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Journal

Applied and Environmental Microbiology, 80(10)

ISSN

0099-2240

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Publication Date

2014-05-15

DOI

10.1128/aem.04151-13

Peer reviewed

AEM Journals.ASM.org

MINIREVIEW

Examining the Role of Membrane Lipid Composition in Determining the Ethanol Tolerance of *Saccharomyces cerevisiae*

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Yeast (*Saccharomyces cerevisiae*) has an innate ability to withstand high levels of ethanol that would prove lethal to or severely impair the physiology of other organisms. Significant efforts have been undertaken to elucidate the biochemical and biophysical mechanisms of how ethanol interacts with lipid bilayers and cellular membranes. This research has implicated the yeast cellular membrane as the primary target of the toxic effects of ethanol. Analysis of model membrane systems exposed to ethanol has demonstrated ethanol's perturbing effect on lipid bilayers, and altering the lipid composition of these model bilayers can mitigate the effect of ethanol. In addition, cell membrane composition has been correlated with the ethanol tolerance of yeast cells. However, the physical phenomena behind this correlation are likely to be complex. Previous work based on often divergent experimental conditions and time-consuming low-resolution methodologies that limit large-scale analysis of yeast fermentations has fallen short of revealing shared mechanisms of alcohol tolerance in *Saccharomyces cerevisiae*. Lipidomics, a modern mass spectrometry-based approach to analyze the complex physiological regulation of lipid composition in yeast and other organisms, has helped to uncover potential mechanisms for alcohol tolerance in yeast. Recent experimental work utilizing lipidomics methodologies has provided a more detailed molecular picture of the relationship between lipid composition and ethanol tolerance. While it has become clear that the yeast cell membrane composition affects its ability to tolerate ethanol, the molecular mechanisms of yeast alcohol tolerance remain to be elucidated.

In recent times, the global energy crisis has spurred a renewed interest in the production of ethanol as a replacement for petroleum-based liquid fuels for transportation (1). Ethanol derived from various raw materials, such as sugar or starch (e.g., grains) and lignocellulosic material, is considered to be one viable, sustainable source for these fuels (1–3). However, efficient bioethanol production must address a problem common to many industrial-scale fermentation processes utilizing *Saccharomyces cerevisiae* to produce alcohol, namely, tolerance of the inhibitory effect of self-produced ethanol (4).

FERMENTATION AND *SACCHAROMYCES CEREVISIAE*

The primary goal of alcoholic fermentation is the conversion of sugars, such as glucose and fructose, to ethanol and carbon dioxide. This is typically carried out in an anaerobic environment. However, yeast (*S. cerevisiae*) preferentially ferments in the presence of oxygen when sugar levels are above 9 g/liter, a phenomena known as the Crabtree effect (5). During the beginning phases of fermentation, the yeast cell population does not experience significant increases in ethanol concentration. In the first time period, known as the latent or lag phase, yeast cells are utilizing sugar and other nutrients, particularly nitrogen, for energy and to initiate cell growth; however, they are also adapting to the new environmental stresses and conditions (5). Upon adaptation, the yeast enters the exponential growth phase, where it begins to grow at a high rate that is primarily limited by the concentration of nitrogen (6–8). During this same growth period, dissolved oxygen in the fermentation medium is utilized by yeast to produce ergosterol, the major sterol in yeast, and unsaturated fatty acids that will be incorporated into diacylglycerol (DAG), the precursor to all of the phospholipids that compose the yeast cell membrane (5). Once the available nitrogen has been depleted, the yeast cells begin to transition into stationary-phase metabolism, where the yeast cell population achieves its highest density. During early stationary-phase metabolism, significant amounts of sugar are converted to ethanol and cell viability remains relatively high, though fermentation begins to slow (5–7). As the sugar concentration continues to fall (and ethanol levels rise), the viable yeast cell population does not change significantly until virtually all of the sugar has been utilized, and the yeast cells then begin to die.

