Growth and Lipid Composition of Some Dematiaceous Hyphomycete Fungi Grown at Different Salinities

By J. L. MULDER,* M. A. GHANNOUM, L. KHAMIS AND
K. ABU ELTEEN

Department of Botany and Microbiology, Kuwait University, PO Box 5969,
Safat 13060, Kuwait

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The growth of the dematiaceous hyphomycetes (darkly pigmented microfungi) *Alternaria phragmospora*, *Alternaria chlamydospora* and *Ulocladium chartarum* was altered by increasing concentrations of NaCl in the growth medium. *U. chartarum* demonstrated the greatest sensitivity to salinity changes: chlamydospora-like structures were common at NaCl concentrations > 5%. Lipid analyses of these fungi are presented. Steryl esters and fatty acids were the main lipid classes in the apolar fractions. Phosphatidylethanolamine, phosphatidylglycerol and phosphatidylcholine were the main polar compounds. Most phospholipids decreased at higher salinity. The major fatty acid was oleic acid (18:1) which decreased at 10% NaCl. A similar pattern was observed for palmitic acid (16:0). Myristic acid (14:0) and myristoleic acids (14:2, 14:1) were detected in all isolates cultured at 10% NaCl.

INTRODUCTION

Grant & Long (1981) stated that an extreme environment is one in which some organisms are successful and a majority are excluded. This is clearly demonstrated when considering obligate organisms. The situation is less clear when dealing with a changing environment supporting both obligate and facultative organisms. Kushner (1978) emphasized the importance of the facultative organism in elucidating the possible adaptive features and mechanisms which assist in the exploitation of a changing and stressful environment.

Solute-tolerant micro-organisms demonstrate different structural and biochemical responses to alterations in the solute concentration. Brown (1972, 1976) reported that the most important feature in sugar-tolerant yeasts was the accumulation of polyols in the cell. Similarly, Avron & Ben-Amotz (1979) reported on the increasing levels of glycerol in the alga *Dunaliella* with an increase in NaCl concentration in the growth medium. In the fungus *Dendryphiella salina*, Wethered *et al.* (1985) reported increased levels of polyols when the fungus was grown in saline media.

Numerous investigations have concentrated on the structure and physiology of the halophilic and halotolerant bacteria, whereas the filamentous fungi have been relatively neglected. Combs *et al.* (1968) confined their studies to the effect of NaCl on the lipid content of the pathogenic yeast *Candida albicans*. Tresner & Hayes (1971) reported on the tolerance of terrestrial fungi to NaCl in their environment, but did not explain what were the actual responses of these genera to increasing salinity. Apart from the study on *Dendryphiella salina* by Paton & Jennings (1988) little information is available on the responses of dematiaceous hyphomycetes (darkly pigmented microfungi) to salinity changes even though as a group they are frequently isolated from saline soils and salt-marshes (Pugh, 1962; Moustafa, 1975; Radwan *et al.*, 1984). Moustafa (1975), in a study of osmophilous (osmotolerant) fungi found in the salt-marshes of Kuwait, recorded fluctuating salinities ranging from 1.2% to 24.2% where crusts were frequently formed. Similarly, Al-Hasan *et al.* (1987) reported on the seasonal changes in salt-marshes. An organism

from such an ecological niche may possess specific characteristics which help it to adapt to the changing environment. Thus the main objective of the present study was to investigate the effects of increasing concentrations of NaCl on the growth and structure of three dematiaceous hyphomycetes, which are present in naturally occurring environments with a fluctuating salt concentration as well as in the desert soils of Kuwait.

METHODS

Isolation of the microfungi. *Ulocladium chartarum*, *Alternaria chlamydospora* and *Alternaria phragmospora* were isolated from soil samples collected from the salt-marsh and surroundings in the Al-Khiran area situated south of Kuwait city. Isolation media included sea-water agar (SWA) (Norris *et al.*, 1970) and potato-dextrose agar (PDA). Standard soil dilution techniques were used and isolates were purified on PDA plates. Pure isolates were maintained on PDA slants and stored at 4 °C.

Growth studies. PDA was used in all growth studies. The varying saline concentrations were prepared by addition of appropriate amounts of NaCl to the media to yield final concentrations of 5.0%, 7.5%, 10.0%, 12.0% and 14.0%. NaCl was not present in the control agar growth plates.

Radial growth. Triplicate plates for each concentration and isolate were incubated at 25 °C. Colony diameters were determined daily for a period of 21 d.

