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Ethanol-Induced Changes in Glycolipids of *Saccharomyces cerevisiae*

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Abstract

Total glycolipid content of *Saccharomyces cerevisiae* cells increased in ethanol-treated yeast cells. Sialic acid and hexosamine contents of glycolipids from ethanol-treated cells decreased, whereas those of hexoses increased. Increased sialidase activity in the presence of ethanol may be responsible for the decrease in sialic acid content of glycolipids. The saccharide moieties of glycolipids of *S. cerevisiae* consisted of fucose, mannose, galactose, and glucose. Ethanol treatment of yeast cells caused an increase in glucose and a decrease in galactose content of glycolipids. The changes in glucose content can be related to changes in β-glucosidase activity under alcohol stress. The content of cerebrosides, sulfatides, and monoglucosyldiglycerides was enhanced following ethanol treatment. An increase in cerebroside as well as in sulfatide content during alcohol stress might play an important role in stabilizing the membrane both physically and structurally. Such variations in glycolipid content and composition of *S. cerevisiae* cells may represent an adaptive response to ethanol stress.

Index Entries: *Saccharomyces cerevisiae*; glycolipids; sugars; ethanol.

Introduction

Under anaerobic conditions, yeasts produce ethanol, which is accumulated in the medium and causes environmental stress. Ethanol retards the growth rate of yeast and its viability, modifies fermentative ability, alters membrane permeability, and decreases membrane integrity *(1–3)*. The primary target of alcohols is the plasma membrane. Microbial cells adjust their membrane lipid composition to tolerate ethanol stress. Adaptation of membrane lipid composition has frequently been interpreted in

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terms of necessity to maintain membrane lipid fluidity within some undefined boundaries *(4)*. Glycolipids are a structurally diverse group of membrane components that are found in species ranging from bacteria to humans. Glycolipids appear to be ubiquitous in animal cells, but their content varies among different cell types *(5,6)*. Besides being structural components of the cell membrane, they also play an important role in cellular functions such as in cell–cell communication and act as receptor components, anchors for proteins, and regulators of signal transduction *(7,8)*. Since glycolipids have a high transition temperature and show extensive hydrogen-bonding capacity, they are known to impart structural integrity to the membranes of organisms living in harsh environmental conditions *(9)*. Under heat-stress conditions, glycolipid content as well as glycosylation patterns of *Saccharomyces cerevisiae* has been reported to vary *(10)*.

In the present investigation, we studied changes in the glycolipids of the yeast *S. cerevisiae* under ethanol stress.

Materials and Methods

Yeast Strain

S. cerevisiae strain MTCC827 was procured from the Institute of Microbial Technology, Chandigarh (Punjab).

Growth of Yeast Cells

S. cerevisiae cells were grown on yeast peptone dextrose medium in 500-mL Erlenmeyer flasks containing 100 mL of medium *(11)* at 27 ± 1°C for 24 h. The composition of the medium was 20 g/L of glucose, 20 g/L of peptone, and 10 g/L of yeast extract. During growth, cultures were shaken on a rotary shaker at 250 rpm and 27 ± 1°C. The cells were harvested by centrifuging at 5000*g* for 15 min and were washed with 0.01 *M* phosphate buffer (pH 7.0) to remove adhering metabolites and unused ingredients in the medium.

To study the effect of alcohol, yeast cells grown to stationary phase were suspended in 0.01 *M* phosphate buffer (pH 7.0) containing 0.5% dextrose and different concentrations of ethanol (0–12% [v/v]) and incubated for 4 h. Control cells were suspended in the same buffer containing 0.5% dextrose but no ethanol.

Extraction of Lipid

Total lipids were extracted from the yeast cells by the method of Folch et al. *(12)*. Pure lipids obtained were dissolved in chloroform and stored at –4°C.

Separation of Glycolipids

Glycolipids were isolated from total lipids by silicic acid chromatography *(13)*. The column was eluted with the solvents chloroform, acetone,

and methanol, respectively. Glycolipids were eluted in the acetone fraction. Excessive solvent was evaporated on a rotary evaporator. Glycolipids were estimated on the basis of their total sugar content. Total sugar content was determined by the method of Dubois et al. *(14)*.

