Effect of ethanol on the phospholipid and fatty acid content of *Schizosaccharomyces pombe* membranes

ANNA I. KOUKOU, DEMOKRITOS TSOUKATOS and CONSTANTIN DRAINAS*

Sector of Organic Chemistry and Biochemistry, Department of Chemistry, University of Ioannina, 451 10 Ioannina, Greece

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Ethanol at concentrations up to 5% (v/v) had no effect on the growth of *Schizosaccharomyces pombe*, whereas concentrations over 7.5% were inhibitory. The major membrane phospholipids in *S. pombe* cells growing aerobically in the absence of added ethanol were phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine. Oleic acid (18:1) was the main fatty acid. When ethanol (7.5%) was added to aerobically growing cultures, the phosphatidylinositol content increased, whereas the 18:1 content decreased. Similar changes were observed in the membrane phospholipids of cells grown anaerobically without ethanol. However, the presence of ethanol in anaerobically growing cultures had an opposite effect on fatty acids, as the 18:1 content increased. The results support the idea that ethanol tolerance in *S. pombe* may be connected with a high content of 18:1 fatty acids, and with the ability to maintain a high rate of phospholipid biosynthesis.

Introduction

Microbial cells respond rapidly to alterations of their chemical environment. A significant contribution to this response is attributed to biochemical mechanisms which control membrane structure and function. Consequently, studying the changes in the content of membrane phospholipids and their fatty acid composition which follow such environmental alterations could help in understanding microbial adaptation to chemicals such as ethanol.

It has been reported that the presence of alcohols in the environment of growing cells usually results in changes in their membrane structure (Ingram & Buttke, 1984; Dombek & Ingram, 1984; Ingram, 1976). Resistance to alcohols has been connected with fatty acid composition (Casey & Ingledew, 1986; Amore & Stewart, 1987). Thus ethanol provides an excellent system for studying environmental adaptation and its relation to structural changes of microbial membranes. Although several reports have described the effect of ethanol on the structure of various microbial cell membranes, the available data on the content of phospholipids and their fatty acid composition are not consistent. For example, ethanol was reported to give rise to an increase in phosphatidylglycerol and a decrease in phosphatidylethanolamine in membranes of *Escherichia coli* (Ingram, 1976). Under similar conditions, a decrease in phosphatidylglycerol, lysophosphatidylglycerol and phosphatidyl-

ethanolamine was observed in *Bacillus subtilis* (Rigomier *et al.*, 1980). In contrast, ethanol was found to cause an increase in phosphatidylethanolamine in *Bacillus cereus* membranes (Kates *et al.*, 1962). In *Tetrahymena pyriformis* the presence of ethanol led to an increase in phosphatidylethanolamine and a decrease in 2-aminoethylphospholipid (Nandini-Kishore *et al.*, 1979). In the bacterium *Zymomonas mobilis*, which produces large amounts of ethanol, the presence of ethanol caused an increase in cardiolipin and a decrease in phosphatidylglycerol and phosphatidylethanolamine (Carey & Ingram, 1983). As far as the fatty acid content of phospholipids is concerned, ethanol induced an increase in the proportion of 18:1 at the expense of 16:0 fatty acids in *E. coli* (Ingram, 1976; Buttke & Ingram, 1978), and in *Saccharomyces cerevisiae* (Beaven *et al.*, 1982). In *T. pyriformis* an increase of 18:2 and a decrease of 16:1 and 16:2 fatty acids was observed in the presence of ethanol (Nandini-Kishore *et al.*, 1979). In contrast, the presence of ethanol did not affect the fatty acid composition of the membranes of *Z. mobilis* (Carey & Ingram, 1983).

The fission yeast *Schizosaccharomyces pombe*, which is a member of the wine flora, produces low amounts of ethanol. It grows up to the late phase of the wine fermentation (Amerine *et al.*, 1972) and is thus expected to be highly resistant to ethanol. These features have attracted considerable scientific attention and some reports have already described membrane phospholipids

and their total fatty acid composition in *S. pombe* (White & Hawthorne, 1970; Johnston & Coddington, 1984; Fernandez *et al.*, 1986). The present report gives detailed qualitative and quantitative data on the membrane phospholipids of *S. pombe* and it describes for the first time the effects of ethanol on the growth of *S. pombe*. The effect of ethanol on the content of membrane phospholipids and their fatty acid composition under aerobic and anaerobic conditions is also reported.