While many yeasts, such as *S. cerevisiae*, have an innate ability to withstand ethanol concentrations that would prove growth inhibiting or lethal to other organisms, under certain circumstances, yeast will stop fermenting even though there are high levels of residual sugar remaining; this phenomenon is known as a "stuck" fermentation (9). There are a number of known factors that contribute to a stuck fermentation, among which are the yeast strain, nitrogen availability, sugar concentration, and fermentation temperature. Ultimately, it is the yeast's inability to adapt to and tolerate increasing levels of ethanol that leads to fermentation arrest (9–11).

Yeasts have evolved numerous mechanisms that permit them to grow and thrive in the extraordinarily hostile and stressful environment of an alcoholic fermentation. Throughout its fermentation life cycle, *Saccharomyces cerevisiae* is exposed to drastic changes or extremes in osmotic pressure, pH, and nutrient levels. Furthermore, the cells may be exposed to heat or cold shock and will experience increasing concentrations of ethanol and other

Published ahead of print 7 March 2014

Editor: V. Müller

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doi:10.1128/AEM.04151-13

toxic compounds, such as acetic acid or sulfite (7). To adapt to these dynamic physical and physiological stresses, yeasts have evolved stress adaptation mechanisms that sense changes in the yeast cell physiology and its environment that subsequently induce the expression of the group of genes known as the stress response elements (STRE) (12, 13). The stress response elements that respond to heat shock and ethanol exposure demonstrate significant genetic and functional overlap, particularly for those that stabilize membrane-associated proteins (14, 15).

Ethanol concentrations of approximately 13 volume percent (~10% [wt/vol]) will reduce the fermentative activity of yeast by approximately 50% (16). However, for most glycolytic enzymes, significant denaturation is not observed at ethanol concentrations of 15% (wt/vol), and complete denaturation does not occur until ethanol concentrations exceed 40% (wt/vol) (16). Conversely, ethanol has been shown to enhance the passive proton flux through the yeast cell membrane, leading to depolarization of membrane potential and resulting in inhibition of nutrient uptake (e.g., amino acids and ammonium) but not of glucose uptake due to its transport being electroneutral (16). Furthermore, exposure of yeast cells to ethanol fluidizes the cell membrane (17). The ability of the yeast cell membrane to maintain its fluidity in a high-ethanol environment has been correlated with ethanol tolerance (18, 19). Finally, exposure of the yeast cell plasma membrane to ethanol has been shown to modulate the activity of membrane proteins, such as Pma1, which is the primary H^+-ATPase responsible for maintaining intracellular pH and plasma membrane potential in *S. cerevisiae* (20). These examples highlight the accumulating evidence that the yeast cell membrane is the primary target of the toxic effect of ethanol (9, 10, 16).

COMPOSITION OF MEMBRANE BILAYERS IN YEAST

The yeast biological membrane is composed primarily of phospholipids, glycosphingolipids, ergosterol, and proteins (21). Phospholipids are the primary structural component of the membrane and are essential to the viability of the cell. The fundamental structure of phospholipids is based on a glycerol-3-phosphate backbone with two fatty acid chains esterified to positions 1 (*sn1*) and 2 (*sn2*). The phospholipid class is defined by the molecule bonded to the phosphate group at the *sn3* position on the glycerol backbone. Due to the amphipathic nature of these molecules, they spontaneously form bilayers, and in biological membranes, there are embedded integral and peripheral membrane proteins involved with nutrient transport, ionic and pH homeostasis, and cellular signaling and transduction. Furthermore, certain classes of lipids act as second messengers in a number of cellular signaling pathways in yeast (22, 23). Phospholipids are distributed asymmetrically in the bilayer leaflets of the yeast cell membrane, and this asymmetry is necessary to maintain membrane surface potential and membrane protein activity (24). Maintenance of lipid asymmetry is maintained by a class of proteins called lipid translocases, and local or global disruption of lipid asymmetry is important to cell cycle progression, endocytosis, and cell polarity (25). The major phospholipids in yeast are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidic acid (PA), with minor amounts of cytidinediphosphate-diacylglycerol (CDP-DAG) (26). The most common fatty acids esterified to the glycerol moiety of yeast phospholipids are palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1}$), stearic acid ($C_{18:0}$), and oleic acid ($C_{18:1}$). The major sterol in yeast is ergosterol, with minor amounts of lanosterol and squalene (27). The relative amounts of these phospholipids depend upon a number of factors, including the yeast strain, nutrient availability, temperature, and growth phase (26, 28–31). Furthermore, molecular oxygen is required during the biosynthesis of saturated fatty acid and ergosterol, and adequate levels of dissolved oxygen early in fermentation are critical to fermentation success (9).