Biomass. Triplicate nutrient broth shake cultures for each isolate and salinity were incubated at room-temperature. After a 7 d period mycelial mats were harvested, washed and dried for determination of the total dry weights. This provided another measure of the growth rate at different salinities.

Light microscopy. Slide mounts, using lactophenol or occasionally lactophenol cotton blue, were made from colonies grown at the different NaCl concentrations. All observations and photography were done with an Opton research microscope.

Scanning electron microscopy (SEM). Samples were taken from both broth and agar cultures and fixed in 2.5% (v/v) glutaraldehyde, pH 7.4. The specimens were rinsed in 0.1 M-sodium cacodylate buffer, pH 7.4, and dehydrated by passing through an ethanol series. To guard against osmotic shock fixation was done in the growth medium by adding glutaraldehyde to give a final concentration of 2.5%; the fixation period was 16 h at 4 °C (Cole, 1986). After critical-point drying and coating with palladium-gold [250 Å (25 nm) layer], the specimen was examined in a Novascan 30 stereoscan electron microscope.

Lipid extraction and analysis. Broth cultures at 0 and 10.0% NaCl were incubated at 25 °C for 14 d; 10.0% NaCl was chosen on the basis of the evidence obtained in the growth studies. After 14 d all isolates were in the late exponential growth phase. There is no evidence to suggest that the lipid compositions change during this growth phase of the mycelium. At 10.0% NaCl growth was less but there was still sufficient fungal mycelium for lipid extraction. Mycelium was harvested by centrifugation. Total lipids were extracted with chloroform/methanol (2:1, v/v) and purified by standard methods (Folch *et al.*, 1957). Non-polar lipids were resolved by chromatography on silica gel plates using hexane/diethyl ether/acetic acid (90:10:1, by vol.) as the solvent system (Mangold & Malins, 1960). Polar lipids were resolved by two-dimensional TLC, using chloroform/methanol/7 M-ammonium hydroxide (65:30:4, by vol.) as the solvent system in the first direction, and chloroform/methanol/-acetic acid/water (170:25:25:4, by vol.) in the second direction (Nichols, 1964).

Lipid fractions were detected by iodine vapour or by charring at 220 °C after spraying with aqueous 50% (v/v) H₂SO₄. Fractions were identified by comparison with authentic samples and by the use of specific reagents (Dittmer & Lester, 1964; Siakotos & Rouser, 1965; Vioque, 1984). Lipids were quantified densitometrically using a Beckman R-112 densitometer.

Methanolysis of total lipids was achieved by heating at 90 °C with 2% (v/v) H₂SO₄ in absolute methanol (Chalvardjian, 1964). Following extraction with hexane, the resulting fatty acid methyl esters were purified by TLC and were analysed by GLC using a Pye-Unicam 204 chromatograph with a glass column (1.83 m × 4 mm i.d.) packed with 15% (w/w) DEGS on Anakron D (100–120 mesh) at 170 °C with nitrogen as the carrier gas.

RESULTS

Radial extension and production of biomass

With increasing salinity above 5%, a decrease in the growth rate was observed both for the linear extension and the biomass of all three isolates (Fig. 1*a–d*). The *Alternaria* spp. produced the greatest biomass as well as linear extension with the linear extension of *A. chlamydospora* being inhibited more at higher salinities. *U. chartarum* was consistently the most affected by increasing salinity. Slight growth was recorded for the *Alternaria* spp. on 15% NaCl media after a 7 d incubation period. A longer incubation period (21 d) resulted in significant growth of *A.*

Fig. 1. Relative growth at different NaCl concentrations. (a) *A. phragmospora*; (b) *A. chlamydospora*; (c) *U. chartarum*. □, No NaCl; △, 5% (w/v) NaCl; ○, 10%, ▽, 15%. (d) Production of biomass after 7 d; ■, *A. phragmospora*; □, *A. chlamydospora*; ▨, *U. chartarum*.

phragmospora (58 mm) and *A. chlamydospora* (36 mm). These values are much lower compared to the growth of *A. phragmospora* (90 mm) and *A. chlamydospora* (65 mm) in 10% NaCl. *U. chartarum* produced a 20 mm colony at 15% NaCl whilst at 10% a diameter of 63 mm was recorded.