Methods

Growth of cultures. *Schizosaccharomyces pombe* strain 975 h⁺ was used in all experiments. It was maintained on yeast extract agar slants (1% w/v, yeast extract; 3% w/v, glucose; 2% w/v, agar). Cultures were grown aerobically in 400 ml of a mineral medium (Gutz *et al.*, 1974), containing 3% glucose and 0.5% yeast extract, shaken on an orbital shaker (New Brunswick, 300 r.p.m.) at 30 °C. For reduction of growth rate, 40% glucose or 3% xylose were used as alternative carbon sources, as described in the text. Growth was followed by measuring OD₆₀₀. Cells were harvested by centrifugation (1800 g) in either the late exponential phase (OD₆₀₀ = 0.5) or the early stationary phase (OD₆₀₀ = 0.9). Ethanol was added to sterile medium after cooling and before inoculation, at concentrations indicated in the text. For anaerobic conditions, conical flasks containing growth medium were bubbled with pure nitrogen for 45 min after sterilization and inoculation, then sealed by rubber stoppers on which water plugs were adjusted. This set-up allowed efflux of CO₂ while excluding any influx of air. The sealed flasks were incubated as above, with occasional gentle shaking by hand (Kovak *et al.*, 1967). Anaerobically growing cells were harvested at early stationary phase (OD₆₀₀ = 0.9) as above. Cells from fresh overnight yeast extract agar slant-cultures suspended in 5 ml liquid medium were used as inocula in all cases. Starting cultures contained 10⁶ cells ml⁻¹.

Ethanol estimations. Ethanol was estimated in clear medium filtrates at the end of culture growth by gas-liquid chromatography as described before (Koutinas *et al.*, 1988).

Extraction of total lipids. Cell pellets harvested as described above from 1200 ml cultures were washed twice in 30 ml 1.2 M-sorbitol and resuspended in the same solution (30 ml per g cell wet weight). Cells were converted to spheroplasts by digestion with Novozyme (Novo Industry, Denmark) essentially as described by Dickinson & Isenberg (1982); they were incubated with 0.09% (w/v) Novozyme at 30 °C for 6 min. The yield of spheroplasts was 100%. Spheroplasts were harvested by low-speed centrifugation (750–800 g), and washed twice by resuspension in 10 ml 1.2 M-sorbitol and centrifugation as above. The total membrane fraction was obtained as a pellet after osmotic lysis of the spheroplasts in 10 ml 0.9% NaCl and centrifugation at 29000 g for 20 min at 4 °C (Schibeci *et al.*, 1973). Over 99% of the spheroplasts were lysed by this method. The membrane fraction was extracted in chloroform/methanol (2:1, v/v) as described by Folch *et al.* (1957). The total lipids were dissolved in chloroform/methanol (2:1) and used for phospholipid and fatty acid analysis. Samples were stored under nitrogen gas at -20 °C. Spheroplasting offers better recovery of at least the major lipids, as compared with extraction by other established methods (Atkinson *et al.*, 1980; Greenberg *et al.*, 1983; Fernandez *et al.*, 1986).

Phospholipid analysis. The phospholipid fraction was separated from other lipid classes by thin-layer chromatography (TLC) on plates of silica gel 60 F₂₅₄ (0.5 mm thick) using a solvent system of hexane/

diethyl ether/glacial acetic acid (70:30:1, by vol.). The phospholipid band (*R_F* = 0) was scraped off the plates and silica gel was extracted by the method of Bligh & Dyer (1959). Phospholipid extract (20 µl, containing 80 µg phospholipids) was separated by HPLC (Varian LC 5000) using a Micropak Si-10 column. The peaks were compared to those of standard phospholipids using a UV detector. The column was eluted isocratically at a flow rate of 1 ml min⁻¹ with acetonitrile/methanol/75% phosphoric acid (130:5:1.5, by vol.). Fractions of 1 ml were collected, extracted according to Bligh & Dyer (1959) and the chloroform phase was washed three times with the upper phase to eliminate phosphoric acid (Jackson *et al.*, 1984). The different HPLC fractions were further characterized by TLC using the following solvent systems: (I) chloroform/glacial acetic acid/methanol/water (72:25:5:1.5, by vol.) and (II) chloroform/methanol/ammonia/water (65:35:5:2, by vol.). Spots were visualized by iodine vapour and phosphate spray reagent (Vaskovsky & Kostetsky, 1968). Spots containing amino groups were detected by spraying with ninhydrin reagent (Marinetti, 1962). To identify the phospholipids, standards were run along with the samples on the TLC plates. The phospholipid bands were scraped off the plates and phosphorus was determined as described by Bartlett (1959) and modified by Marinetti (1962). Cells from 2 ml samples of each culture were collected by centrifugation as above, washed with distilled water, extracted in 0.5 ml 0.5 M-NaOH, and used for total protein estimations by the Lowry method.

