Residual mitochondrial transmembrane potential decreases unsaturated fatty acid level in sake yeast during alcoholic fermentation

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ABSTRACT

Oxygen, a key nutrient in alcoholic fermentation, is rapidly depleted during this process. Several pathways of oxygen utilization have been reported in the yeast *Saccharomyces cerevisiae* during alcoholic fermentation, namely synthesis of unsaturated fatty acid, sterols and heme, and the mitochondrial electron transport chain. However, the interaction between these pathways has not been investigated. In this study, we showed that the major proportion of unsaturated fatty acids of ester-linked lipids in sake fermentation mash is derived from the sake yeast rather than from rice or koji (rice fermented with *Aspergillus*). Additionally, during alcoholic fermentation, inhibition of the residual mitochondrial activity of sake yeast increases the levels of unsaturated fatty acids of ester-linked lipids. These findings indicate that the residual activity of the mitochondrial electron transport chain reduces molecular oxygen levels and decreases the synthesis of unsaturated fatty acids, thereby increasing the synthesis of estery flavors by sake yeast. This is the first report of a novel link between residual mitochondrial transmembrane potential and the synthesis of unsaturated fatty acids by the brewery yeast during alcoholic fermentation.

Subjects Agricultural science, Biotechnology, Microbiology

Keywords Sake yeast, Mitochondria, Unsaturated fatty acid, Alcoholic fermentation, Oxygen, Anaerobiosis

INTRODUCTION

Sake, the traditional rice wine of Japan, has a history of more than 1,100 years (Kitagaki & Kitamoto, 2013). Sake is manufactured by mixing koji (rice fermented with *Aspergillus*, which catalyzes the breakdown of starch), steamed rice, water, and the sake yeast *Saccharomyces cerevisiae*, and brewing the mash for 20–30 days. Sake is brewed at 12–20 °C and thus sake yeast grows well at these temperatures as compared to other yeasts. However, sake yeast also grows well at higher temperatures (30 °C), both aerobically and anaerobically. Refined sake, which contains abundant estery flavors such as those of...
isoamylacetate and ethylcaproate, is preferred worldwide. The estery flavors in sake are produced during fermentation by sake yeasts. The augmentation of estery flavors by sake yeasts is key for the production of high quality sake (Kitagaki & Kitamoto, 2013). During sake brewing, oxygen is rapidly depleted within 1–2 days due to the consumption of oxygen and production of CO$_2$ by sake yeasts (Nagai et al., 1992); however, a limited amount of oxygen may be added by stirring the fermentation mash with a rod at 1–2 day intervals.

Lipid components, such as unsaturated fatty acids (Fujii et al., 1997), sterols (Ohta & Hayashida, 1983), and glucosylceramide (Sawada et al., 2015) affect the fermentation profiles of yeast. In particular, the presence of unsaturated fatty acid results in a decrease in the production of isoamylacetate by brewery yeasts via repression of the alcohol acetyltransferase gene ATF1 (Yoshimoto et al., 1998; Fujii et al., 1997; Fujiwara et al., 1998). Therefore, unsaturated fatty acid content is targeted to control isoamylacetate production by sake yeasts. Additionally, unsaturated fatty acids regulate ethanol tolerance (You, Rosenfield & Knipple, 2003). To date, however, efforts to regulate the synthesis of unsaturated fatty acid have been focused solely on molecular oxygen content (Fujii et al., 1997; Nakagawa, Sugioke & Kaneko, 2001) and acyltransferase activity (De Smet et al., 2012). Alternative factors that potentially regulate the content of unsaturated fatty acids in sake yeast remain unknown.