THE BIOPHYSICAL EFFECTS OF ETHANOL ON LIPID BILAYERS

The interaction of ethanol with lipid membranes has been a topic of considerable controversy over the mechanism of anesthesia for decades (32). Some investigators have concluded that it is the interaction of ethanol with the lipid membrane that induces structural changes in membrane-associated proteins (33), while others contend that it is the interaction of ethanol with the membrane proteins that is responsible for its effect (the interested reader should consult Mihic et al. [34]). Investigations of the interaction of ethanol with membrane bilayers utilizing nuclear magnetic resonance (NMR) spectroscopy are in agreement with the former (35). By examining the cross-relaxation rates (i.e., the contact probabilities) of ethanol with phospholipid bilayers, it has been demonstrated that ethanol has the largest cross-relaxation rate with the glycerol backbone and is therefore localized in the lipid-water interface of the bilayer (35). These results have been corroborated by similar investigations of ethanol-phosphatidylcholine bilayer interactions by comparing the measured cross-relaxation rate profile to profiles generated using molecular dynamic simulations (36). Therefore, the experimental evidence indicates that ethanol partitions in the lipid-water interface by forming hydrogen bonds with lipid molecules and, to a lesser degree, by the hydrophobic effect (35). At relatively low ethanol concentrations, ethanol will partition into membrane bilayers composed of phosphatidylcholine, reducing its main melting transition (37). Effectively, low ethanol concentrations fluidize the membrane due to increased lipid head group spacing from the steric effects of ethanol intercalating in the lipid water interface. At a higher ethanol concentration, the membrane bilayer will experience a dramatic reduction in thickness as it transitions into the interdigitated phase (37, 38). The interdigitated phase occurs when the fatty acyl chains cross the bilayer midplane and ethanol shields the fatty acid methyl groups from the aqueous phase. The result of lipid interdigitation is a reduction of up to 30% in membrane thickness (37).

In biological membranes, ethanol-induced membrane perturbations could have a number of potential effects. A significant reduction in membrane thickness due to lipid interdigitation would likely have a profound effect on membrane protein conformation and function. Biological membranes act as both a solvent and cofactor for membrane-bound proteins (39). As a cofactor, some lipids, referred to as annual lipids, surround or are localized to a specific portion of the membrane-associated protein and are considered integral to their function (39). As a solvent, the fatty acyl tails of the lipids ensure a hydrophobic match for the embedded membrane proteins, stabilizing them in the membrane. Changes in membrane thickness can result in exposure of hydrophobic amino acid residues in integral membrane proteins and a phenomenon known as hydrophobic mismatch (39). Subsequently, hydrophobic mismatch could lead to membrane protein aggregation to minimize the exposure of hydrophobic portions of

membrane proteins to an aqueous environment (40). Hydrophobic mismatch could also produce a conformational change in the membrane protein, referred to as tilt (40). This conformational change would likely alter the activity of the membrane protein. Indeed, Ca^{2+}-ATPases embedded in interdigitated bilayers exhibited changes in protein conformation and rapid loss of enzyme activity (41). Furthermore, ethanol-induced fluidization and interdigitation of the lipid bilayer have been shown to increase the ion permeability of membranes (42). Therefore, ethanol-induced thinning in biological membranes could result in "leaky" membranes, protein aggregation, and changes in the activity of membrane proteins.