Estimation of Sugar Content in Glycolipids

Purified glycolipids were assayed for hexoses, sialic acid, and hexosamine content. Hexoses were estimated using phenol/sulfuric acid method *(14)*, sialic acid by the method of Svennerholm *(15)*, and hexosamine by the Elson-Morgan method as described by Dische et al. *(16)*.

Analysis of Sugars

Different sugars of glycolipids were separated and estimated by gas-liquid chromatography (GLC). Glycolipids were hydrolyzed in 1 *N* anhydrous methanolic HCl at 100 ± 2°C in sealed vials for 18 h, and the resulting monosaccharides were converted into trimethylsilyl (TMS) derivatives *(17)*. TMS derivatives were analyzed on a Shimadzu GS-17C gas chromatograph equipped with a PTE-5 (5% diphenyl, 95% dimethylsiloxane) column (Shimadzu, Kyoto, Japan). Detector and injector temperatures were maintained at 280 and 250°C, respectively, and the flow rate of the carrier gas, nitrogen, was at 42 mL/min. The oven temperature was programmed from 150 to 250°C at 7°C/min. Analysis was monitored with a flame ionization detector.

Fractionation of Glycolipids by Column Chromatography

Glycolipids were fractionated by DEAE-cellulose ion-exchange column chromatography *(13)*. The purity of glycolipid subclasses was checked by thin-layer chromatography (TLC) on silica gel G plates using chloroform/methanol/water (65/25/4 [v/v/v]) as the solvent system *(18)*. Specific spray reagents were used to identify neutral glycolipids, sulfatides, ceramide polyhexosides, cerebrosides, and glycosyldiglycerides. Various glycolipids were then quantified on the basis of their hexose content estimated by the method of Roe *(19)*.

Enzymatic Assays

β-Glucosidase and β-galactosidase activity were assayed using *p*-nitrophenyl-β-D-glucopyranoside and *p*-nitrophenyl-β-D-galactopyranoside as substrates, respectively. These substrates yield *p*-nitrophenol, which absorbs maximally at 420 nm *(20)*. Sialidase activity was assayed by using fetuin as substrate to yield sialic acid as the product, which absorbs at 549 nm *(21)*. One unit of enzyme activity was defined as the amount of enzyme producing 1 μ*M* product/h. The specific activities of the enzymes were expressed as units/milligram of the protein. Protein content was determined by the method of Lowry et al. *(22)*.

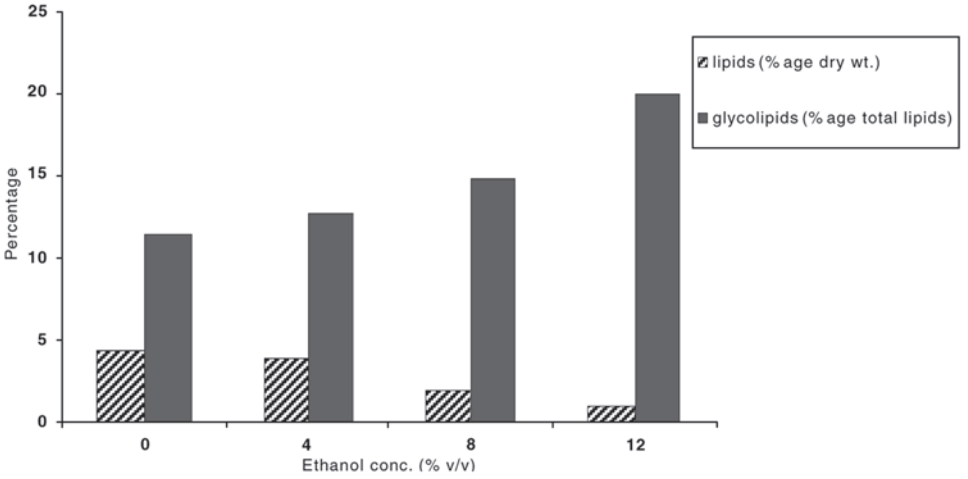

Fig. 1. Effect of ethanol on total lipids and glycolipids of *S. cerevisiae*.