The biomass values for each isolate after a 7 d incubation period are compared in Fig. 1(d). Increasing salinity resulted in the expected decrease in biomass production in all three isolates, but this reduction was more gradual than that observed for radial extension. As for radial extension, *U. chartarum* exhibited greater sensitivity than the other two isolates, with a 75% biomass reduction at 14% NaCl, whereas the reductions in the biomass of *A. phragmospora* and *A. chlamydospora* were 52% and 48% respectively.

Cell structure

Light microscopy and SEM studies revealed a substantial alteration in the overall structure and morphology of the isolates. There was a progressive trend from the typical thin-walled sporulating mycelium in the absence of NaCl to the thick-walled, chlamydosporic mycelial form with few or no conidia. The mycelial cells became larger with increasing salinity, whilst septation became more pronounced and more frequent. This resulted in the formation of large thick-walled cells in cultures grown in 14% NaCl (Figs 2 and 3). Conidiogenesis, which is usually prolific in these isolates, was greatly inhibited by concentrations of NaCl > 5% (data not shown). The SEM studies confirmed the increased frequency of septation (Fig. 4).

Lipid composition

The composition of the lipid classes in the isolates grown in varying salinities are shown in Table 1 and in Figs 5 and 6. The lipid classes in the apolar fractions were mainly steryl esters and fatty acids, followed by sterols, hydrocarbons and diglycerides. The latter were not, however,

Fig. 2. Photomicrographs of mycelium of *U. chartarum* grown at different concentrations of NaCl. (a) No NaCl – thin-walled mycelium with typical dictyospores; (b) 5% (w/v) NaCl; (c) 7.5%; (d) 10%; (e) 12%; (f) 14%. Note the increasing frequency of septation resulting in mycelium with inflated cells. Bar, 10 µm.

Fig. 3. Photomicrographs of *A. phragmospora* (a, b) and *A. chlamydospora* (c, d). Cultures were grown without NaCl (a, c) or at 10% NaCl (b, d). Bar, 10 μ m.

detected in *A. chlamydospora*. Similarly, 1- and 2-monoglycerides were present only in trace amounts in the latter species, whereas in *A. phragmospora* and *U. chartarum* significant increases were recorded in 10% NaCl. The sterols in all the isolates increased at the higher salinity. The main polar compounds recorded at significant levels in the three isolates were phospholipids including phosphatidylethanolamine, phosphatidylglycerol and phosphatidylcholine. All phospholipids decreased in the isolates grown at the higher salinity, except for phosphatidylcholine which increased slightly in *A. phragmospora*. Ceramide monohexoside was the major glycolipid present. Cardiolipin was detected in all the isolates and at different salinities. Monogalactosyldiacylglycerol and digalactosyldiacylglycerol occurred in small amounts.

The constituent fatty acids of the total lipids from each isolate grown at the different salinities are given in Table 2. In all isolates oleic acid (18:1) was the major fatty acid, and its proportion decreased at higher salinity. A similar pattern was observed for palmitic acid (16:0). Linoleic acid (18:2) in *A. chlamydospora* and *U. chartarum* increased very markedly at higher salinity, whereas in *A. phragmospora* there was a decrease. Myristic acid (14:0) and myristoleic acids (14:1, 14:2) were detected in all isolates at the higher salinity, but only in trace amounts in the absence of NaCl.

The results in Table 3 show some lipid classes and their fatty acid patterns. The predominant fatty acid for the individual classes was 18:1 when the organisms were cultured in the absence of NaCl. The saturated 16:0 and polyunsaturated 18:2 fatty acids also occurred in significant amounts in the lipid classes. In most lipid classes 18:1 decreased with the increase in salinity.

Fig. 4. Scanning electron micrographs of the fungal isolates demonstrating increased septation at 10% NaCl. (a, b) *A. phragmospora*, no NaCl and 10% NaCl; (c, d), *A. chlamydospora*, no NaCl and 10% NaCl; (e, f) *U. chartarum*, no NaCl and 10% NaCl.

Table 1. Composition of lipid classes from the fungal isolates grown at different salinities

Values are expressed as the percentage (w/w) of the total amount of lipid and are the means $\pm$ SD of three determinations. ND, Not detected; Tr., trace; Control, no NaCl.