Investigations of model membrane systems have demonstrated that ethanol can induce lipid interdigitation; however, this is not the entire story. Sterols are an essential component of cell membranes and are found in virtually all eukaryotic cells (43). Ergosterol is the major sterol in yeast, and inclusion of this sterol in synthetic bilayers mitigates the membrane-thinning effect of ethanol. In giant vesicles composed of dipalmitoylphosphatidylcholine (DPPC) and ergosterol, varying the concentration of ergosterol in the vesicles could delay the onset of interdigitation to higher ethanol concentrations (44, 45). Tierney et al. (45) demonstrated that at membrane ergosterol concentrations above 20 mol%, the induction of interdigitation required significantly more ethanol than in vesicles composed of pure DPPC, indicating a potential route through which lipid composition could modulate ethanol tolerance in yeast. Extending this work, Vanegas et al. (46) demonstrated a similar membrane-protective effect of ergosterol by using supported lipid bilayers and measuring changes in bilayer thickness using atomic force microscopy (AFM). Exposing pure DPPC bilayers to 15 vol% ethanol, a concentration common to industrial fermentations, induced the interdigitated phase. Similar to the observations of Tierney et al. (45), the inclusion of 20 mol% ergosterol in the DPPC bilayers significantly reduced the amount of lipid interdigitation (46). Atomistic molecular dynamic simulations of DPPC-ergosterol bilayers indicate that when ethanol partitions in the lipid-water interface of the membrane, it results in competition between ethanol and ergosterol to hydrogen bond with the carbonyl group of DPPC (47). Subsequently, ergosterol moves toward the bilayer midplane due to the steric effects of ethanol in the membrane interface, increasing the density of ergosterol toward the terminal ends of the fatty acyl chains of DPPC. This movement of ergosterol prohibits lipid interdigitation by compensating for the favorable Van der Waals forces that are diminished due to the increased head group spacing. Furthermore, the addition of the unsaturated lipid dioleoylphosphatidylcholine (DOPC) in synthetic bilayers demonstrated a similar protective effect to ergosterol (48). The lipid composition in yeast biomembranes is vastly more complex than the two- and three-component systems used in these studies. However, these simplified studies with model membrane systems demonstrate that changes in lipid composition can mitigate the membrane-perturbing effect of ethanol and may provide clues as to the mechanisms yeast have evolved to cope with the high ethanol levels they encounter during fermentation.

THE EFFECTS OF ETHANOL ON YEAST CELL MEMBRANES

Analysis of ethanol's effect on biological membranes has indirectly confirmed many of the hypotheses from investigations of model lipid bilayers exposed to alcohol. Madeira et al. (49) measured the fluxes of water and protons through the plasma membrane of *Saccharomyces cerevisiae* when exposed to ethanol concentrations greater than 10 vol%. They reported that ethanol enhanced the passive water transport, while it inhibited the mediated water transport. Furthermore, proton diffusion was increased significantly at higher temperatures and ethanol concentrations. By isolating the plasma membrane of several yeast species and exposing this fraction to ethanol, Aguilera et al. (20) demonstrated that the activity of Pma1 H^+-ATPase is modulated by ethanol and lipid composition and is most active in highly ethanol-tolerant species. These studies support the notion that higher ethanol concentrations can compromise the yeast membrane, making them leaky to protons and, potentially, other ions, resulting in yeast cell depolarization.

Membrane permeability is not the only consequence of exposing biological membranes to ethanol. In the synaptic membranes of ethanol-sensitive rats, lipid interdigitation, followed by protein aggregation, was observed when these membranes were exposed to ethanol (50). Furthermore, the synaptic membranes from ethanol-tolerant or ethanol-insensitive rats did not exhibit protein aggregation or lipid interdigitation upon exposure to ethanol, indicating that biomembranes can develop resistance to the perturbing effect of ethanol. Additionally, ethanol has been shown to modulate the activity of G protein-coupled receptors (GPCR) via a lipid-mediated mechanism such that ethanol-induced membrane fluidization inhibited metarhodopsin II formation (33) and membrane-mediated endocytosis of plasma membrane-associated proteins in *Saccharomyces cerevisiae* (51). Therefore, ethanol has considerable potential to disrupt membrane-associated protein distribution, function, and turnover via lipid-mediated mechanisms in biological membranes.

