Ethanol Induces Autophagy Regulated by Mitochondrial ROS in *Saccharomyces cerevisiae*

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**Introduction**

In order to remove dysfunctional and unneeded constituents and recycle intracellular nutrients, macro-autophagy (hereinafter referred to as autophagy) encapsulates and transfers cytoplasmic material to the vacuole or lysosome for degradation [1]. Autophagy-related genes (ATGs) originally were identified in the budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) [2]. The most important of these ATGs was the ubiquitin-like protein ATG8 that attached to lipid phosphatidylethanolamine (PE) on the outside of emerging phagophores. The ATG8-PE adduct contributed to expand and seal the vesicle and recruit specific cargo [3,4]. Therefore, upregulation of *ATG8* expression is implicated in the induction of autophagy [5]. Moreover, the initiation of autophagy is regulated by Ser/Thr kinase *ATG1* and its accessory regulator *ATG13* in fungi [6–10]. It has been proven that the expression of *ATG1* is also an indicator of autophagy levels [11].

During the early stage of fermentation, sufficient nitrogen helps the proliferation and growth of yeast. The further consumption of nitrogen then results in sugar fermentation. Therefore, nitrogen limitation has been very common in winemaking. It was also well known that nitrogen starvation could induce autophagy [12,13]. However, autophagy was induced early in wine fermentation in a nitrogen-replete environment [14]. About the function of autophagy during fermentation, it has been reported that recycling of iron via autophagy is critical for the transition from glycolytic to respiratory growth [15]. Therefore, the function of autophagy and the exact factors inducing it are still unknown during fermentation.
More and more studies showed that reactive oxygen species (ROS) were associated with fermentation [16–19]. ROS were a possible part of the yeast response to a variety of stress factors occurring during most fermentations [20]. ROSs, mainly including the superoxide anion (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$), are generated by cells during normal metabolism [21]. Many studies have shown that ROS play a vital role in regulating autophagy [21–23]. However, which molecule of ROS regulated autophagy was still being debated. For example, it had been proven that H$_2$O$_2$ had the ability to activate autophagy [21]. However, Chen et al. showed that O$_2^-$ could induce autophagy [24]. In addition, other ROS except O$_2^-$ possibly induced autophagy [12]. Therefore, the exact ROS molecule regulating autophagy still needed to be researched in the future.

Ethanol production is likely to remain one of the most important biotechnological products well into the future, with the continued manufacture of spirits, wine, sake, beer, and so on [25]. But ethanol accumulation in the culture broth is still a significant stress factor during fermentation. Although S. cerevisiae is highly ethanol tolerant high ethanol inhibits cell growth and viability [26–28], and the mechanism of ethanol tolerance is still unclear. The aim of the current study was to obtain new insight into the response of yeast under ethanol stress conditions and to clarify the complicated relationship between ROS and autophagy during fermentation. To achieve these aims, ROS production and the expression of ATG8 or ATG1 genes were evaluated in wild type and mutant yeast strains grown on a medium with ethanol.

Materials and Methods

Strain and Maintenance Medium

The wine yeast strain BY4742 (S. cerevisiae) was supplied by Pro. Zhiwei Huang of East China University. The mutants atg1 and atg8 were purchased from Invitrogen (Carlsbad, CA). The mutants were constructed by hygromycin stripe homologous replacement of the mutation genes of ATG1 or ATG8 and selected by yeast peptone dextrose agar (YPD) medium with 200 μg/ml G418. Wild-type yeasts could not grow on YPD medium containing G418 whereas mutant cells could survive. The mutants were preserved on YPD medium containing G418 in order to prevent back mutation. The wild-type yeast maintained at 4°C overnight in 250-ml flasks containing 100 ml of YPD medium. The flasks were incubated at 30°C in an orbital shaker set at 180 rpm. The experimental cultures were inoculated with 5 × 10$^5$ CFU/ml of starter culture. Fermentation was carried out in 500-ml flasks filled to two-thirds of their volume and maintained at 30°C in an orbital shaker at 180 rpm, according to the methodology [12]. With distilled water as a control, 10% ethanol made by adding absolute ethyl alcohol was used as ethanol stress. 100 mM 2-methoxyestradiol (2-ME) was made by adding absolute ethyl alcohol was used as ethanol stress. 100 mM 2-methoxyestradiol (2-ME) was stored in dimethylsulfoxide (DMSO) and final concentration was 100 μM. 1 M 3-amino-1,2,4-triazole (3-AT) was stored in double distilled water and final concentration was 2 mM. 10 mM rapamycin (Rapa) dissolved in ethanol and stored in TritonX-100 and final concentration was 5 μM. 3-methyladenine (3-MA) was stored in PBS solution and final concentration was 10 μM. 2.5 mM glutathione (GSH) and 10 mM N-acetyl-cysteine (NAC) were both dissolved in double distilled water. 100 mM antimycin A (Anti A) and rotenone (Rote) were both dissolved in DMSO and final concentrations for both were 5 mM. Each flask was closed with a rubber stopper. Prior to sampling, the flasks were stirred to ensure homogeneity.

