Chapter 8
Nutrient Stress Responses of the Bottom-Fermenting Yeast

Satoshi Yoshida and Hiroyuki Yoshimoto

Abstract Beer is an alcoholic beverage that is made by yeast fermentation of the raw ingredients malt and hops. Since the mid-1990s, varieties of alcoholic beverages such as low-malt beer and no-malt brews made from soybean proteins or peptides instead of malt, and liquid sugar, have been launched. In the process of developing these alcohol beverages, a number of problems that have not previously been encountered in beer production have arisen. To solve these problems, therefore, it is necessary to investigate the physiological state of brewing yeast under various stressed conditions. In this chapter, we describe cellular responses to stress caused by different sugars, by nutrients other than sugars, and by mineral and vitamin deficiency, in addition to other environmental stresses that are predicted to be related to genes specific to bottom-fermenting yeast.

Keywords Beer • Low-malt • Mineral • No-malt • *Saccharomyces pastorianus* • SICD • Stress • Sugar • Vitamin • Yeast

8.1 Introduction

Beer is an alcoholic beverage that is made by yeast fermentation of the raw ingredients malt and hops. In Japan, rice and corn are sometimes used as auxiliary materials to give a mild and fresh taste. In standard beer, malt is milled and mashed to make wort. The sugar in wort is then converted to alcohol by yeast fermentation. Since the mid-1990s, a low-malt beer, happoushu, which is made from wort containing liquid sugars and a smaller amount of malt as raw materials, and no-malt brews, made from soybean proteins or peptides instead of malt and liquid sugars, have been produced. These worts contain sugars and nitrogen sources including amino acids, but their ratio of sugars to nutrients other than sugars (hereafter "nonsugar nutrients") is higher than that of standard beer wort. Therefore, yeast is

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exposed to many stresses other than the high concentration of sugar, high osmolarity, and high alcohol content produced during fermentation, which are known to exert stress on yeast during beer production.

Brewing yeasts for beer are largely divided into two groups: top-fermenting yeast and bottom-fermenting yeast. The former is used to brew top-fermenting beer including ale-type beers, whereas the latter is used to brew bottom-fermenting beer including pilsner-type beer. Almost all top-fermenting yeasts belong to the species *Saccharomyces cerevisiae*. This type of yeast floats to the surface of the tank at the end of the fermentation. In contrast, the bottom-fermenting yeast flocculates and settles at the bottom of the tank when the main fermentation has finished. All bottom-fermenting yeasts belong to the species *Saccharomyces pastorianus*, which is a natural hybrid formed from aneuploidy between *S. cerevisiae* and *Saccharomyces eubayanus* based on their genome sequences (Libkind et al. 2011). Regarding *S. pastorianus*, the whole genome sequence has been determined (Nakao et al. 2009), transcriptomic analysis of the genes has been performed (Yoshida et al. 2007a), and the structure and regulation of the expression of orthologous genes have been investigated (Minato et al. 2009).