Oxygen is required for various biosynthetic pathways of yeast, including those involved in the synthesis of unsaturated fatty acids (Mitchell & Martin, 1995), sterols (Fornairon-Bonnefond et al., 2003), heme synthesis (Maines, 1988), oxidation of lipids by reactive oxygen radicals (Salmon et al., 2000), cell wall protein expression (Kitagaki, Shimoi & Itoh, 1997), and the expression of diauxic shift-related genes (Kitagaki et al., 2009). However, oxygen is depleted in the very early phase of alcoholic fermentation. As a result, the availability of molecular oxygen is limited during alcoholic fermentation. The utilization of oxygen during alcoholic fermentation via 2 major pathways, fatty acid desaturation and sterol synthesis, has been precisely investigated (Rosenfeld & Beauvoit, 2003a; Rosenfeld et al., 2003b). In addition to these pathways, the mitochondrial electron transport chain which utilizes molecular oxygen (O’Connor-Cox, Lodolo & Axcell, 1996) and nonclassical mitochondrial electron transport chain activity which produces nitric oxide from NO$_2^-$ (Castello et al., 2008) have been reported. However, there are few reports on the interactions of the mitochondrial electron transport chain and other pathways during alcoholic fermentation.

In previous studies, we have demonstrated that mitochondrial activities, morphologies or degradation of sake yeast affect fermentation characteristics such as malic acid, pyruvic acid productivity and carbon flux (Kitagaki et al., 2008; Kitagaki, 2009; Horie et al., 2010; Motomura, Horie & Kitagaki, 2012; Shiroma et al., 2014; Kitagaki & Takagi, 2014; Agrimi et al., 2014; Obi et al., 2014). Based on these studies, we hypothesize that the residual mitochondrial electron transport chain activity of brewery yeasts is the determinant of unsaturated fatty acid production efficiency.

In the present study, we show that the major proportion of fatty acids which are ester-linked to glycerophospholipids and neutral lipids in the fermentation mash is
derived from sake yeast, not rice or koji, and the synthesis of the unsaturated fatty acids in
sake yeast increases when the activity of the mitochondrial electron transport chain is
inhibited. To our knowledge, this is the first report indicating that residual mitochondrial
activity is essential for regulating the content of unsaturated fatty acids in fermentation
mash, providing a valuable insight into the relationship between mitochondrial activity
and the ester-producing ability of brewery yeasts.

MATERIALS AND METHODS

Strains and media
Sake yeast RAK1536 K7 his3/his3 + pRS413-GPDmitoGFP (Kitagaki et al., 2008;
Hashimoto et al., 2005) and laboratory yeast CEN.PK2 + pRS413-GPDmit obtained from
Euroscarf (Entian & Kotter, 1998) were used in this study. For culturing of these yeasts,
CSM (-HIS) medium (0.67% Difco™ Yeast Nitrogen Base w/o Amino Acids and
Ammonium Sulfate, 0.08% Complete Supplement Mixture Drop-out: -HIS, and
2% glucose) was used.

Analysis of unsaturated fatty acid level
In order to analyze the amount of fatty acids ester-linked to glycerophospholipids and
neutral lipids in the fermentation mash, 30 µl of 0.2 mg/ml heptadecanoic acid was added
to the extracted solution as an internal control.

For preparation of the fermentation mash, 12.6 g pregelatinized rice (Tokushima seiko,
Co. Ltd., Awa, Japan) with 30% of its surface polished and removed, 4.8 g pregelatinized
koji (Tokushima seiko, Co. Ltd., Awa, Japan) with 30% of the surface of rice polished and
removed, and 42 ml distilled water were mixed. In order to prepare the fermentation mash
with yeast, yeast was added to the mash at 1 × 10⁷ cells/ml and incubated at 30 °C for
7 days. For preparation of the fermentation mash without yeast, the mash was directly
frozen without adding yeast. The mash was freeze-dried and 20 mg, 80 mg, or 320 mg of
the freeze-dried samples were subjected to fatty acid analysis. For analysis of the fatty acid
composition ester-linked to glycerophospholipids and neutral lipids of yeast cells, cells
were collected after incubation by centrifugation at 16,900 g for 1 min, washed twice with
distilled water, and freeze-dried. An aliquot (20 mg) of the sample was subjected to
fatty acid analysis. Degradation of the ester bond of lipids, methylation of the fatty acids
and purification by silica gel chromatography was performed according to the
manufacturer’s protocol. The derivatized fatty acids were analyzed by gas chromatography
(Shimadzu GC-2014; initial temperature 240 °C, hold 5 min, 4 °C/min, maximum 240 °C,
hold 5 min, sampling time 1 min, nitrogen gas, pressure 85.3 kPa) equipped with a
DB-WAX column (length 30.0 m, 0.25 mm ID, film thickness 0.25 μm), an AOC-20
autosampler (sampling volume 1 μl, split ratio 1:50), and Supelco FAME standard
(Sigma Aldrich, St. Louis, MO, USA) as a reference.