Assessment of Intracellular H$_2$O$_2$ and O$_2^-$

H$_2$O$_2$ was monitored with 2,7-dichlorodihydrofluorescein diacetate (DCHF-DA) (Sigma) essentially as described elsewhere [30], with minor adaptations. Briefly, yeast (10$^7$ cells/ml) was stained by 20 μM PI for 10 min at 37°C and then observed by fluorescence microscope. The fluorescence intensity was measured by fluorescence 96-well microplate (excitation wavelength 540 nm, emission wavelength 590 nm). The death of cells was with high red fluorescence.

Assessment of Intracellular H$_2$O$_2$ and O$_2^-$

DCFH-DA didn’t fluoresce but could transmembrane freely. The dye could be hydrolyzed by esterase to 2, 7-dichlorofluorescin (DCFH) which was arrested in an actively respiring cell. DCFH was oxidized by ROS to a fluorescent compound DCF in the yeast cells overnight in 250-ml flasks containing 100 ml of YPD.
qRT-PCR

Wild-type yeast was treated with a different reagent from ethanol. RNA was obtained from the sample which was periodically collected. RNA was extracted using Trizol (Invitrogen, Carlsbad, USA) standard procedures. Heat shock treatment (15 min at 42°C followed by 3 min at 95°C) was for cellular disruption. Total RNA (250 ng) was reverse transcribed using a SuperScript III Platinum Two-Step Real-Time Quantitative RT-PCR Kit with SYBR green from Invitrogen. One microliter of the reverse-transcribed RNA was used as a template to amplify the genes, using primers to the ATG8 gene (sense, 5-TTGCTGACAGGTAAGAATAGG-3; antisense, 5-ATCAACGCGAGTAAAGG-3), ATG1 gene (sense, 5-TACTGTGCTCTTGGGGACCTA-3; antisense, 5-CCGACGCTAACTGCTGGAGT-3) and the ACT1 gene (sense, 5-GGATTCTGAGGTTGCTGCTT-3; antisense, 5-TGACCCATA CCGACCATGATAACG-3). The expression of the ATG8 and ATG1 genes was assessed by qRT-PCR in a StepOnePlus system (ABI). Results were normalized to the reference gene ACT1. The data were analyzed by applying the Livak method or the 2^ΔΔCT method [31]. The method was as follows: ΔΔCT = (CT(target gene) − CT(reference gene)) test − (CT(target gene) − CT(reference gene)) calibrator, where CT is the threshold cycle.

Statistical Analysis

Data were reported as mean values of at least three independent assays and presented as means ± standard deviations (SD). Statistical analyses were carried out using a Student’s t-test. P values of less than 0.05 or 0.01 were considered statistically significant, or very significant which were shown as ** and *** at the tops of the columns in the figures.