Various environmental stress responses of laboratory *S. cerevisiae* yeast have been investigated. Because *S. cerevisiae* is the simplest eukaryote, it has also been used extensively for basic research. On the basis of the cellular response of yeast to antibiotics, the working mechanisms of antibiotics in higher eukaryotes including mammals have become evident and have facilitated the analysis and application of related cellular functions. For example, by analysis of response to the immunosuppressant rapamycin, function of the TOR (*To*lerant to *r*apamycin) signal transduction pathway related to nutrient signal response has been elucidated (Schmelzle and Hall 2000). In addition, analysis of response to the protein kinase C (PKC) inhibitor staurosporine has facilitated elucidation of the PKC-phosphatidylinositol (PI) signal transduction pathway related to regulation of cell growth and cell wall biosynthesis (Yoshida et al. 1992). Similarly, the PI 3-kinase signal transduction pathway has been investigated by analysis of the response to wortmannin (Zewail et al. 2003). On the other hand, during the process of brewing beer, yeast encounters various important environmental stresses, including higher sugar concentration, higher water pressure, and greater alcohol and organic acids content. With respect to osmolarity, yeast has a two-component system related to the HOG (*h*igh *o*smolarity *g*lycerol production) signal transduction pathway, which has been investigated in detail (Saito and Posas 2012). High-gravity brewing has been investigated from the aspect of saving costs and energy. In addition, structure and composition of the plasma membrane, including ergosterol and transcription factors, are known to be involved in tolerance to alcohol (Ding et al. 2009; Alper et al. 2006); in particular, sake yeast *MSN2/4* and *RIM15* have been shown to be related to alcohol tolerance in a comparison of sake yeast with laboratory yeast (Watanabe et al. 2012). With respect to organic acids, a mechanism for tolerance to weak organic acid (Piper et al. 2001) and lactic acid (Suzuki et al. 2012) has been reported. Among these various stress responses, the nutrient stress responses of brewing yeast relative to sugars and minerals are the main focus of this chapter.

8.1.1 Cellular Response to Nonsugar Nutrient Starvation in Bottom-Fermenting Yeast

Laboratory yeast *S. cerevisiae* cells are known to arrest at G_1 phase under conditions of carbon starvation (Thevelein 1992). In addition, under conditions of thiamine starvation, the activity of enzymes requiring thiamine diphosphate as a coenzyme is decreased, resulting in metabolic deficiency (Hohmann and Meacock 1998). When the balance between sugar and nonsugar nutrients is disturbed, for example, the level of nonsugar nutrients is markedly reduced, yeast cells are likely to die (Granot and Snyder 1991). Moreover, the senescence of cells is also involved in the nutrient stress response, providing a link between sugar consumption and cell death.

8.1.1.1 Cell Senescence

With respect to the senescence of yeast cells, the gene *SIR2* (*s*ilent *i*nformation *r*egulator 2) encoding an NAD-dependent histone deacetylase, sirtuin, has been isolated. This gene was initially discovered in yeast, but shortly afterward homologous genes were discovered in mammals, and *SIR2* has been found to be conserved among various organisms. Disruption of *SIR2* was found to result in a 50 % decrease in the lifespan of cells, whereas an increase in *SIR2* gene expression elongated the lifespan by 1.3-fold. In addition, when the concentration of glucose (Glc) in the medium was decreased from 2 to 0.5 %, the lifespan of the yeast cells was increased by 1.3-fold, showing that calorie restriction elongates lifespan in yeast cells as in mammals (Bitterman et al. 2003). In humans, activation of this gene might be used to control aspects of senescence, such as the elimination of reactive oxygen molecules, fat burning, muscle strengthening, control of arteriosclerosis and high blood sugar, prevention of diabetes, and control of cancer. That is, the lifespan of humans might be increased by activation of *SIR2* via moderate calorie restriction, a diet with moderate nutrient balance, and avoidance of overeating. Indeed, it has been reported that resveratrol, which is present at high levels in red wine, is an activator of Sir2p, and there is a relationship between red wine and senescence (Chen and Guarente 2006). It follows that the lifespan of the yeast cells might be elongated via the activation of this gene, for example, by providing growth media with a moderate nutrient balance.

8.1.1.2 Sugar-Induced Cell Death (SICD)

Notably, laboratory yeast cells die immediately when they are transferred to D-Glc solution, but they live when they are transferred to a synthetic minimum medium containing YNB (yeast nitrogen base without amino acid) and D-Glc, L-Glc solution, or water. This phenomenon is called SICD (*s*ugar-*i*nduced *c*ell *d*eath) and is caused by a deficiency of nonsugar nutrients (Granot and Snyder 1991).