Results and Discussion

Alcohols, products of microbial fermentation, pose an environmental stress to micro-organisms *(23)*. Ethanol, which diffuses freely from the cell into external medium, is toxic for its producers as well *(24,25)*. However, the micro-organisms adapt to such stress by bringing about changes in their membrane and physiology.

Effect of Ethanol on Lipids and Glycolipids of S. cerevisiae

Lipids, the main constituents of plasma membrane, play an important role in the adaptation of organisms to adverse conditions. Total lipids of *S. cerevisiae* cell membrane decreased from 4.53 to 1.09% of their dry weight on an increase in ethanol concentration to 12% (v/v) (Fig. 1). A decrease in total lipid content of the yeast cells and *Mucor fragilis* CCMI 142 in the presence of ethanol has also been reported *(3,26)*. The decrease in total lipid content of ethanol-treated cells may be owing to enhanced lipid peroxidation *(3)*, and/or alcohol-induced changes in membranes may be activating the enzymes involved in the hydrolysis of lipids. The reduction in lipid content along with dehydration caused by alcohol may alter the specific interactions between lipid and membrane proteins essential for the maintenance of membrane integrity *(27)*. The total lipid content of *S. cerevisiae* cells decreased in the presence of ethanol, whereas total glycolipid content increased from 11.58% of the lipids to 20.09% when they were incubated in 12% ethanol for 4 h (Fig. 1). Chronic ethanol exposure is reported to affect the glycosylation patterns of microvillous membrane of ethanol-fed rats *(5)*. Glycolipids have the capability to undergo interlipid hydrogen bonding via glycosyl head groups and thus provide structural stability to membranes. Therefore, the relative higher content of glycolipids in ethanol-treated yeast cells may represent an adaptive response to ethanol stress.

Table 1
Effect of Ethanol on Sugar Composition of Glycolipids of *S. cerevisiae*[a]

Ethanol concentration (% [v/v])	Hexoses (μg/mg glycolipid)	Sialic acids (μg/mg glycolipid)	Hexosamine (μg/mg glycolipid)
0	124.43 ± 0.35	30.22 ± 0.10	65.6 ± 0.23
4	117.3 ± 0.26	25.8 ± 0.23	71.2 ± 1.36
8	127.9 ± 0.38	23.4 ± 0.19	63.6 ± 0.54
12	170.1 ± 0.52	11.95 ± 0.14	33.3 ± 0.42

[a]Results are the mean ± SD of three independent experiments.

Table 2
Effect of Ethanol on Glycolipid-Hydrolyzing Enzyme Activities of *S. cerevisiae* Cells[a]

Ethanol concentration (% [v/v])	Sialidase (U/mg protein)	β-Glucosidase (U/mg protein)	β-Galactosidase (U/mg protein)
0	14.76 ± 0.07	1.45 ± 0.09	6.52 ± 0.10
4	19.93 ± 0.05	1.49 ± 0.11	6.17 ± 0.08
8	21.18 ± 0.10	2.57 ± 0.06	5.97 ± 0.10
12	35.44 ± 0.06	1.19 ± 0.05	4.93 ± 0.09

[a]Results are the mean ± SD of three independent experiments.