Lipid class	Relative amounts (% w/w)					
	<i>A. phragmospora</i>		<i>A. chlamydospora</i>		<i>U. chartarum</i>	
	Control	10% NaCl	Control	10% NaCl	Control	10% NaCl
<i>Apolar</i>						
Hydrocarbon	2.8 $\pm$ 0.1	13.9 $\pm$ 1.0	0.1 $\pm$ 0.0	2.4 $\pm$ 0.1	2.4 $\pm$ 0.0	4.3 $\pm$ 0.1
Steryl ester	11.5 $\pm$ 0.8	11.4 $\pm$ 0.9	17.2 $\pm$ 1.8	18.0 $\pm$ 1.5	15.1 $\pm$ 1.2	12.8 $\pm$ 0.7
Triacylglycerol	2.6 $\pm$ 0.1	1.1 $\pm$ 0.0	ND	1.8 $\pm$ 0.0	2.9 $\pm$ 0.1	3.2 $\pm$ 0.0
Fatty acid	10.9 $\pm$ 0.7	9.6 $\pm$ 0.8	2.7 $\pm$ 0.0	9.1 $\pm$ 0.6	9.5 $\pm$ 0.9	19.4 $\pm$ 1.4
1,3- and 1,2-diglyceride	8.0 $\pm$ 0.3	6.1 $\pm$ 0.5	ND	ND	5.1 $\pm$ 0.2	7.0 $\pm$ 0.5
Sterol	6.8 $\pm$ 0.4	12.5 $\pm$ 1.3	5.8 $\pm$ 0.1	12.3 $\pm$ 1.9	8.0 $\pm$ 0.5	12.2 $\pm$ 0.7
1- and 2-monoglyceride	0.9 $\pm$ 0.02	9.2 $\pm$ 0.4	Tr.	Tr.	4.9 $\pm$ 0.1	9.7 $\pm$ 0.3
<i>Polar</i>						
Monogalactosyldiacylglycerol	1.2 $\pm$ 0.1	1.1 $\pm$ 0.1	ND	ND	ND	ND
Steryl glycoside	0.9 $\pm$ 0.0	9.2 $\pm$ 0.9	3.6 $\pm$ 0.2	Tr.	1.6 $\pm$ 0.0	Tr.
Ceramide monohexoside	6.9 $\pm$ 0.5	2.4 $\pm$ 0.1	7.1 $\pm$ 0.4	10.1 $\pm$ 1.9	3.9 $\pm$ 0.2	0.9 $\pm$ 0.0
Unidentified (1)	11.7 $\pm$ 1.0	0.9 $\pm$ 0.0	ND	ND	ND	ND
Phosphatidylethanolamine	8.6 $\pm$ 0.7	3.5 $\pm$ 0.1	22.1 $\pm$ 2.3	7.5 $\pm$ 0.8	12.5 $\pm$ 1.2	5.1 $\pm$ 0.1
Phosphatidylglycerol	11.8 $\pm$ 0.9	1.5 $\pm$ 0.1	11.7 $\pm$ 0.9	8.2 $\pm$ 0.9	9.7 $\pm$ 1.0	4.7 $\pm$ 0.1
Phosphatidylcholine	9.8 $\pm$ 0.3	11.3 $\pm$ 1.4	23.6 $\pm$ 2.1	20.5 $\pm$ 1.5	14.4 $\pm$ 1.7	12.6 $\pm$ 1.1
Digalactosyldiacylglycerol	0.8 $\pm$ 0.0	0.2 $\pm$ 0.0	ND	ND	ND	0.3 $\pm$ 0.0
Phosphatidylserine	0.8 $\pm$ 0.0	0.3 $\pm$ 0.0	1.9 $\pm$ 0.1	4.3 $\pm$ 0.4	3.8 $\pm$ 0.2	2.1 $\pm$ 0.0
Phosphatidylinositol	0.5 $\pm$ 0.0	0.9 $\pm$ 0.1	2.0 $\pm$ 0.0	1.2 $\pm$ 0.0	5.0 $\pm$ 0.1	2.3 $\pm$ 0.0
Phosphatidic acid	0.8 $\pm$ 0.0	Tr.	0.9 $\pm$ 0.0	1.0 $\pm$ 0.0	Tr.	1.0 $\pm$ 0.3
Cardiolipin	2.7 $\pm$ 0.1	4.9 $\pm$ 0.9	1.3 $\pm$ 0.0	3.6 $\pm$ 0.5	1.2 $\pm$ 0.0	2.4 $\pm$ 0.8

Table 2. Constituent fatty acids of total lipids from the fungal isolates grown at different salinities

Values are expressed as the percentage (w/w) of the total amount of lipid and are the means $\pm$ SD of three determinations. ND, Not detected; Tr., trace; Control, no NaCl.