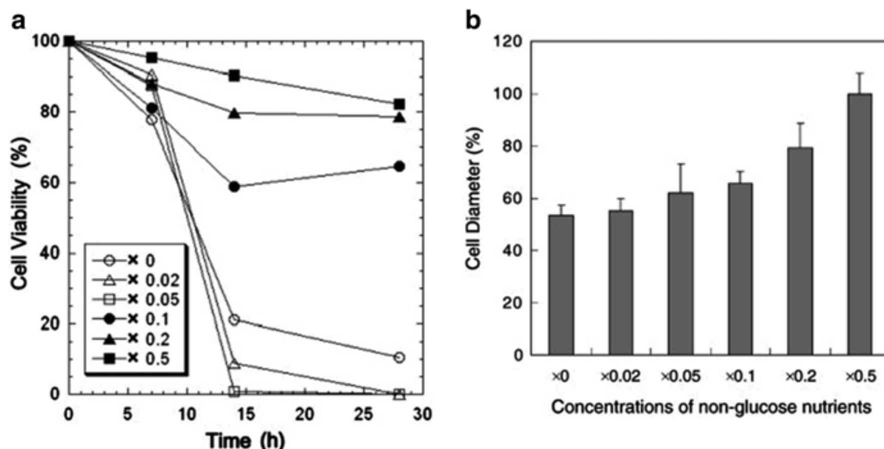

Fig. 8.1 Changes in cell viability and morphology are influenced by the balance between Glc and non-Glc nutrients. Fermentation tests were carried out in synthetic medium containing various concentrations of nonsugar nutrients in the original basal medium containing a fixed amount of sugars. X0, X0.02, X0.05, X0.1, X0.2, and X0.5 indicate 0-, 0.02-, 0.05-, 0.1-, 0.2-, and 0.5-fold concentrations of nonsugar nutrients, respectively. **a** Cell viability was measured after 7, 14, and 28 h of incubation. **b** The diameter of yeast cells was calculated for cells after 28 h of incubation. Data are mean ± SD of at least three determinations

SICD is partially suppressed by the addition of a nitrogen source, suggesting that it is also related to a deficiency of nutrients other than nitrogen. SICD is induced in a solution of fermentable sugars including fructose (Frc) and sucrose in addition to D-Glc. SICD also occurs in nonfermentable sugars such as glycerol, galactose, acetic acid, and ethanol. In addition, laboratory yeast cells that cannot use maltose (Mal) do not undergo SICD in a solution containing 2 % Mal, just as in water.

Collectively, these observations show that yeast cells die when they consume sugars in the absence of nonsugar nutrients (Granot and Snyder 1993). Yeast cells that have died from SICD show DNA and RNA degradation, plasma membrane damage, nuclear fragmentation, and cell shrinkage, all of which are typical of apoptosis. That is, SICD is a type of apoptosis, in which the cells die and release nutrients including nitrogen sources and trace elements such as minerals and vitamins into the medium. These nutrients are then used by surrounding cells to survive. Under the conditions of SICD, reactive oxygen species (ROS) are produced, and addition of the antioxidant agent ascorbate suppresses cell death (Granot et al. 2003).

Yeast cells tend to die in low-malt beer fermentation as compared with normal beer fermentation. Therefore, we investigated whether SICD occurs during the fermentation of bottom-fermenting yeast under anaerobic conditions (Yoshimoto et al. 2009). We found that the viability of bottom-fermenting yeast cells decreased as the ratio of sugars to nonsugar nutrients increased (Fig. 8.1a). At the same time, the diameter of the cells decreased as the ratio of sugars to nonsugar nutrients increased, suggesting that cell-cycle control is destroyed during SICD (Fig. 8.1b). In other words, it is important to regulate nonsugar nutrients for suitable fermentation.

Cell death caused by an imbalance between sugars and nonsugar nutrients should be an important consideration in the fermentation of low-malt beer and no-malt brews. Taking account of the concentration of D-Glc for the lifespan previously described, we suggest that a balance between sugars and nonsugar nutrients is a key factor for life events such as growth, fermentation, and longevity.