Effect of Ethanol on Sugar Composition of Glycolipids of S. cerevisiae

Ethanol treatment of *S. cerevisiae* affected not only the content of glycolipids but the composition of carbohydrate moieties as well. The hexose content of glycolipids increased whereas that of sialic acid content decreased from ethanol-treated cells. The hexosamine content of glycolipids increased when the cells were treated with 4% ethanol but decreased at higher concentrations of ethanol (Table 1). Similar changes in hexose, sialic acid, and hexosamine content in intestinal mucous membrane of ethanol-fed rats have been reported *(28)*. A decrease in the content of sialic acid in brain membrane of rats exposed to ethanol has been reported as well *(29,30)*. The variation in the content of these monosaccharides may be owing to either altered rate of glycosylation and sialylation of glycolipids or removal of the sugars or both. It is suggested that it is not carbohydrate content but ethanol that, when administered chronically, greatly impairs the glycosylation machinery of rat liver *(31)*. Ethanol is also known to affect membrane enzyme activities *(32)*. The activity of sialidase was markedly enhanced in *S. cerevisiae* cells exposed to ethanol (Table 2). This increase in sialidase activity during ethanol exposure may be one of the factors responsible for the corresponding decrease in sialic acid content.

Table 3
Effect of Ethanol on Hexose Composition of Glycolipids of *S. cerevisiae*[a]

Ethanol concentration (% [v/v])	Fucose (relative% age)	Mannose (relative% age)	Galactose (relative% age)	Glucose (relative% age)
0	5.38	35.20	55.19	4.20
4	3.31	36.62	50.46	9.66
8	6.44	38.95	49.07	5.52
12	3.85	41.53	49.53	5.09

[a]Results are the mean ± SD of three independent experiments.

Effect of Ethanol on Hexose Composition of Glycolipids of S. cerevisiae

Various hexoses present in glycolipids were separated and identified by GLC. Fucose, mannose, galactose, and glucose were identified as major hexoses in *S. cerevisiae* glycolipids (Table 3). Fungal cerebrosides have been reported to have glucose or galactose as their carbohydrate moieties *(33)*. Although fucose was a quantitatively minor component of the yeast *S. cerevisiae* (Table 3), fucose-containing glycoconjugates on the cell surface are known to be important in cell-cell recognition. Fucose content of glycolipids in yeast cells treated with 8% (v/v) ethanol increased but decreased significantly at 12% (v/v) ethanol (Table 3). Glucose was also a quantitatively minor component of glycolipids. Its content became more than double at 4% (v/v) ethanol, but this increase was only 31% in 8% (v/v) ethanol-treated cells. The change in glucose content is related to changes in β-glucosidase activity under alcohol stress (Table 2). Enhanced glucosylation of the lipids when yeast cells were treated with 4% ethanol might be an adaptive response. Mannose is a major saccharide of glycolipids, which constitutes 35% of monosaccharide moieties in glycolipids of untreated yeast cells. It increased to 41% when the yeast cells were treated with ethanol (Table 3). Yeast cells are known to produce mannose-rich surface glycans *(34)*. The concentration-dependent increase in mannose content of glycolipids in ethanol-treated cells suggests that this monosaccharide may contribute to the adaptation of yeast to ethanol. Galactose, which constitutes 55% of the saccharide moiety of the glycolipids from untreated yeast cells, decreased when cells were treated with ethanol (Table 3). The decreased galactose content cannot be explained on the basis of β-galactosidase activity. The change in the concentration of these sugar residues may be an important target, directly or indirectly, in the biologic actions of ethanol.

Effect of Ethanol on Glycolipid Fractions of S. cerevisiae

Glycolipids from *S. cerevisiae* were fractioned by DEAE- cellulose ion-exchange chromatography. Five different fractions were obtained and identified as cerebrosides, ceramide polyhexosides, sulfatides, monogluco-

Table 4
Effect of Ethanol on Glycolipid Fractions of *S. cerevisiae*[a]

Ethanol concentration (% [v/v])	Cerebrosides (μg/mg glycolipid)	Ceramide polyhexosides (μg/mg glycolipid)	Sulfatides (μg/mg glycolipid)
0	129.69 ± 0.06	376.14 ± 0.19	270.33 ± 0.47
4	194.22 ± 0.80	242.53 ± 0.60	350.80 ± 0.83
8	158.33 ± 0.17	108.73 ± 0.27	564.17 ± 0.82
12	176.48 ± 0.28	171.92 ± 0.64	164.63 ± 1.2

[a]Results are the mean ± SD of three independent experiments.