Analysis of yeast physiology following oxygen shutoff
Yeast cells (K7 his3/his3 RAK1536 + pRS413-GPDmitoGFP and CEN.PK2+pRS413-
GPDmit) were precultured in selective medium, inoculated into 1 ml of CSM medium at
1.0 × 10^6 cells/ml with a layer of liquid paraffin on top of the culture medium, and incubated statically at 30 °C for 4 h, 12 h, and 24 h, with or without 0.004% w/v resalurin (Sigma-Aldrich, St. Louis, MO, USA). Formaldehyde (3.7% v/v) was added and the culture was incubated at room temperature for 30 min. Cells were collected by centrifugation, washed twice with distilled water, and visualized under a fluorescent microscope.

**Statistical analysis**

Fermentation experiments were performed in triplicate from independent starter cultures. Significant differences between the averages of the data were calculated by unpaired two-tailed Student's \( t \)-test.

**RESULTS**

**Sake yeast is the main source of unsaturated fatty acids in fermentation mash**

First, in order to elucidate the source of unsaturated fatty acids in the fermentation mash, their composition was investigated. As koji (catalyst of starch), rice pre-fermented with *A. oryzae*, rice, and yeast are used for sake brewing, the same amount of koji and rice was fermented with or without yeast, and its fatty acid composition was analyzed. We found that the oleic acid composition of fermentation mash with yeast was 136-fold higher than that of fermentation mash without yeast, palmitic acid composition of fermentation mash with yeast was 137-fold higher than that of fermentation mash without yeast and polyunsaturated fatty acids such as linoleic acid and \( \alpha \)-linolenic acid that are contained in rice and koji were not detected (Table 1). These results clearly indicate that sake yeast, which contains palmitic acid and oleic acid, but not rice or koji, which contains linoleic acid and \( \alpha \)-linolenic acid, is the main source of unsaturated fatty acids of the sake mash.

**Mitochondrial morphology remains tubular after oxygen shutoff**

During alcoholic fermentation on an industrial scale, molecular oxygen is depleted after 1–2 days of fermentation (*Nagai et al., 1992*) due to consumption by yeast and the production of a large quantity of CO\(_2\), which is heavier than air. Therefore, after 1–2 days, yeast is exposed to extreme anaerobiosis. In order to simulate industrial fermentation, we designed a culture system to deplete molecular oxygen during alcoholic fermentation. Immediately after the start of fermentation, a layer of liquid paraffin, which functions as an oxygen valve, was added on top of the culture media, and fermentation was carried out by sake yeast. The concentration of oxygen after oxygen shutoff was monitored using the oxidoreductive stain resarulin. The color of the culture indicated that oxygen is depleted within 8 h of the start of fermentation when incubated with sake and laboratory yeasts (Figs. 1A–1L). In order to investigate the mitochondrial status of sake yeast under these conditions, the mitochondrial morphology of sake yeast was examined. Mitochondrial morphology is an indicator of mitochondrial transmembrane potential
It was found that most of the mitochondria of sake yeast were tubular at 4 h after oxygen shutoff, but gradually fragmented 12 h after the start of fermentation; however, a certain portion of mitochondria stayed tubular at 24 h after start of the culture (Figs. 1M–1R). This result indicates that oxygen is depleted during the early phase of alcoholic fermentation upon oxygen shutoff; however, mitochondrial electron potential is retained during anaerobic stages of alcoholic fermentation.
Inhibition of mitochondrial electron potential elevates synthesis of unsaturated fatty acids