We found that the bottom-fermenting yeast died in D-Glc solution under both aerobic and anaerobic conditions (Yoshimoto et al. 2009). Next, we investigated whether respiratory function is required for SICD. We isolated pet$^-$ mutants from wild-type bottom-fermenting yeast and showed that these mutant yeast cells died rapidly in D-Glc solution. These results suggest that mitochondrial respiratory function is not required for SICD (Yoshimoto et al. 2009).

8.1.1.3 Pathways Involved in SICD

It is important to determine how yeast cells die in SICD. A microarray analysis was performed on bottom-fermenting yeast cells incubated in 10 % D-Glc solution or water at 20 °C for 24 h. It was found that genes related to the Ras/cAMP signal transduction pathway were more highly induced in cells incubated in D-Glc solution than in those incubated in water. Genes that function upstream of the glycolysis pathway and TCA cycle were also highly induced in D-Glc solution. By contrast, expression of the genes functioning downstream of the glycolysis pathway and those related to protein synthesis was lower in cells incubated in D-Glc solution than in those incubated in water. In addition, the expression of many genes involved in cell-cycle control was affected (Fig. 8.2). On the basis of the transcriptome analysis, in the presence of consumable sugars, the yeast cells took in these sugars and started glycolysis. Although genes functioning upstream of glycolysis and TCA cycle were induced, genes functioning downstream of glycolysis were not induced, resulting in an imbalance in sugar metabolism. Under these conditions, although protein synthesis was decreased, some cell-cycle regulatory genes, including those in the Ras/cAMP signal transduction pathway, were activated and cell division was induced. As a result, the yeast cells would die because of an imbalance at the cell-cycle checkpoint.

Yeast has other degradation systems: namely, the ubiquitin/proteosome and autophagy systems. The ubiqutin/proteosome system is used for protein recycling, as well as protein degradation. Autophagy, which is induced by nitrogen starvation, is used to degrade proteins in the vacuole; the amino acids from the degraded protein are then used for yeast cell growth. Autophagy also includes mitophagy, which is a dynamic degradation system by which organella including mitochondria are degraded in the vacuole. It is possible that autophagy is one of the pathways of SICD. Moreover, formic acid and acetic acid induce apoptosis-like cell death (Du et al. 2008; Guaragnella et al. 2011), and phosphate and succinic acid have been reported to inhibit SICD (Lee et al. 2011), suggesting that there is a relationship between organic acids and SICD.