Numerous studies have analyzed the relationship between lipid composition and ethanol tolerance in *S. cerevisiae*. Early studies investigating Sake yeast strains demonstrated that these strains had significantly higher tolerance to ethanol when grown in medium supplemented with unsaturated fatty acids (52). In two studies, an *S. cerevisiae* strain grown in medium enriched with unsaturated fatty acids and sterols was exposed to a buffered (pH 4.5) 1 to 1.5 M (3.6 to 5.6 wt%) solution of ethanol during mid-exponential growth, a technique referred to as ethanol shock (53, 54), and experienced decreased biomass and cell viability. Mishra and Prasad (55, 56) similarly exposed exponential-growth-phase *S. cerevisiae* to a buffered (pH 4.5) 2 M (7.3 wt%) ethanol solution to ascertain ethanol tolerance via L-alanine uptake and proton flux through the membrane. Based on the conclusions of these studies, they proposed that increased levels of phosphatidylserine promoted a favorable anion/zwitterion ratio in the plasma membrane that increased ethanol tolerance (55). They also reported that cells with membranes enriched in unsaturated fatty acids exhibited higher ethanol tolerance based upon their L-alanine uptake, proton efflux, and fermentation rate (56). Castillo Agudo (57) concluded that *S. cerevisiae*, when exposed to various concentrations of ethanol (4 to 12 vol%) during exponential growth, does not necessarily require unsaturated fatty acids for viability and tolerance of the ethanol but may increase its ergosterol levels to maintain membrane fluidity. Conversely, You et al. (58) reported that the ethanol tolerance of desaturase-deficient *S. cerevisiae* strains could be restored by supplementation with oleic acid (Δ^9Z-$C_{18:1}$) and *cis*-vaccenic acid (Δ^{11}Z-$C_{18:1}$) but not pal-

mitoleic acid (Δ^9Z-$C_{16:1}$) and that oleic acid is critical in maintaining membrane fluidity in yeast exposed to ethanol. In one very interesting study, Aguilera et al. (20) exposed five yeast species to ethanol during growth and subsequently analyzed their fatty acid and ergosterol composition, as well as the H^+-ATPase activity. Using multiple linear regression and principal component analysis, they determined that oleic acid, palmitoleic acid, and ergosterol were highly correlated with H^+-ATPase activity and ethanol tolerance (20). Furthermore, the highest concentrations of ergosterol were observed in the two *S. cerevisiae* species used in the study, which were the most ethanol tolerant of the species analyzed.

Studies analyzing the effect of ethanol shock on phospholipid class concentration in *S. cerevisiae* indicate that elevated ergosterol-to-phospholipid ratios and the incorporation of longer-chain unsaturated fatty acids are associated with more ethanol-tolerant strains (59). However, Chi et al. (60) reported that the addition of inositol to synthetic fermentation medium increased phosphatidylinositol levels and decreased both phosphatidylcholine and phosphatidylethanolamine during stationary-phase metabolism. Yeast fermenting in synthetic medium lacking inositol also exhibited elevated phosphatidylinositol levels upon transitioning to stationary phase, albeit at lower concentrations than those observed in inositol-supplemented ferments. The absence of inositol in the fermentation medium resulted in lower CO_2 liberation rates, while its presence resulted in slightly higher final ethanol concentrations, indicating that this nutrient is important for ethanol production at some concentration (60). Furthermore, inositol supplementation resulted in yeast cells that were more resistant to ethanol shock in experiments at high ethanol concentrations (18 vol%) (60). It should be noted, however, that during exponential growth and in the presence of exogenous inositol, as well as other nutrients, phosphatidylcholine synthesis is enhanced while phosphatidylinositol levels are suppressed (26). Upon entering stationary-phase metabolism, the opposite trend is observed, such that phosphatidylcholine levels decrease while phosphatidylinositol levels rise (26).