syldiglycerides (MGDGs), and diglucosyldiglycerides (DGDGs). The purity of these fractions was checked by TLC. Different glycolipid fractions were identified by using specific spray reagents and comparing their R_f values with those of authenticated standards (data not given). The effect of ethanol treatment on the yeast cells was studied on these fractions of glycolipids. The cerebroside content of *S. cerevisiae* glycolipids increased by 49.75% in 4% (v/v) ethanol-treated yeast cells (Table 4). A higher concentration of ethanol also caused an increase in cerebrosides, but to a lesser extent. Alcohol intoxication has been reported to raise the content of cerebrosides in rat brain *(35–37)* as well as microvillous membranes *(38)*. The changes in glucose and cerebroside content of the glycolipids of alcohol-treated *S. cerevisiae* cells indicate that glucose might be the major carbohydrate moiety of the cerebrosides. The presence of cerebrosides in membrane stabilizes the interaction between lipids and proteins *(39)*. Therefore, an increase in cerebroside content during alcohol stress might play an important role in stabilizing the membrane both physically and structurally.

Sulfatide content of the glycolipids also increased to more than double at 8% (v/v) ethanol, but a further increase in ethanol concentration to 12% (v/v) reduced the sulfatide content significantly (Table 4). The increase in the concentration of sulfatides in rat brain membrane has also been reported in the presence of ethanol *(36)*. It was reported that sulfatides and cerebrosides are involved in carbohydrate–carbohydrate interactions between lipid head groups, which results in the stabilization of membranes *(40)*. Thus, an increase in the content of cerebrosides and negatively charged sulfatides in the presence of ethanol suggested that these glycolipids may help in the stabilization of the yeast membrane under stress conditions.

Ceramide polyhexosides decreased drastically in glycolipids of *S. cerevisiae* cells when treated with an ethanol concentration up to 8% (v/v), but a further rise in ethanol concentration to 12% (v/v) resulted in a lesser decrease (Table 4). In contrast to yeast cells, ceramide polyhexoside content of rat brain membrane exposed to ethanol increases *(36)*. The alterations in the ceramide polyhexoside content are in consonance with the changes in galactose content of glycolipid of ethanol-treated yeast cells (Table 3), sug-

Table 5
Effect of Ethanol on MGDG and DGDG Content of Glycolipids of *S. cerevisiae*[a]

Ethanol concentration (% [v/v])	MGDG (μg/mg glycolipid)	DGDG (μg/mg glycolipid)
0	64.01 ± 0.10	164.92 ± 0.10
4	155.56 ± 0.93	108.23 ± 0.53
8	87 ± 0.54	81.92 ± 0.94
12	71.50 ± 0.90	158.77 ± 0.42

[a]Results are the mean ± SD of three independent experiments.

gesting that galactose may be the major saccharide component in ceramide polyhexosides.

MGDG and DGDG contents of the *S. cerevisiae* cell glycolipids were also affected by ethanol treatment. When the cells were treated with 4% ethanol, MGDG content in glycolipids increased more than two times, but a high concentration of alcohol caused a significant decrease in the content of this glycolipid (Table 5). An increase in MGDG has also been reported in brain membrane of rats exposed to ethanol *(37)*. Variation in the glucose content of glycolipids in ethanol-treated *S. cerevisiae* cells matched the variation in the MGDG. There was a decrease in DGDG content with an increase in ethanol concentration up to 8% (v/v) ethanol. However, when the cells were treated with 12% (v/v) ethanol, DGDG content increased significantly (Table 5). The structure of the lipid bilayer is maintained by the regulated ratio of the nonbilayer forming MGDG and bilayer forming DGDG glycolipids *(41)*, thus allowing a flexible response to various triggering events.

Conclusion

The results revealed that although ethanol caused a membrane-fluidizing effect in yeast cell membrane, the changed glycolipid content and composition may provide stability and integrity to the membrane and, therefore, changes in glycosylation patterns may help the yeast *S. cerevisiae* cells to adapt to ethanol stress.

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