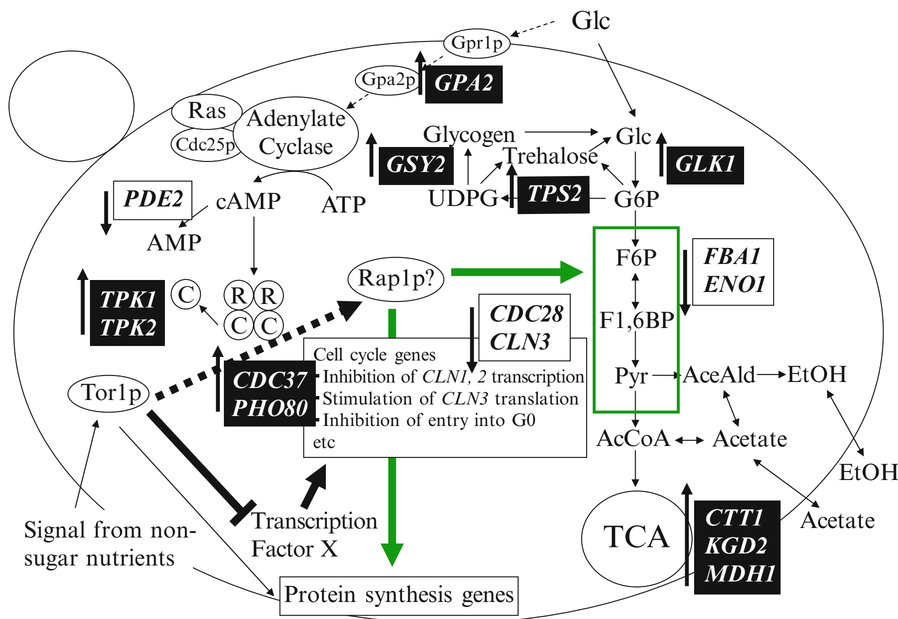

Fig. 8.2 Cartoon of metabolic pathways in yeast summarizing the results from microarray analysis of gene expression in yeast cells incubated in Glc10 and water. Genes described in *italic* and *outlined characters* with *upward arrow* show increased expression in Glc10 as compared with water, and genes described in *italic font* and *parentheses* with *downward arrow* show decreased gene expression

8.1.2 Sugars in the Wort and Fermentative Ability of Bottom-Fermenting Yeast

Beer wort contains various kinds and concentrations of sugars (Table 8.1). In this section, we describe the sugar fermentation ability, genomic structure, and environmental stress responses of the bottom-fermenting yeast *S. pastorianus*. By a comparison of the genome and ESTs between bottom-fermenting yeast and the laboratory yeast S288C, bottom-fermenting yeast was found to possess the *MAL* gene cluster involved in transport and utilization of Mal at subtelomeric regions (Jespersen et al. 1999), in addition to bottom-fermenting yeast-specific genes encoding transporters of Frc (Goncalves et al. 2000) and maltotriose (Dietvorst et al. 2005), which are not present in the genome of S288C. These results are consistent with the fact that beer wort contains abundant Mal and maltotriose as compared with Glc, and bottom-fermenting yeasts that use Mal and maltotriose efficiently to produce beer have been selected until now.

When different kinds of sugars are present in the media, all yeast including bottom-fermenting yeast first utilize monosaccharides such as Glc and sucrose,

Table 8.1 Sugar composition of a typical wort

Sugar	Amount (%)
Hexose	7–9
Sucrose	3
Maltose	43–47
Maltotriose	11–13
Dextorin (low MW)	6–12
Dextorin (high MW)	19–24
Pentosan	3–4
β-Glucan	0.2–0.3

which are easy to ferment. Next, Mal and maltotriose are used; however, oligosaccharides that are more complex than tetrasaccharides are barely fermented. With respect to monosaccharides, wort mainly contains Glc, although some Frc is present. These monosaccharides are transported into the cells by hexose transporters (HXT), and are used to produce ethanol through glycolysis and a large amount of ATP through the TCA cycle and respiratory chain reaction. Frc is also transported into the cells via a transporter encoded by *FSY1* (Goncalves et al. 2000). With respect to sucrose utilization, either invertase is secreted and degrades sucrose to Glc for uptake, or sucrose is transported into the cell by an Agt1p transporter that dose not transport Glc and degraded to Glc intracellularly (Alves et al. 2008). The *MAL* gene cluster that comprises *MALX1*, *MALX2*, and *MALX3* encoding maltase, a Mal repressor, and a Mal transporter, respectively, is related to Mal utilization (Jespersen et al. 1999). In addition, maltotriose is transported by Mtt1p (Dietvorst et al. 2005), and Agt1p (Alves et al. 2008). Mtt1p also transports Mal at low affinity. Bottom-fermenting yeast cannot utilize oligosaccharides, probably for lack of an oligosaccharide transporter rather than the lack of a degradative enzyme (Zastrow et al. 2001).