Experiments utilizing ethanol shock to ascertain a yeast strain's ability to tolerate ethanol have produced a number of contradictory results. Some potential reasons for these conflicting results are the often-divergent experimental conditions used in these studies, including different yeast strains, growth phases and temperatures, ethanol concentrations, and nutrients in the fermentation media. Taken as a whole, the conclusions from these studies indicate that elevated concentrations of longer-chain unsaturated fatty acids and ergosterol are necessary to increase the ability of yeast to tolerate high ethanol concentrations. Furthermore, elevated phosphatidylinositol levels may also contribute to this organism's ability to tolerate an ethanol challenge during growth. However, it is critical to note here that, while interesting and important, ethanol shock during cell growth may not be relevant to normal yeast fermentation conditions, as yeast ethanol production is largely not growth associated (6, 7). That is, during alcoholic fermentations, exponential growth is generally complete prior to significant accumulation of ethanol. In this case, a more relevant challenge would be the addition of ethanol after the growth phase and during active conversion of sugar to ethanol, using a wide range of *Saccharomyces cerevisiae* strains.

LIPID COMPOSITION IN YEAST ALCOHOLIC FERMENTATIONS

Fewer studies have been published that investigate how yeast cell lipid composition changes over the course of fermentation as the levels of ethanol slowly rise as the yeast converts sugar to ethanol. Analysis of *Saccharomyces cerevisiae* grown anaerobically in a chemostat at sugar concentrations of 12% (wt/vol) indicates that the growth rate does not have any effect on the phospholipid or sterol composition of fermenting yeast (61). This study also indicated that, as the yeast adapts to self-produced ethanol, they exhibit increasing ergosterol levels in their membrane, with higher ratios of phosphatidylinositol-to-phosphatidylcholine and larger amounts of $C_{18:0}$ fatty acids relative to the amounts of $C_{16:0}$ fatty acids (61). A study of the gene expression patterns, lipid composition, and fermentation ability of two wine yeast strains fermented under enological conditions (~24% sugar [wt/vol]) and compared at two growth phases, at inoculation and during late exponential growth, reported that the strain that became stuck had lower levels of palmitic acid (C_{16}) and palmitoleic acid ($C_{16:1}$) (62). The results from this study also demonstrated that, early in fermentation, the strain that completed fermentation had higher concentrations of oleic acid, but by late exponential phase, both strains had equivalent levels of this fatty acid. Furthermore, the strain that failed to complete fermentation had higher levels of sterol, particularly ergosterol, toward the end of exponential growth (62), in contrast to the studies on yeast using ethanol challenge during growth. Mannazzu et al. (63) analyzed the fermentative abilities of three *S. cerevisiae* strains in sugar- and nitrogen-rich (~24% sugar [wt/vol]) but lipid nutrient-limited fermentations. A lack of lipid nutrients in conjunction with an anaerobic environment produces stressful growth conditions that would likely negatively affect membrane biosynthesis and, subsequently, cell viability. Therefore, they also monitored the lipid composition, membrane integrity, and cell viability throughout the approximately 25-day fermentation. They reported that the strain that utilized the most sugar, produced the highest quantities of ethanol, maintained the lowest membrane permeability, and had the greatest viability exhibited higher C_{16} fatty acid-to-total fatty acid (C_{16}/TFA) and unsaturated fatty acid-to-total fatty acid (UFA/TFA) levels throughout fermentation (63). They also observed that ergosterol levels were inversely related to the levels of ethanol produced, and they concluded that ergosterol was not essential to cell viability and membrane integrity during alcoholic fermentation.

Both ethanol shock and fermentation experiments have led the investigators to conclude that elevated unsaturated fatty acids are essential to *S. cerevisiae* viability, membrane integrity, H^+-ATPase function, and ethanol tolerance. Elevated phosphatidylinositol levels have also been associated with increased ethanol production, though it is unclear from these experiments what growth phase the yeast was in when the levels of this lipid were measured, as it would be expected that phosphatidylinositol levels would be higher during stationary phase (28). Overall, the conclusions from these studies regarding the role of ergosterol in ethanol tolerance in yeast are unclear. While some studies have concluded that it is essential to tolerance, others have found that ergosterol levels did not increase appreciably during fermentation and, therefore, must not be critical to alcohol tolerance.