Fatty acid analysis. The total lipid extracts or phospholipid extracts were concentrated under nitrogen. Fatty acid methyl esters were prepared by refluxing with BF₃ (20% w/v, in methanol; Merck), as described by Morrison & Smith (1964). Fatty acid methyl esters were analysed by gas-liquid chromatography (Varian 3700) on a 15% DEGS column (2 m) supported on Chromosorb W, AW, 80/100 at 175 °C. Injector and detector temperatures were 240 and 270 °C respectively. The flow rate of the carrier gas (N₂) was 30 ml min⁻¹. Fatty acids were identified using authentic standards (fatty acid methyl esters obtained from Poly Science Corporation). Percentage fatty acid compositions were calculated by multiplying the retention time by the peak height on the trace.

Results and Discussion

Phospholipid composition of *S. pombe* membranes

The major phospholipids in *S. pombe* membranes were phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine (Table 1). These results, in general, were similar to those reported by Fernandez *et al.* (1986). There were no significant differences in the major phospholipid content of membrane extracts between cells harvested at the late exponential and the early stationary phase (results not shown). However, significant differences in the major membrane phospholipid content were observed between cells grown under aerobic and anaerobic conditions (Table 1). Under anaerobic conditions the total phospholipids (expressed as µmol phosphorus per mg protein) were increased. Similar results have been reported for *B. cereus* (Kates *et al.*, 1962). The percentage content of phosphatidylinositol was increased, whereas that of phosphatidylcholine was decreased (Table 1). An elevation of the cardiolipin content and a reduction of the phosphatidylethanol-

amine content were also observed. Phosphatidylinositol is probably the main constituent of this elevation. Phosphatidylinositol is produced by a side route of the phospholipid biosynthetic pathway in *S. pombe*, where the common precursor is phosphatidic acid (Fernandez *et al.*, 1986). Phosphatidylinositol biosynthesis does not require energy (White & Hawthorne, 1970) and this could account for its increased level in *S. pombe* membranes under anaerobic conditions.

Fatty acid composition of membrane lipids in *S. pombe*

The fatty acid composition of total lipids, neutral lipids, total phospholipids and the major phospholipid fractions from membrane extracts of *S. pombe* cells growing aerobically are summarized in Table 2. Oleic acid (18:1) was the main constituent, representing 81.8% of the total fatty acids. Palmitic acid (16:0), stearic acid (18:0) and palmitoleic acid (16:1) represented 10.4%, 5.5% and 1.1% respectively. A number of shorter-chain fatty acids such as lauric acid (12:0) and myristic acid (14:0) were also identified, but in small amounts (0.4% and 0.3% respectively). Proportionally, more oleic acid and palmitic acid were found in the phospholipid fraction than in the neutral lipids fraction. Similarly, oleic acid was higher in the phosphatidylcholine fraction. The phosphatidylinositol and phosphatidylserine fractions contained higher levels of palmitic acid and stearic acid than the other phospholipid fractions. There were no significant differences in the fatty acid composition of the membrane lipids between cells harvested at the late

exponential and early stationary growth phases. Other reports on the fatty acid composition of total lipids in *S. pombe* have also indicated oleic acid to be the main fatty acid constituent, followed by palmitic acid. However, the levels of fatty acids reported by others were significantly different (White & Hawthorne, 1970; Johnston & Coddington, 1984).

The fatty acid composition of lipid fractions and subfractions obtained from membrane extracts of *S. pombe* cells grown anaerobically is shown in Table 3. Under these conditions the proportion of saturated fatty acids was increased, and that of unsaturated fatty acids decreased. This indicates that the principal route for biosynthesis of mono-unsaturated fatty acids in *S. pombe* could be an oxidative mechanism which requires oxygen and NADH, as originally proposed by Bloomfield & Bloch (1958) and Jollow *et al.* (1968) for *Saccharomyces cerevisiae*.