On the basis of the above results, we hypothesized that residual mitochondrial electron transport chain activity results in the utilization of the low levels of molecular oxygen available, thereby limiting the availability of molecular oxygen for fatty acid desaturation within endoplasmic reticulum in sake yeast. In order to verify this hypothesis, the mitochondrial uncoupler, carbonyl cyanide m-chlorophenylhydrazone, was added to the medium during alcoholic fermentation, and the composition of fatty acids was monitored. We found that the level of unsaturated fatty acids (oleic acid and palmitoleic acid) were increased in response to mitochondrial uncoupler (Figs. 2B and 2D, p < 0.05), whereas the level of saturated fatty acid, stearic acid was unaffected (Fig. 2C, p > 0.05) and that of palmitic acid was decreased (Fig. 2A, p < 0.05) in response to the...
mitochondrial uncoupler. This result is consistent with our hypothesis that residual mitochondrial electron transport chain activity inhibits the synthesis of unsaturated fatty acids.

DISCUSSION

The interactions of the multiple pathways of oxygen utilization during alcoholic fermentation are poorly understood, especially in the case of sake brewing, where oxygen-derived substances from rice and koji coexist with sake yeast. The present study is the first to report that sake yeast is the main source of unsaturated fatty acids in fermentation mash, and that unsaturated fatty acid content of the fermentation mash is affected by mitochondrial activity of sake yeast. This novel insight suggests that the content of unsaturated fatty acid in the fermentation mash may be decreased by augmenting the mitochondrial activity of yeast during alcoholic fermentation.

Although shrinkage of mitochondria during anaerobiosis has been reported ([Plattner & Schatz, 1969](#)), the time scale of this shrinkage and its effect of other metabolic processes during alcoholic fermentation remain obscure. Our research group has previously shown that mitochondrial activity during sake brewing affects the brewing characteristics, such as malate and pyruvate production efficiencies and carbon flux, of sake yeast ([Oba et al., 2014; Shiroma et al., 2014; Agrimi et al., 2014; Kitagaki & Takagi, 2014; Motomura, Horie & Kitagaki, 2012; Horie et al., 2010; Kitagaki, 2009; Kitagaki et al., 2008](#)). However, the role of mitochondria of sake yeast on fatty acid desaturation has not been elucidated to date. The present study is the first to show that, in contrast to the rapid depletion of oxygen during alcoholic fermentation, as indicated by resarulin staining, mitochondrial transmembrane electron potential, as revealed by mitochondrial morphology, is maintained for a certain period of time. Additionally, we demonstrate that the residual mitochondrial activity decreases the content of unsaturated fatty acid of sake yeast. Our findings suggest that the utilization of sake yeasts exhibiting decreased mitochondrial activity should result in increased levels of unsaturated fatty acids during fermentation, thereby reducing estery flavors. This hypothesis requires further verification.

The utilization of oxygen involves pathways other than the mitochondrial transport chain and fatty acid desaturation. The stoichiometric relationship between these two mechanisms reported in this study suggests that they represent one of the main pathways of oxygen utilization; however, synthesis of ergosterol requires 9 moles of O₂ whereas desaturation of fatty acid requires only 1 mole ([Espenshade & Hughes, 2007; Bloch, 1969](#)) and inhibition of mitochondrial activity also affected sterol synthesis ([Adams & Parks, 1969](#)). Moreover, several steps of the synthesis of heme inside the mitochondria also requires oxygen. For example, during the synthesis of heme, coproporphyrinogen III reenters the mitochondrion, where the oxygenase converts it to protoporphyrin IX (Protoporphyrinogen IX oxidase (EC 1.3.3.4) using molecular oxygen ([Maines, 1988](#))). Therefore, it is considered that mitochondrial electron transport chain, mitochondrial alternative respiration pathway, ergosterol synthesis, heme synthesis and fatty acid desaturation compete for the residual oxygen during alcoholic fermentation, although
the quantitative ratio among these pathways seems to depend on the conditions (Salmon, Forniron & Barre, 1998).