The ambiguity in the literature regarding how lipid composi-

tion contributes to ethanol tolerance and fermentation completion is due in large part to considerable variation in the experimental conditions used in these studies, as well as the limited number of yeast strains analyzed. Furthermore, none of the investigations discussed here provided structural data on the lipids, which would be critical to understanding how specific lipid species contribute to ethanol tolerance and to elucidate potential mechanisms by which the membrane mitigates the toxic effects of ethanol. Analyses of lipid compositional changes that occur during fermentation in numerous yeast strains with various levels of ethanol tolerance utilizing modern tools of lipidomics and chemometrics are providing much greater insight into how this organism's membrane adapts to ethanol (63–65).

Recently, we developed an analytical methodology utilizing high-performance liquid chromatography coupled online to atmospheric pressure ionization mass spectrometry (API-MS) that facilitates rapid quantitative analysis of yeast lipid extracts (64). This method was used in conjunction with multivariate statistical analysis to ascertain how the lipid composition in 22 *Saccharomyces cerevisiae* strains correlated with yeast cell growth and ethanol production over the course of alcoholic fermentation (65). Partial least-squares regression modeling of the correlation of the lipid composition data to fermentation kinetic data indicated that both ethanol production and maximum yeast cell density were highly correlated with yeast cell lipid composition. In particular, yeast strains that converted more sugar to ethanol had higher levels of specific phosphatidylcholine species that have previously been demonstrated to stabilize model membrane bilayers in a high-ethanol milieu (48). Strains that were unable to complete fermentation had higher concentrations of phosphatidylinositol during the early stages of fermentation (65). Interestingly, ergosterol was not significantly correlated with ethanol production or yeast cell growth, in agreement with the observations of previous studies analyzing yeast lipid composition during alcoholic fermentation (20, 63, 64). While it seems likely that ethanol tolerance per cell is related to the biophysical effects discussed here, the reason for the strong correlation between maximum yeast cell density and cell lipid composition is less clear.

In another recent study, our group examined the effects of temperature extremes on fermentation kinetics, membrane fluidity, and yeast cell lipid composition (66), as we had previously shown that yeast cell inactivation is likely an exponential function of temperature (7). The results from this analysis confirmed that the fermentation temperature had a profound effect on fermentation kinetics and yeast cell lipid composition. Furthermore, these data revealed that stuck fermentations at high temperatures (35°C) and low-temperature fermentations (15°C) had significantly different lipid compositions (65). Specifically, all of the yeast strains analyzed in this study that experienced fermentation arrest at elevated temperatures had higher concentrations of several phosphatidylinositol species than at other temperatures. Conversely, low-temperature fermentations had the highest concentrations of phosphatidylethanolamine and phosphatidylcholine with medium-chain fatty acids (66). Ergosterol did not appear to have any protective effect, regardless of fermentation temperature, on any of the strains analyzed in this study.

CONCLUSIONS

The relationship between yeast ethanol tolerance and lipid composition has been well studied over the past 4 decades. Analyses of model membrane systems have demonstrated that ethanol partitions in the lipid-water interface of the membrane and that ethanol can induce large structural changes in the lipid bilayer and membrane-associated proteins. Genetic analysis of yeast has indicated that a number of genes involved in stress response, including membrane protein stabilization, experience increased expression during fermentation. Lipid membrane components that have been shown to correlate with ethanol tolerance in yeast depend strongly on the experimental conditions used to evaluate alcohol tolerance. For example, the membrane levels of ergosterol have been demonstrated to be significantly higher in yeast strains resistant to ethanol shock, while strains that produce higher levels of ethanol during fermentation have little correlation with membrane ergosterol levels. API-MS provided detailed molecular structure information that is necessary to ascertain both physical and physiological effects of differences in lipid composition between strains exhibiting varied levels of ethanol tolerance. While it is clear that the cell membrane composition is critical in determining ethanol tolerance, a detailed molecular mechanism for this tolerance remains to be elucidated.

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