Results

Ethanol Stress Induced Autophagy

Ethanol was the main metabolite of S. cerevisiae during the fermentation process [25]. In order to confirm the effect of ethanol stress on autophagy, the expression levels of the ATG1 and ATG8 genes were measured by qRT-PCR. Based on the preliminary experiment, the peak expression value of ATG8 and ATG1 was at 4 h. Therefore, expression of the ATG8 and ATG1 genes was detected at 4 h. The results showed that the expression levels of the ATG1 and ATG8 genes were very significantly increased during ethanol stress (p < 0.01) (Fig. 1). In detail, the expression levels of

Fig. 1. Ethanol-induced expression of the ATG1 and ATG8 genes. Normalized fold expression levels of the ATG1 and ATG8 genes were evaluated by qRT-PCR in S. cerevisiae treated with 10% ethanol for 4 h. ACT1, encoded actin, was used as internal reference. Values indicated mean ± standard deviation (n = 3). Statistical significance (**, p < 0.01) was determined by a Student’s t-test and was shown as the statistical significance between ethanol with control. Con: control, Eth: treated with ethanol.

Fig. 2. Cell death of mutants was higher than that of wild type under ethanol stress. Wild type, atg1 and atg8 treated with 10% ethanol for 2 h were stained with PI. Then all strains were analyzed by fluorescence microscope (A) and fluorescence microplate reader (B). Values indicated mean ± standard deviation from (n = 6). Statistical significance (**, p < 0.01) was determined by a Student’s t-test and was shown as the statistical significance between ethanol with control. Con: control, Eth: treated with ethanol.
ATG1 and ATG8 were increased to 153.5% and 252.6% by ethanol stress, respectively (Fig. 1). Therefore, we confirmed that ethanol stress indeed induced autophagy.

**Autophagy Contributed to Survival of S. cerevisiae from Ethanol Stress**

The above results revealed that ethanol stress markedly induced autophagy. In order to clarify the role of autophagy during ethanol stress, the death rates of the wild type, atg1 and atg8 strains were assessed by PI staining. The results showed that there was no difference between the wild type and the mutant strains during their growth on YPD medium (Fig. 2). However, ethanol stress increased the cell death rate of all strains (Fig. 2). Compared with wild-type yeast, the cell death of atg1 and atg8 mutants was dramatically enhanced by ethanol stress. Therefore, this indicated that autophagy protected yeast cells from ethanol stress.

**Ethanol Induced Production of H$_2$O$_2$ and O$_2^-$ in S. cerevisiae**

To evaluate whether ROS accumulation was accompanied by ethanol stress, ROS production was measured by fluorescence staining and flow cytometry. Production of H$_2$O$_2$ and O$_2^-$ reached highest at 2 h according to the preliminary experiment. Therefore, production of H$_2$O$_2$ and O$_2^-$ was detected at 2 h. The production of H$_2$O$_2$ and O$_2^-$ was induced in both cases by ethanol stress (Fig. 3A). H$_2$O$_2$ production in atg1, atg8 mutants was higher than that in wild type yeast under ethanol treatment for 24 h. In addition, the atg1 mutant had the highest H$_2$O$_2$ concentration (Fig. 3B). Similarly, the O$_2^-$ content in mutant cells was also higher than that in wild type yeast cells (Fig. 3C). However, the difference in O$_2^-$ production between wild type and mutants was not greatly different than that observed in H$_2$O$_2$. Therefore, these results demonstrated that ethanol stress contributed to the accumulation of ROS and autophagy had the ability to eliminate ROS.

![Fig. 3. Ethanol induced more H$_2$O$_2$ and O$_2^-$ in mutants than in wild type.](image-url)

Production of H$_2$O$_2$ and O$_2^-$ in wild type treated with 10% ethanol for 2 h were stained by DCFH or DHE (A). Production of H$_2$O$_2$ (B) and O$_2^-$ (C) in wild type, atg1 and atg8 treated with 10% ethanol for 24 h were stained by DCFH (B) or DHE (A). All strains were analyzed by flow cytometry. Black bars represent 10 µm.
Autophagy Induced by Ethanol Stress Depended on ROS

The above results show that ethanol not only induced the expression of ATG1 and ATG8 but also enhanced the production of H$_2$O$_2$ and O$_2^\cdot$. In addition, accumulating evidence shows that moderate ROS as signal molecules regulated autophagy [12, 21, 23]. Therefore, in order to clarify the relationship between autophagy and ROS, reducing agents were used to change ROS levels. Obviously, both GSH and NAC reducing agents decreased very significantly the production of H$_2$O$_2$ and O$_2^\cdot$ under ethanol stress (Figs. 4A and 4B). The production of H$_2$O$_2$ was reduced to 55.4% and 44.0% by GSH and NAC (Fig. 4B). GSH and NAC decreased the production levels of O$_2^\cdot$ to 43.5% and 29.2% (Fig. 4B). Simultaneously, GSH and NAC markedly decreased the gene expression of ATG1 and ATG8 (Fig. 4C). For example, expression of ATG1 and ATG8 was decreased to 54.7% and 38.8% by GSH. NAC lowered ATG1 and ATG8 expression to 55.6% and 19.3%. Moreover, in wild type, GSH and NAC also decreased the production of H$_2$O$_2$ and O$_2^\cdot$ in atg1 and atg8 mutants (Fig. S1).