In conclusion, we demonstrate, for the first time, that residual mitochondrial electron transmembrane potential decreases the synthesis of unsaturated fatty acid within endoplasmic reticulum. This novel finding should provide valuable insights into metabolic regulation and engineering of brewery yeasts.

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Competing Interests
The authors declare that they have no competing interests.

Author Contributions
- Kazutaka Sawada performed the experiments, wrote the paper, reviewed drafts of the paper.
- Hiroshi Kitagaki conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper, fatty acid analysis, fermentation experiments.

Data Deposition
The following information was supplied regarding data availability:
Raw data is available in the Supplemental Information.

Supplemental Information
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REFERENCES

Adams BG, Parks LW. 1969. Differential effect of respiratory inhibitors on ergosterol synthesis by Saccharomyces cerevisiae during adaptation to oxygen. *Journal of Bacteriology* 100(1):370–376.

Agrimi G, Mena MC, Izumi K, Pisano I, Germinario L, Fukuzaki H, Palmieri I, Blank LM, Kitagaki H. 2014. Improved sake metabolic profile during fermentation due to increased mitochondrial pyruvate dissimilation. *FEMS Yeast Research* 14:249–260 DOI 10.1111/1567-1364.12120.

Benard G, Bellance N, James D, Parrone P, Fernandez H, Letellier T, Rossignol R. 2007. Mitochondrial bioenergetics and structural network organization. *Journal of Cell Science* 120:838–848 DOI 10.1242/jcs.03381.

Bloch K. 1969. Enzymic synthesis of monounsaturated fatty acids. *Accounts of Chemical Research* 2(7):193–202 DOI 10.1021/ar50019a001.

Castello PR, Woo DK, Ball K, Wojcik J, Liu L, Poyton RO. 2008. Oxygen-regulated isoforms of cytochrome c oxidase have differential effects on its nitric oxide production and on hypoxic signaling. *Proceedings of the National Academy of Sciences of the United States of America* 105(24):8203–8208 DOI 10.1073/pnas.0709461105.

De Smet CH, Vittone E, Scherer M, Houweling M, Liebisch G, Brouwers JF, de Kroon AI. 2012. The yeast acyltransferase Sct1p regulates fatty acid desaturation by competing with the desaturase Ole1p. *Molecular Biology of the Cell* 23(7):1146–1156 DOI 10.1091/mbc.E11-07-0624.

Entian K-D, Kotter P. 1998. Yeast mutant and plasmidcollections. *Methods in Microbiology* 26:431–449 DOI 10.1016/S0580-9517(08)70344-1.

Espenshade PJ, Hughes AL. 2007. Regulation of sterol synthesis in eukaryotes. *Annual Review of Genetics* 41:401–427 DOI 10.1146/annurev.genet.41.110306.130315.

Fujii T, Kobayashi O, Yoshimoto H, Furukawa S, Tamai Y. 1997. Effect of aeration and unsaturated fatty acids on expression of the *Saccharomyces cerevisiae* alcohol acetyltransferase gene. *Applied and Environmental Microbiology* 63:910–915.

Fujiwara D, Yoshimoto H, Sone H, Harashima S, Tamai Y. 1998. Transcriptional co-regulation of *Saccharomyces cerevisiae* alcohol acetyltransferase gene, ATF1 and delta-9 fatty acid desaturase gene, OLE1 by unsaturated fatty acids. *Yeast* 14:711–721 DOI 10.1002/(SICI)1097-0061(19980615)14:8<711::AID-YEA263>3.0.CO;2-8.