Fatty acid	Relative amounts (% w/w)					
	<i>A. phragmospora</i>		<i>A. chlamydospora</i>		<i>U. chartarum</i>	
	Control	10% NaCl	Control	10% NaCl	Control	10% NaCl
<14	0.4 $\pm$ 0.0	2.0 $\pm$ 0.1	0.6 $\pm$ 0.0	1.2 $\pm$ 0.0	0.65 $\pm$ 0.1	0.4 $\pm$ 0.0
14:0	3.9 $\pm$ 0.3	8.0 $\pm$ 0.7	0.8 $\pm$ 0.1	2.6 $\pm$ 0.0	0.14 $\pm$ 0.0	0.1 $\pm$ 0.0
14:1	Tr.	4.3 $\pm$ 0.0	2.1 $\pm$ 0.0	3.2 $\pm$ 0.0	Tr.	4.6 $\pm$ 0.2
14:2	ND	4.7 $\pm$ 0.0	Tr.	2.3 $\pm$ 0.1	Tr.	3.2 $\pm$ 0.1
16:0	19.3 $\pm$ 1.0	11.2 $\pm$ 0.9	21.1 $\pm$ 1.1	17.6 $\pm$ 1.3	29.6 $\pm$ 1.9	17.3 $\pm$ 1.3
16:1	Tr.	7.4 $\pm$ 0.8	0.2 $\pm$ 0.0	3.7 $\pm$ 0.1	Tr.	7.1 $\pm$ 1.0
18:0	5.2 $\pm$ 0.0	6.3 $\pm$ 0.1	5.5 $\pm$ 0.0	3.5 $\pm$ 0.5	3.6 $\pm$ 0.2	0.5 $\pm$ 0.1
18:1	56.9 $\pm$ 3.1	42.0 $\pm$ 2.3	65.1 $\pm$ 4.7	25.5 $\pm$ 1.8	45.1 $\pm$ 3.1	26.5 $\pm$ 1.8
18:2	10.6 $\pm$ 0.9	3.6 $\pm$ 0.1	1.2 $\pm$ 0.0	34.3 $\pm$ 2.0	12.6 $\pm$ 0.9	30.3 $\pm$ 2.1
18:3	2.2 $\pm$ 0.0	1.7 $\pm$ 0.1	0.2 $\pm$ 0.0	1.5 $\pm$ 0.0	8.3 $\pm$ 0.2	10.0 $\pm$ 1.1
>18	1.5 $\pm$ 0.0	5.8 $\pm$ 0.1	3.3 $\pm$ 0.3	4.6 $\pm$ 0.1	Tr.	Tr.

This trend was reversed for 18:2 which increased in the major polar compounds (phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine), the one exception being phosphatidylcholine in *A. phragmospora*. Significant decreases in the saturated fatty acid 16:0 were recorded in phosphatidylglycerol in *U. chartarum* and *A. chlamydospora* as well as in

Fig. 5. Thin-layer chromatographic separation of the apolar lipids of *U. chartarum*, *A. phragmosporea* and *A. chlamydosporea* grown at the indicated NaCl concentration. Lipids were visualized by exposure to iodine vapour. H, hydrocarbon; SE, steryl esters; TG, triacylglycerols; FA, fatty acids; DG, diglycerides; S, sterols; MG, monoglycerides; PL, polar lipids.

phosphatidylethanolamine in *A. phragmosporea* and *U. chlamydosporea*. The unsaturated 16:1 occurred in trace amounts or was not detected in the major polar compounds of the isolates grown without NaCl, but appeared in significant amounts in the isolates grown at the higher salinity.

Fig. 6. Two-dimensional thin-layer chromatograms showing the composition of polar lipid classes for the three isolates grown without NaCl and at 10% NaCl. (a, b) *U. chartarum*, no NaCl and 10% NaCl; (c, d) *A. chlamydosporea*, no NaCl and 10% NaCl; (e, f) *A. phragmosporea*, no NaCl and 10% NaCl. TLC was done using silica gel plates and the following solvent systems: chloroform/methanol/7 M-ammonium hydroxide (65:30:4, by vol.) in the first dimension and chloroform/methanol/acetic acid/water (170:25:25:4, by vol.) in the second dimension. The lipid fractions were visualized by charring after spraying with 50% (v/v) H_2SO_4 . MGDG, monogalactosyldiacylglycerol; SG, steryl glycoside; CMH, ceramide monohexoside; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid; CL, cardiolipin.