Notably, *FSY1* is conserved among various *Saccharomyces* species (de Sousa et al. 2004), in addition to *Kluyveromyces* and *Zygosaccharomyces* species (Diezemann and Boles 2003; Pina et al. 2004). Because these yeast species would use smaller amount of sugar as rapidly as possible under severe wild conditions, they have evolved to possess these Frc transporters to survive

8.1.3 *The Effect of Differences in Sugar on Ethanol Fermentation of Bottom-Fermenting Yeast*

In the production of low-malt alcohol beverages, liquid sugars are usually used to supplement the lack of carbohydrates. Because liquid sugars composed of monosaccharides such as Glc and Frc, and disaccharides including Mal, are used under

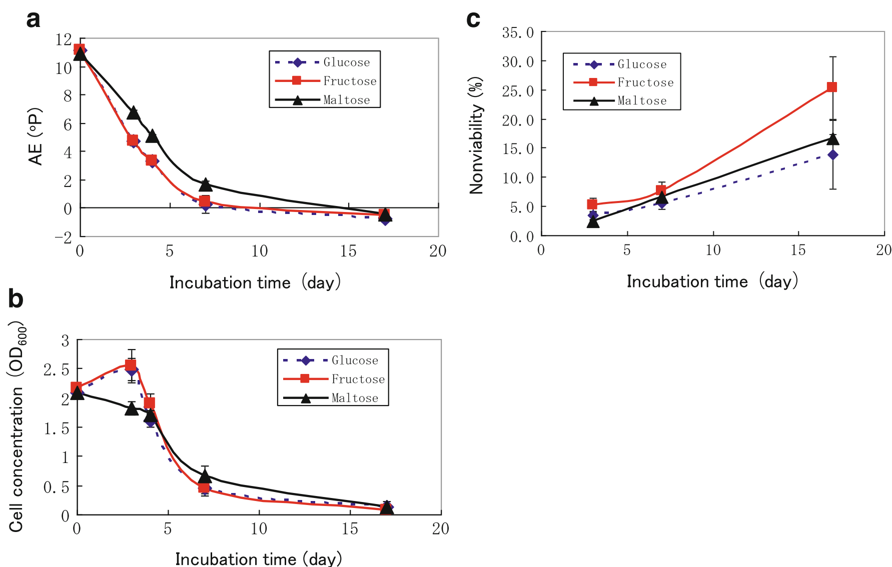

Fig. 8.3 Fermentation of the bottom-fermenting yeast in the YNB medium containing Glc, Frc, or Mal. **a** Apparent extract (AE) of the medium; **b** optimal density at 600 nm yeast cells; **c** nonviability of yeast cells floating in the tube. Yeast cells were incubated in 1 1 0.67 % YNB medium containing 10 % Glc (*diamonds*), Frc (*squares*), or Mal (*triangles*) in the EBC tube at 20 °C. Data are mean ± SD of at least three determinations

some conditions, we investigated the effect of these sugars on the fermentation of bottom-fermenting yeast (Yoshida et al. 2013). The fermentation ability and viability of the yeast cells were investigated in YNB medium containing Glc, Frc, or Mal as the sole carbon source. There was no significant difference in the fermentation ability of the bottom-fermenting yeast between the Glc and the Frc media, but its fermentation ability in the Mal medium was lower than that in Glc medium. By contrast, the viability of the yeast cells was lower in the Frc medium than in the Glc or Mal medium (Fig. 8.3). Furthermore, the bud size of the yeast cells was smaller in the Frc medium. Next, we carried out a microarray analysis of the yeast cells in the Glc and Frc media (Table 8.2). Many genes related to sugar metabolism including glycolysis were upregulated in the Frc medium as compared with the Glc medium. In addition, genes related to ergosterol biosynthesis, ribosome biogenesis, and transcription, many of which are essential genes, were downregulated in the Frc medium (Table 8.2). These results indicate that, as the expression level of these essential genes is lowered and sugar metabolism is activated in the Frc medium, the balance of cell-cycle control is destroyed and the viability of the cells is decreased (Yoshida et al. 2013). On the other hand, because the fermentation rate was lower in the Mal medium than in the Glc medium, the metabolic rate of the cells and the

Table 8.2 Microarray analysis of yeast cells incubated in medium containing various sugars