In spite of moderate ROS acting as a signal, excess ROS oxidized nearby biological macromolecules including DNA, proteins and lipids [32–36]. In order to protect themselves from oxidative damage, cells used enzymes to eliminate ROS, such as catalase (CAT) in cytoplasm and manganese superoxide dimutase (Mn-SOD) in mitochondria [37–39]. Therefore, 2-ME and 3-AT were used as the inhibitors of Mn-SOD and CAT, to increase of O$_2^\cdot$ and H$_2$O$_2$ levels in cells. The results showed that 2-ME increased the production of H$_2$O$_2$ and O$_2^\cdot$ (Figs. 5A and 5B). 3-AT only induced the levels of O$_2^\cdot$ to 159.0% but had no effect on the levels of H$_2$O$_2$ (Fig. 5B). 2-ME and 3-AT both activated the expression of ATG1 and ATG8 genes under ethanol stress (Fig. 5C). In detail, the expression of ATG1 and ATG8 was elevated to 216% and 183.9% by 2-ME. 3-AT increased the expression levels of ATG1 and ATG8 to 185.6% and 152.1%, respectively. The production of H$_2$O$_2$ and O$_2^\cdot$ in atg1 and atg8 mutants was also induced by 2-ME and 3-AT (Fig. S2).

To further verify that ROS regulated autophagy in yeast under ethanol stress, the inhibitor of autophagy (3-MA) and the inducer of autophagy (Rapa) were added in the medium under ethanol stress. The results showed that Rapa very significantly promoted the production of H$_2$O$_2$ and O$_2^\cdot$ in wild-type yeast (Figs. 6A and 6B) and evoked the expression of ATG1 and ATG8 (Fig. 6C). On the contrary, 3-MA dramatically decreased only the content of H$_2$O$_2$ (Figs. 6A and 6B) and reduced the expression of ATG1 and ATG8 (Fig. 6C). Therefore, autophagy was dependent on levels of H$_2$O$_2$ under ethanol stress. In mutants, Rapa

![Fig. 4](image)

**Fig. 4.** GSH and NAC decreased production of H$_2$O$_2$ and O$_2^\cdot$ and expression of ATG1 and ATG8 genes in *S. cerevisiae* under ethanol stress.

(A) Production of H$_2$O$_2$ and O$_2^\cdot$ in wild type treated with 10% ethanol for 2 h was stained by DCFH or DHE, respectively. Black bars represent 10 µm. Relative fluorescence of DCFH and DHE is shown in (B). Values indicated mean ± standard deviation (n = 6). GSH or NAC decreased expression of ATG1 and ATG8 (C). Normalized fold expression levels of ATG1 and ATG8 were evaluated by qRT-PCR in *S. cerevisiae* under ethanol stress for 4 h. ACT1, encoded actin, was used as internal reference. Values indicated mean ± standard deviation (n = 3). Statistical significance (**, p < 0.01) was determined by a Student’s t-test.
Fig. 5. 2-ME and 3-AT increased production of H$_2$O$_2$ and O$_2^{-}$ and expression of ATG1 and ATG8 genes in *S. cerevisiae* under ethanol stress. (A) Production of H$_2$O$_2$ and O$_2^{-}$ in wild type treated with 10% ethanol for 2 h was stained by DCFH or DHE, respectively. Black bars represent 10 um. Relative fluorescence of DCFH and DHE is shown in (B). Values indicated mean ± standard deviation ($n = 6$). 2-ME and 3-AT increased expression of ATG1 and ATG8 (C). Normalized fold expression levels of ATG1 and ATG8 were evaluated by qRT-PCR in *S. cerevisiae* under ethanol stress for 4 h. ACT1, encoded actin, was used as internal reference. Values indicated mean ± standard deviation ($n = 3$). Statistical significance (**, $p < 0.01$) was determined by a Student’s *t*-test.