Fornairon-Bonnefond C, Aguera E, Deytieux C, Sablayrolles JM, Salmon JM. 2003. Impact of oxygen addition during enological fermentation on sterol contents in yeast lees and their reactivity towards oxygen. *Journal of Bioscience and Bioengineering* 95(6):675–678 DOI 10.1263/jbb.105.675.

Hashimoto S, Ogura M, Aritomi K, Hoshida H, Nishizawa Y, Akada R. 2005. Isolation of auxotrophic mutants of diploid industrial yeast strains after UV mutagenesis. *Applied and Environmental Microbiology* 71:312–319 DOI 10.1128/AEM.71.1.312-319.2005.

Horie K, Oba T, Motomura S, Isogai A, Yoshimura T, Tsuge K, Koganemaru K, Kobayashi G, Kitagaki H. 2010. Breeding of a low pyruvate-producing sake yeast by isolation of a mutant resistant to ethyl alpha-transcyanocinnaminate, an inhibitor of mitochondrial pyruvate transport. *Bioscience, Biotechnology, and Biochemistry* 74(4):843–847 DOI 10.1271/bbb.90373.

Kitagaki H. 2009. Mitochondrial-morphology-targeted breeding of industrial yeast strains for alcohol fermentation. *Biotechnology and Applied Biochemistry* 53(3):145–153 DOI 10.1042/BA20090032.

Kitagaki H, Kato T, Isogai A, Mikami S, Shimoi H. 2008. Inhibition of mitochondrial fragmentation during sake brewing causes high malate production in sake yeast. *Journal of Bioscience and Bioengineering* 105(6):673–678 DOI 10.1263/jbb.105.675.
Kitagaki H, Cowart LA, Matmati N, Montefusco D, Gandy J, de Avalos SV, Novgorodov SA, Zheng J, Obeid LM, Hannun YA. 2009. ISC1-dependent metabolic adaptation reveals an indispensable role for mitochondria in induction of nuclear genes during the diauxic shift in S. cerevisiae. The Journal of Biological Chemistry 284:10818–10830 DOI 10.1074/jbc.M805029200.

Kitagaki H, Kitamoto K. 2013. Breeding researches of sake yeasts in Japan: History, recent technological advances, and future perspectives. Annual Review of Food Science and Technology 4:215–235 DOI 10.1146/annurev-food-030212-182545.

Kitagaki H, Shimoi H, Itoh K. 1997. Identification and analysis of a static culture-specific cell wall protein, Tir1p/Srp1p in Saccharomyces cerevisiae. European Journal of Biochemistry 249(1):343–349 DOI 10.1111/j.1432-1033.1997.t01-1-00343.x.

Kitagaki H, Takagi H. 2014. Mitochondrial metabolism and stress response of yeast: Applications in fermentation technologies. Journal of Bioscience and Bioengineering 117(4):383–393 DOI 10.1016/j.jbiosc.2013.09.011.

Nagai H, Kondo K, Mishima H, Takemura S. 1992. Effects of dissolved oxygen on sake brewing. Hakkokogaku Kaishi 70(5):361–369 DOI 10.1016/0922-338x(92)90123-c.

Nakagawa Y, Sugioka S, Kaneko Y, Harashima S. 2001. O2R, a novel regulatory element mediating Rox1p-independent O(2) and unsaturated fatty acid repression of OLE1 in Saccharomyces cerevisiae. Journal of Bacteriology 183:745–751 DOI 10.1128/JB.183.2.745-751.2001.

Maines MD. 1988. Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. The FASEB Journal 2(10):2557–2568.

Mitchell AG, Martin CE. 1995. A novel cytochrome b5-like domain is linked to the carboxyl terminus of the Saccharomyces cerevisiae delta-9 fatty acid desaturase. The Journal of Biological Chemistry 270:29766–29772 DOI 10.1074/jbc.270.50.29766.