Gene name	Frc/Glc	Glc/Sor	Function
SUC2	1.71	9.17	Invertase
HXK1	1.68	18.38	Hexokinase
MAL12	1.56	10.03	α-Glucosidase
HXK2	1.49	9.83	Hexokinase
DAK2	1.49	2.40	Dihydroxyacetone kinase
YNR073C	1.48	8.79	Putative mannitol dehydrogenase
IMA2	1.40	3.79	Isomaltase
IMA3	1.40	3.84	Isomaltase
GSY1	1.37	5.49	Glycogen synthase
YFL052W	1.29	3.68	Putative zinc cluster protein
PGM2	1.26	11.69	Phosphoglucomutase
GLC3	1.24	6.59	Glycogen branching enzyme
YPR196W	1.23	3.06	Putative transcription factor
RTC2	1.23	2.66	Restriction of telomere capping
EMI2	1.19	7.03	Early meiotic induction
BDH1	1.19	6.66	Butanediol dehydrogenase
ADH4	1.40	0.43	Alcohol dehydrogenase
AST1	1.12	0.33	ATPase-stabilizing
*ERG1**	0.66	1.96	Ergosterol biosynthesis
ERG5	0.66	2.56	Ergosterol biosynthesis
*ERG25**	0.70	2.48	Ergosterol biosynthesis
CYB2	0.72	18.76	Cytochrome B
ERG6	0.79	2.03	Ergosterol biosynthesis
ARA1	0.86	3.16	Arabinose dehydrogenase
*ERG9**	0.89	3.83	Ergosterol biosynthesis
CMR3	0.77	0.55	Putative zinc finger protein
JID1	0.80	0.65	DnaJ protein
*YEF3**	0.82	0.16	Translation elongation factor 3
YML108W	0.83	0.59	Unknown
*RRS1**	0.84	0.44	Regulator of ribosome synthesis
YEH1	0.85	0.29	Steryl ester hydrolase
NUP84	0.85	0.58	Nuclear pore complex subunit
*NOP53**	0.88	0.35	Nucleolar protein
HEF3	0.90	0.37	Translational elongation factor EF-3
*RIX1**	0.93	0.25	Ribosome export

Frc/Glc indicates the relative gene expression ratio of yeast cells incubated in YPF10 (1 % yeast extract, 2 % polypeptone, and 10 % fructose) and YPD10 (1 % yeast extract, 2 % polypeptone, and 10 % glucose) at 20 °C for 4 days. Glc/Sor indicates the relative gene expression ratio of yeast cells incubated in 10 % glucose and 10 % sorbitol at 20 °C for 24 h

Asterisk indicates that the gene is essential gene for cell growth in YPD medium

Frc fructose, *Glc* glucose, *Sor* sorbitol

viability of the cells were lower in the Mal medium than in the Glc medium. These results indicate that we should select a kind of liquid sugars depending on the sugar composition and cost.

In a study where yeast cells were incubated in Mal medium, the Mal transporters were immediately degraded via endocytosis and the ubiqutin/proteosome system after the addition of Glc (Hatanaka et al. 2009). This observation was used to select a mutant whose Mal transporter was resistant to degradation caused by the addition of Glc. The mutant was found to be resistant to 2-deoxyglucose, which is a glucose analogue (Hatanaka et al. 2009). This mutant should be useful for brewing beer-like alcoholic beverages in wort containing liquid sugars because it can use both Glc and Mal without delaying fermentation. At present, it is necessary to determine the optimal fermentation temperature and the amount of pitching yeast cells for which the Mal transporters will still be intact after Glc is completely consumed.