Table 3. *Fatty acid patterns of some lipid classes from the fungal isolates grown at different NaCl concentrations*

Tr., trace; ND, Not detected.

Fatty acid	<i>A. phragmospora</i>		<i>A. chlamydospora</i>		<i>U. chartarum</i>	
	0%	10%	0%	10%	0%	10%
(a) <i>Phosphatidylglycerols</i>						
<14	0.5	0.1	Tr.	Tr.	ND	Tr.
14:0	1.1	1.8	0.9	1.3	0.1	0.6
14:1	Tr.	Tr.	1.8	7.8	ND	7.5
14:2	ND	ND	ND	ND	ND	1.1
16:0	6.6	5.8	25.7	1.4	31.5	9.1
16:1	0.1	1.3	ND	23.9	ND	8.0
18:0	0.2	0.3	Tr.	15.7	ND	ND
18:1	76.4	50.7	66.0	ND	34.1	28.0
18:2	12.1	39.3	1.8	23.9	18.6	34.4
18:3	2.3	0.9	ND	26.0	15.7	11.2
>18	0.8	ND	3.6	ND	ND	ND
(b) <i>Phosphatidylethanolamines</i>						
<14	0.6	1.1	Tr.	Tr.	0.2	0.1
14:0	1.7	1.0	1.9	1.5	1.1	0.3
14:1	4.3	0.1	6.3	6.0	ND	4.0
14:2	ND	ND	ND	5.1	ND	1.6
16:0	33.1	25.0	4.1	6.6	21.2	1.5
16:1	Tr.	Tr.	Tr.	20.1	0.5	10.4
18:0	3.1	0.2	Tr.	10.6	ND	ND
18:1	40.6	48.4	74.4	9.7	40.6	30.0
18:2	10.7	14.0	3.5	17.6	28.1	39.0
18:3	5.4	5.1	ND	15.9	8.4	13.2
>18	0.5	6.2	9.8	5.9	ND	ND
(c) <i>Phosphatidylcholines</i>						
<14	0.4	1.2	0.1	Tr.	Tr.	0.2
14:0	1.6	5.3	1.0	0.4	0.3	0.5
14:1	0.6	Tr.	1.4	1.7	ND	ND
14:2	ND	ND	2.8	ND	5.3	8.7
16:0	12.3	15.1	3.6	9.1	22.1	4.0
16:1	Tr.	Tr.	Tr.	ND	ND	15.2
18:0	Tr.	0.5	0.8	0.2	Tr.	2.9
18:1	70.2	47.0	73.5	20.2	41.2	22.9
18:2	13.1	7.7	11.8	65.9	20.1	27.2
18:3	1.7	3.2	ND	2.5	11.0	18.4
>18	0.2	20.0	5.0	ND	ND	ND

DISCUSSION

In response to the halostress created by the addition of different concentrations of NaCl in the media, the fungal isolates lower their growth rates and alter their structure and lipid composition. The growth at increasing salinity indicates the halotolerance of the isolates. The pattern of morphological change, which includes the increase in septation and width as well as the production of chlamydospores, is similar in all three isolates. This may contribute to the ability of these fungi to withstand the osmotic pressures created by the increased NaCl.

The lipid pattern observed was as expected and similar to the patterns reported by Weete (1980) for other similar fungal species. The effects of increased salinity were observed in the reduction of the major fatty acid (18:1) of the total lipids, but the unsaturated fatty acids were greater than the saturated fatty acids. This is consistent with the effects of salinity on lipids of *C. albicans* (Combs *et al.*, 1968). Phosphatidylethanolamine and phosphatidylglycerol decrease and

cardiolipin increases with the increase of NaCl in the growth medium, which may be a specific response to NaCl or may result from changes in growth rate. The scarcity of studies on similar eukaryotic organisms makes comparison difficult. Investigations which are reported are those which deal with prokaryotic organisms. Haloadaptive responses in prokaryotes may be very different to those in eukaryotes, as the organisation of a prokaryote is different. However, Miller (1985) did report a similar response for the halotolerant bacterium *Planococcus*. Adams *et al.* (1987) reported that phosphatidylethanolamine synthesis is reduced after increases in NaCl concentration. These NaCl-induced changes in lipid composition may control the ionic permeability of the organism in high salinity conditions (Ohno *et al.*, 1976, 1979). The similar responses observed in the present study suggest that lipids may play a role in regulating membrane permeability at different salinities.

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