Effect of ethanol on the growth and lipid composition of *S. pombe*

Ethanol is toxic for various micro-organisms, even those which can produce fairly high amounts of ethanol such as the bacterium *Z. mobilis* (Carey & Ingram, 1983) and the yeast *Sacch. cerevisiae* (Beaven *et al.*, 1982). The presence of ethanol in the growth medium of *S. pombe* inhibited growth, in a concentration-dependent fashion (Fig. 1). A concentration up to 0.8 M (5%, v/v) had no effect on growth, whereas some growth inhibition (about twofold) was observed at 1.2 M (7.5%). Growth was significantly

Table 1. Phospholipid composition of *S. pombe* membranes under aerobic or anaerobic growth conditions in the presence or absence of added ethanol

The values are given as percentage of total phosphorus, and are means $\pm$ SE. The standard error was calculated from the analysis of two samples of four different cultures.

Phospholipids*	Aerobic conditions			Anaerobic conditions		
	Control†	+ 0.8 M EtOH	+ 1.2 M EtOH	Control†	+ 0.8 M EtOH	+ 1.2 M EtOH
Polar PL	7.5 $\pm$ 1.5	8.2 $\pm$ 1.5	5.1 $\pm$ 0.6	9.4 $\pm$ 1.6	8.3 $\pm$ 1.5	8.1 $\pm$ 1.6
PI	19.6 $\pm$ 0.7	19.1 $\pm$ 0.7	25.5 $\pm$ 2.3	24.5 $\pm$ 2.1	30.0 $\pm$ 3.1	32.7 $\pm$ 2.3
PC	40.0 $\pm$ 1.4	40.5 $\pm$ 3.4	35.5 $\pm$ 4.3	35.2 $\pm$ 3.6	33.9 $\pm$ 3.4	30.4 $\pm$ 4.5
PS	3.0 $\pm$ 1.2	2.4 $\pm$ 0.9	2.9 $\pm$ 0.5	2.1 $\pm$ 0.3	3.2 $\pm$ 0.5	3.4 $\pm$ 0.3
PE	23.8 $\pm$ 2.1	19.5 $\pm$ 1.5	18.9 $\pm$ 1.8	17.4 $\pm$ 1.8	14.5 $\pm$ 0.8	16.8 $\pm$ 0.9
CL	3.7 $\pm$ 1.4	5.4 $\pm$ 1.2	6.0 $\pm$ 2.6	6.6 $\pm$ 0.6	7.4 $\pm$ 0.9	10.7 $\pm$ 1.1
PA	2.0 $\pm$ 0.5	1.7 $\pm$ 0.5	0.5 $\pm$ 0.1	1.1 $\pm$ 0.3	1.7 $\pm$ 0.4	4.4 $\pm$ 0.6
Unknown	1.7 $\pm$ 0.5	0.9 $\pm$ 0.4	1.1 $\pm$ 0.5	1.6 $\pm$ 0.2	4.7 $\pm$ 1.2	2.4 $\pm$ 0.9
P:protein ratio‡	0.050	0.050	0.054	0.082	0.063	0.060

* PL, phospholipids; PI, phosphatidylinositol; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine; CL, cardiolipin; PA, phosphatidic acid.

† Control: Growth on 3% glucose (DT 125 min), 40% glucose (DT 300 min) or 3% xylose (DT 420 min) in the absence of ethanol (DT, doubling time in aerobic conditions).

‡ μ mol lipid phosphorus in membranes per mg of total protein.

Fig. 1. Effect of added ethanol on the growth of *S. pombe*. Concentration of ethanol: ○, 0.8 M; ▲, 1.2 M; △, 1.6 M; ▼, 2.4 M. ●, No added ethanol.

inhibited by higher concentrations, and ceased at a concentration of 2.4 M (15%). Similar results have been reported for some strains of *Sacch. cerevisiae* (Rose, 1980). *S. pombe* grown in the medium used here (see Methods), containing 3% glucose, produced low amounts of ethanol (0.08 M, or 0.4%). Ethanol production remained constant from the late exponential to the

stationary phase. Under anaerobic conditions, ethanol production increased about threefold, its concentration in the growth medium reaching 0.2 M (1.1%). Ethanol production was not increased when a higher initial glucose concentration (5%, 10% or 20%) was used, under either aerobic or anaerobic conditions. Although *S. pombe* produces only low amounts of ethanol, it appears to be resistant to high ethanol concentrations, as the addition of 5% ethanol did not affect its growth, and 7.5% added ethanol had little effect on growth (Fig. 1).