8.1.4 Effect of Minerals and Vitamins on Alcohol Fermentation

It has been reported that yeast cells alter their metabolism in response to mineral availability. Among the various minerals, cations including zinc, manganese, magnesium, calcium, copper, potassium, and iron are components of enzymatic activity and are essential for cell growth (Eide et al. 2005). Apart from zinc, these cations are usually abundant in beer wort; in low-malt wort, by contrast, yeast cells show inhibition of growth and fermentation because of a deficiency of cations. In particular, with respect to zinc, yeast cells show growth inhibition, delayed fermentation, and an increase of off-flavors including acetaldehyde when the concentration of zinc is less than 0.1 ppm (Jacobsen et al. 1981). Therefore, it is very important to maintain a stable concentration of zinc in wort by altering the way in which wort is made. A deficiency of potassium has also been reported to cause an increase in ROS, outflow of phosphatidylserine, alteration of chromatin structure, and fragmentation of DNA and the nucleus, resulting in cell death (Lauff and Santa-Maria 2010). Moreover, when the concentration of potassium in the medium is decreased, the concentration ratio of intracellular sodium to intracellular potassium increases, and the yeast cells produce more acetic acid as a product of incomplete fermentation (Kawakubo et al. 2012).

With regard to vitamins, most bottom-fermenting yeast strains require biotin for cell growth (Ohsugi and Imanishi 1985). In addition, some top-fermenting yeast strains require thiamine (Hammond 1993). Nearly all vitamins except biotin and thiamine are abundant in normal wort. There are many reports about thiamine in yeast and the enzymes that require thiamine, including enzymes of glycolysis and TCA as a coenzyme (Hohmann and Meacock 1998). If thiamine levels are decreased in brewing, any enzymatic activity requiring thiamine as a coenzyme will be lowered and consequently more pyruvic acid and 2-oxyoglutaric acid will be produced

(Kawakubo et al. 2012). In the process of making of wort, it is most important to maintain the levels of thiamine needed for the fermentation of low-malt beer and beer-like alcoholic beverages.

8.1.5 Bottom-Fermenting Yeast-Specific Genes and Their Relationship to Environmental Stress Responses

Comparison of laboratory yeast with bottom-fermenting yeast shows that many genes are specific to bottom-fermenting strains, including *RTM1*, which provides tolerance to molasses (Ness and Aigle 1995). This gene is conserved among industrial yeasts including bottom-fermenting yeast. It is possible that bottom-fermenting yeast might utilize sugars present in the environment, for example, molasses, as efficiently as possible via the function of Rtm1p. The tyrosine transporter *TAT3* is also a bottom-fermenting yeast-specific gene (Omura et al. 2007). Because few amino acids are available in the environment, yeast cells may have evolved to possess *TAT3* to enable them to use amino acids as efficiently as possible.

The *AMI1* gene has also been isolated by EST analysis of the bottom-fermenting yeast (Yoshida et al. 2007a, b). *AMI1* was deduced to encode a protein with amidase-homologous regions, but with unknown function. On the basis of overexpression of the *AMI1* gene in laboratory yeast, Ami1p was found to be involved in morphogenesis of the vacuole. In addition, metabolomic analysis of yeast cells overexpressing *AMI1* showed that Ami1p is related to the metabolism of amino acids. Because basic amino acids such as histidine, arginine, and lysine are stored in the vacuole, it is possible that Ami1p is also related to vacuolar function. Furthermore, nicotinic acid was found to be dramatically decreased in *AMI1*-overexpressing cells; thus, Ami1p has a relationship with the metabolism of NAD, which is important in the regulation of redox balance. Taken together, these observations suggest that, by breeding yeast cells with a focus on *AMI1*, a yeast strain that is tolerant to stresses and changes in redox balance might be obtained.

8.2 Conclusion

In the brewing of low-malt beer and no-malt brews, the lack of nutrients often causes a delay in fermentation and produces an “off-flavor,” problems that have rarely occurred in standard beer brewing. Yeast cells respond to environmental nutrients, including carbon and nitrogen sources, by regulating their intracellular metabolism. For yeast cells that are sensitive, such nutrient stresses cause an unbalanced metabolism and lead to the production of intracellular ROS. It is highly important to avoid ROS and to reduce the stress load for yeast cells during the production of alcoholic beverages. Thus, to keep nutrient stresses to a minimum and thereby produce beer, low-malt beer, and no-malt beer with a good taste, the cellular responses of yeast cells should be suitably regulated.

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