, cyclopropane fatty acid content is apparently increased by added NaCl at all growth stages (Ohno *et al.*, 1979). The compositional alterations of the cyclopropane fatty acids in *D. halophila*, detected at both the exponential and the stationary phase of growth (Table 2) more closely resemble the alteration observed for the moderate halophile *P. halosaccharolytica* than that for the non-halophile *E. coli*.

Ohno *et al.* (1979) reported that in *P. halosaccharolytica* an increase in either growth temperature or NaCl concentration promoted the formation of cyclopropane fatty acids from the corresponding monounsaturated fatty acids. In the moderate halophile *Flavobacterium halmephilum* (Monteoliva-Sanchez & Ramos-Cormenzana, 1986) only an increase in the salt concentration caused the formation of cyclopropane fatty acids.

The moderate halophile *D. halophila* exhibited phenotypic adaptation to changes in salt concentration. This adaptation process involved changes in the fatty acid composition, especially in the relative amounts of cyclopropane fatty acids. The fact that increased salt concentration led to an increase in cyclopropane fatty acids and a concomitant decrease in the corresponding monounsaturated fatty acids in *D. halophila* suggests that cyclopropane synthetase is activated or induced by high salt concentrations, and could indicate that the intracellular cyclopropane fatty acids are indispensable for Na⁺ permeability in some moderately-halophilic bacteria.

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