Added ethanol at a concentration of 0.8 M (5%) had no significant effect on the membrane phospholipid composition of *S. pombe* cells grown aerobically (Table 1). However, significant changes in the composition of phospholipids were observed when 1.2 M (7.5%) ethanol was added. Phosphatidylinositol and, to a lesser extent, cardiolipin increased, whereas phosphatidylcholine, phosphatidic acid and phosphatidylethanolamine decreased. Similar effects were seen when exogenous ethanol was added to anaerobically growing cells (Table 1). A reduction in growth rate may affect the phospholipid composition of *Sacch. cerevisiae* cells (Hunter & Rose, 1972). To rule out this possibility for *S. pombe*, cells were grown in medium containing 40% glucose or 3%

Table 2. Fatty acid composition (% of total) of *S. pombe* membranes under aerobic growth conditions in the presence or absence of added ethanol

See Table 1 for abbreviations; NL, neutral lipids. The standard error calculated from the analysis of two samples of four different cultures was ~0.1 for values <2.0, and <0.7 for values >2.0. Tr: Trace.

	Total	NL	PL	PI+PS	PC	PE
Control*						
12:0	0.4	0.4	Tr	Tr	Tr	Tr
14:0	0.3	0.3	Tr	Tr	Tr	Tr
14:1	Tr	0.1	Tr	Tr	Tr	Tr
16:0	10.4	6.8	11.7	19.6	8.6	17.6
16:1	1.1	1.7	1.9	1.0	1.3	2.0
18:0	5.5	10.6	4.6	6.7	3.3	2.7
18:1	81.8	80.7	81.8	79.3	86.7	77.9
0.8 M-Ethanol						
12:0	0.3	0.3	Tr	Tr	Tr	Tr
14:0	0.2	0.3	Tr	Tr	Tr	Tr
14:1	Tr	0.4	Tr	Tr	Tr	Tr
16:0	12.2	7.8	13.1	20.5	12.6	20.0
16:1	1.0	1.6	1.8	0.8	1.1	1.4
18:0	8.3	17.8	7.3	11.7	6.8	4.5
18:1	78.1	72.5	77.7	66.8	79.4	73.9
1.2 M-Ethanol						
12:0	0.2	Tr	Tr	Tr	Tr	Tr
14:0	0.1	0.3	Tr	Tr	Tr	Tr
14:1	Tr	0.9	Tr	Tr	Tr	Tr
16:0	13.8	6.2	19.9	23.8	16.8	33.7
16:1	0.7	0.9	1.9	0.7	1.9	1.5
18:0	12.9	20.9	11.6	12.3	12.2	8.7
18:1	72.2	71.5	66.4	63.1	69.1	56.0

* Control: no added ethanol.

xylose as sole carbon source. Under these conditions, in which the growth rate was reduced twofold and threefold, respectively, the phospholipid composition of *S. pombe* membranes remained unchanged (Table 1). It appears that addition of ethanol to aerobically grown cells causes similar alterations to the membrane phospholipid composition of *S. pombe* as does anaerobic growth without the addition of exogenous ethanol (Table 1). A decrease of the intracellular energy pool may account for this phenomenon.

Effect of added ethanol on fatty acid composition

Ethanol, when added to cultures growing aerobically, caused a decrease in the proportion of oleic acid (18:1) and an increase in that of stearic acid (18:0) in all lipid fractions and subfractions (Table 2). This result was quite surprising, as ethanol has been reported to induce an increase in the proportion of unsaturated fatty acids in other micro-organisms (for a review see Ingram & Buttke, 1984). In contrast, an increase in oleic acid at the expense of stearic acid was observed when ethanol was

added to anaerobically growing cultures (Table 3). It is known that phosphatidylinositol, unlike phosphatidylcholine, is considerably deficient in oleic acid and enriched in palmitic acid. However, in the presence of ethanol, changes were observed in the fatty acid proportion of all the fractions of phospholipids. This indicates that the presence of ethanol in the medium promotes a change in fatty acid composition at some point before the synthesis of CDP-diglyceride, which is the last common precursor of all phospholipids. A similar mechanism has been reported for the effects on the composition of *E. coli* lipids when cells are grown in the presence of ethanol (Ingram, 1977).