Fig. 6. Rapa and 3-MA regulated production of H$_2$O$_2$ and O$_2^{-}$ and expression of ATG1 and ATG8 genes in *S. cerevisiae*. (A) Production of H$_2$O$_2$ and O$_2^{-}$ in wild-type yeast with 10% ethanol for 2 h was stained by DCFH or DHE, respectively. Black bars represent 10 µm. Relative fluorescence of DCFH and DHE is shown in (B). Values indicated mean ± standard deviation ($n = 6$). Rapa and 3-MA regulated expression of ATG1 and ATG8 (C). Normalized fold expression levels of ATG1 and ATG8 were evaluated by qRT-PCR in *S. cerevisiae* under ethanol stress for 4 h. ACT1, encoded actin, was used as internal reference. Values indicated mean ± standard deviation ($n = 3$). Statistical significance (**, $p < 0.01$) was determined by a Student’s *t*-test.
and 3-MA both promoted the production of H$_2$O$_2$ and O$_2^\cdot$ under ethanol stress (Fig. S3). Rapa and 3-MA did not change the levels of autophagy in the mutants. Accumulation of more H$_2$O$_2$ and O$_2^\cdot$ by Rapa and 3-MA may be attributed to their alternative function.

**ROS Induced by Ethanol Stress Mainly Originated from Mitochondria**

Since we found that ROS were implicated in autophagy under ethanol stress, the source of ROS was also explored in this study. The respiratory complexes I, II, and III of the mitochondrial electron transport chain (mtETC) were reported to be the major production sites of O$_2^\cdot$ [40, 41]. Anti A and Rote, as the inhibitors of complex III and complex I of the mtETC, were used to increase ROS in mitochondria. The results showed that Anti A and Rote both undoubtedly increased production of H$_2$O$_2$ and O$_2^\cdot$ (Figs. 7A and 7B) and promoted the expression of ATG1 and ATG8 genes under ethanol stress (Fig. 7C). In detail, production of H$_2$O$_2$ was increased to 212.8% and 257.4% by Rote and Anti A, respectively. Rote and Anti A raised production of O$_2^\cdot$ to 225.0% and 299.2%, respectively. Expression of ATG1 and ATG8 was increased to 496.4% and 431.9% by Anti A. Rote raised ATG1 and ATG8 expression to 348.5% and 299.3%. These findings indicated that ROS derived from the mtETC-regulated autophagy under ethanol stress. Therefore, ROS accumulation in yeast attributable to H$_2$O$_2$ and O$_2^\cdot$ derived from the mtETC. In the mutants, Anti A and Rote also increased production of H$_2$O$_2$ and O$_2^\cdot$ (Fig. S4). The results further proved that production of H$_2$O$_2$ and O$_2^\cdot$ was mainly caused by the mtETC under ethanol stress.

**Discussion**

The budding yeast had to escape from nitrogen deficient conditions during most alcoholic fermentations. And it was well known that autophagy could be induced by deficient nitrogen during fermentation [12, 13, 42]. However, Piggott et al. had demonstrated that autophagy was induced at the early stage of wine fermentation in a nitrogen-replete environment [14]. Therefore, it was suggested that autophagy might be triggered by other factors that arose during the early stage of fermentation. Besides that, it had been proven that autophagy was induced in second-fermentation yeasts during sparkling wine production [43].

Cost-effective ethanol production depended on rapid and high-yielding conversion of carbohydrate to ethanol. Therefore, ethanol accumulation in the culture broth...
appeared at the early stage of fermentation. It was well known that the yeast had the property of ethanol tolerance. However, the mechanism of the ethanol tolerance was not clear. In the current study, the results showed that autophagy was induced by ethanol stress (Fig. 1). And compared with atg1 and atg8 mutants, wild type contributed to cell survival from ethanol stress (Fig. 2). Therefore, yeast was likely surviving ethanol injury by means of increasing the levels of autophagy to clear away the damaged organelles.