Motomura S, Horie K, Kitagaki H. 2012. Mitochondrial activity of sake brewery yeast affects malic and succinic acid production during alcoholic fermentation. Journal of the Institute of Brewing 118:22–26 DOI 10.1002/jib.7.

Oba T, Kusumoto K, Kichise Y, Izumoto E, Nakayama S, Tashiro K, Kuhara S, Kitagaki H. 2014. Variations in mitochondrial membrane potential correlate with malic acid production by natural isolates of Saccharomyces cerevisiae sake strains. FEMS Yeast Research 14:789–796 DOI 10.1111/1567-1364.12170.

O’Connor-Cox ESC, Lodolo EJ, Axcell BC. 1996. Mitochondrial relevance to yeast fermentative performance: a review. Journal of the Institute of Brewing 102(1):19–25 DOI 10.1002/j.2050-0416.1996.tb00890.x.

Ohta K, Hayashida S. 1983. Role of tween 80 and monolein in a lipid-sterol-protein complex which enhances ethanol tolerance of sake yeasts. Applied and Environmental Microbiology 46(4):821–825.

Plattner H, Schatz G. 1969. Promitochondria of anaerobically grown yeast 3. Morphology. Biochemistry 8(1):339–343 DOI 10.1021/bi00829a047.

Rosenfeld E, Beauvoit B. 2003a. Role of the non-respiratory pathways in the utilization of molecular oxygen by Saccharomyces cerevisiae. Yeast 20(13):1115–1144 DOI 10.1002/yea.1026.

Rosenfeld E, Beauvoit B, Blondin B, Salmon JM. 2003b. Oxygen consumption by anaerobic Saccharomyces cerevisiae under enological conditions: effect on fermentation kinetics. Applied and Environmental Microbiology 69(1):113–121 DOI 10.1128/AEM.69.1.113-121.2003.

Salmon JM, Fornairon-Bonnefond C, Mazauric J-P, Moutoune M. 2000. Oxygen consumption by wine lees: impact on lees integrity during wine ageing. Food Chemistry 71(4):519–528 DOI 10.1016/S0308-8146(00)00204-1.
Salmon JM, Fornairon C, Barre P. 1998. Determination of oxygen utilization pathways in an industrial strain of *Saccharomyces cerevisiae* during enological fermentation. *Journal of Fermentation and Bioengineering* 86(2):154–163 DOI 10.1016/S0922-338X(98)80054-8.

Sawada K, Sato T, Hamajima H, Jayakody LN, Hirata M, Yamashiro M, Tajima M, Mitsutake S, Nagao K, Tsuge K, Abe F, Hanada K, Kitagaki H. 2015. Glucosylceramide contained in koji mold-cultured cereal confers membrane and flavor modification and stress tolerance to *Saccharomyces cerevisiae* during coculture fermentation. *Applied and Environmental Microbiology* 81(11):3688–3698 Epub 2015 Mar 20 DOI 10.1128/AEM.00454-15.

Shiroma S, Jayakody LN, Horie K, Okamoto K, Kitagaki H. 2014. Enhancement of ethanol fermentation of *Saccharomyces cerevisiae* sake yeast strain by disrupting mitophagy function. *Applied and Environmental Microbiology* 80:1002–1012 DOI 10.1128/AEM.03130-13.

Yoshimoto H, Fujiwara D, Momma T, Ito C, Sone H, Kaneko Y, Tamai Y. 1998. Characterization of the ATF1 and Lg-ATF1 genes encoding alcohol acetyltransferases in the bottom fermenting yeast *Saccharomyces pastorianus*. *Journal of Fermentation and Bioengineering* 86(1):15–20 DOI 10.1016/S0922-338X(98)80027-5.

You KM, Rosenfield CL, Knipple DC. 2003. Ethanol tolerance in the yeast *Saccharomyces cerevisiae* is dependent on cellular oleic acid content. *Applied and Environmental Microbiology* 69:1499–1503 DOI 10.1128/AEM.69.3.1499-1503.2003.