It has been reported for various micro-organisms that the oleic acid content is increased in the presence of ethanol regardless of the presence or absence of oxygen (Nandini-Kishore *et al.*, 1979; Carey & Ingram, 1983; Beaven *et al.*, 1982). Our results indicate that in *S. pombe* this occurs only under anaerobic conditions. This 'opposite' effect of ethanol on the proportion of oleic acid under aerobic conditions was not accompanied by a similar change in the phospholipid composition: in the presence of ethanol phosphatidylinositol appeared to be similarly

Table 3. *Fatty acid composition (% of total) of S. pombe membranes under anaerobic conditions in the presence or absence of added ethanol*

See Table 1 for abbreviations; NL, neutral lipids. The standard error calculated from the analysis of two samples of four different cultures was ~0.1 for values <2.0, and <0.9 for values >2.0. Tr: Trace.

	Total	NL	PL	PI+PS	PC	PE
Control*						
10:0	2.8	4.1	1.2	0.4	1.6	1.8
12:0	3.6	3.8	3.2	1.0	4.3	2.0
14:0	2.8	3.3	2.4	1.9	3.3	1.8
14:1	Tr	0.6	Tr	Tr	Tr	Tr
16:0	24.8	20.5	28.8	36.3	30.9	26.0
16:1	3.1	2.6	3.9	2.6	3.4	5.6
18:0	17.7	27.7	11.5	7.1	14.1	3.7
18:1	45.0	40.3	48.9	50.5	42.4	58.5
0.8 M-Ethanol						
10:0	1.5	2.0	0.5	0.3	0.3	0.5
12:0	2.3	3.0	1.8	0.4	3.6	0.8
14:0	2.0	2.6	1.6	1.1	2.8	1.7
14:1	Tr	0.5	Tr	Tr	Tr	Tr
16:0	23.6	17.4	30.0	36.9	27.7	28.3
16:1	2.4	1.7	2.1	1.8	1.8	4.3
18:0	17.1	24.6	10.3	6.5	14.5	3.9
18:1	50.8	48.0	53.4	53.1	48.8	60.2
1.2 M-Ethanol						
10:0	0.5	1.4	Tr	0.2	0.1	0.5
12:0	1.3	2.0	0.6	0.3	1.9	0.9
14:0	1.3	1.7	1.0	0.5	1.6	1.2
14:1	Tr	0.3	Tr	Tr	Tr	Tr
16:0	20.4	14.0	30.9	37.7	24.4	27.1
16:1	1.1	1.3	1.2	0.9	1.7	2.8
18:0	21.8	29.4	9.0	6.7	17.6	3.3
18:1	53.4	50.0	57.6	53.9	53.0	64.1

* Control: no added ethanol.

increased and phosphatidylcholine similarly decreased under aerobic and anaerobic conditions. A similar phenomenon, as far as the opposite effect of ethanol on the fatty acid proportion is concerned, has been reported for *Mycobacterium smegmatis* (Ingram & Buttke, 1984), but it was related to the growth temperature. In this case, an increase in the proportion of unsaturated fatty acids in the presence of ethanol was observed only when the growth temperature was lower than the physiological one (27 instead of 37 °C); at 37 °C, the presence of ethanol caused a decrease in the unsaturated fatty acid content (Ingram & Buttke, 1984). The effect of ethanol on the proportion of oleic acid under anaerobic conditions may be based on a pathway other than the oxidative one, which produces mono-unsaturated fatty acids, as has been proposed for *E. coli* (Buttke & Ingram, 1978). Such a pathway has not, so far, been reported for a yeast. This second route for the biosynthesis of 18:1 fatty acids under anaerobic conditions, which is possibly affected by ethanol, is under further investigation.

The dependence of ethanol tolerance upon the structure of fatty acids, longer-chain fatty acids (either mono-unsaturated or branched-chain) contributing to ethanol tolerance, has been proposed by many researchers (Carey & Ingram, 1983; Beaven *et al.*, 1982; Casey & Ingledew, 1986; Ingram, 1976). The ability of the cells to increase the proportion of 18:1 fatty acids at the expense of saturated ones in the presence of ethanol is considered to be the main mechanism of ethanol tolerance in yeast (Beaven *et al.*, 1982). Our results indicate that ethanol tolerance in *S. pombe* may be connected with the above mechanisms. First, *S. pombe* membranes had a high content of 18:1 fatty acids. Second, the cells maintained a high rate of phospholipid biosynthesis, as shown by the phosphorus/protein ratio: this ratio increased under anaerobic conditions, or under aerobic conditions in the presence of ethanol, evidently via the increased biosynthesis of phosphatidylinositol. Third, they were able to increase the proportion of 18:1 fatty acids under anaerobic conditions in the presence of ethanol.

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