**Ethanol Stress Induced ROS in S. cerevisiae**

CTT1, encoding a kind of cytosolic CAT, was found to be highly expressed only in ethanol-tolerant sake yeast mutants or after exposure to ethanol [44]. Therefore, eliminating of ROS by CTT1 helped sake yeast survive ethanol stress. Otherwise, ROS were normally induced during fermentation [18, 19]. However, the exact relationship between ROS and ethanol stress was still unclear. In this study, ROS production in wild-type yeast (Figs. 4–7) and mutant cells (Figs. S1, S2, and S4) was induced by ethanol stress. However, the role of ROS was still unknown under ethanol stress.

Compared to wild-type yeast, the atg1 and atg8 mutants had higher production of H$_2$O$_2$ and O$_2^-$ under ethanol stress (Fig. 3). These results were in agreement with previous reports. For instance, it had been shown that the mutants atg2 and atg5 accumulated high levels of H$_2$O$_2$ [45]. Thus, autophagy was in favor of eliminating ROS under ethanol stress. In addition, atg1 and atg8 mutants had a higher cell death rate than wild-type yeast (Fig. 2). These findings suggest that wild-type yeast markedly decreased cell death under ethanol by eliminating ROS by autophagy.

**ROS Derived from mtETC Regulated Autophagy under Ethanol Stress**

Mounting evidence suggested that ROS might play a role in the control of autophagy [21–23, 46, 47]. And we had proven that ethanol activated production of H$_2$O$_2$ and O$_2^-$ (Fig. 3A) and autophagy (Fig. 1). In addition, GSH and NAC decreased autophagy by decreasing the release of H$_2$O$_2$ and O$_2^-$ under ethanol stress (Fig. 4). In agreement, NAC had been reported to reduce ethanol-induced autophagy [48] and also to decrease salinomycin-induced autophagy [49]. On the contrary, in our study 2-ME induced autophagy by means of increasing production of H$_2$O$_2$ and O$_2^-$ whereas 3-AT increased autophagy probably just through production of O$_2^-$ (Fig. 5). Therefore, our results revealed that H$_2$O$_2$ and O$_2^-$ regulated autophagy levels in the yeasts under ethanol stress.

Moreover, Rapa promoted the production of H$_2$O$_2$ and O$_2^-$ and increased the expression of the ATG1 and ATG8 genes (Fig. 6). However, 3-MA decreased the levels of H$_2$O$_2$ and lowered the expression of the ATG1 and ATG8 genes (Fig. 6). The difference was that both Rapa and 3-MA enhanced production of H$_2$O$_2$ and O$_2^-$ in atg1 and atg8 mutants under ethanol stress (Fig. S3). Rapa and 3-MA did not affect the level of autophagy in mutants. Alternative functions of Rapa or 3-MA maybe associated with the increased accumulation of H$_2$O$_2$ and O$_2^-$.

Although ROS have been previously reported to be implicated in autophagy, specifically which ROS were playing this crucial regulatory role was still disputed. Chen et al. considered that O$_2^-$ was the main kind of ROS inducing autophagy [24]. However, Mendes-Ferreira et al. reported that all kinds of ROS except O$_2^-$ had vital roles in regulating autophagy [12]. There were also some reports showing that autophagy was induced by H$_2$O$_2$ [21, 46]. In our study, H$_2$O$_2$ and O$_2^-$ both participated in regulating autophagy in yeast under ethanol stress.

Mitochondrion was the main source of ROS [40, 41]. In the current study, Anti A and Rote also increased the production of H$_2$O$_2$ and O$_2^-$ in atg1 and atg8 mutants under ethanol (Fig. S4). The results proved that H$_2$O$_2$ and O$_2^-$ were mainly derived from the mtETC under ethanol. Anti A and Rote enhanced the production of H$_2$O$_2$ and O$_2^-$ and activated the expression of the ATG1 and ATG8 genes in wild type (Fig. 7). Therefore, ROS derived from mitochondria had vital roles in regulating autophagy under ethanol stress. Many studies have shown that ROS from mitochondria regulated autophagy or mitophagy [39, 50–52]. For example, MITA expression modulated autophagy flux through enhancing mitochondrial ROS by increasing complex-I activity [51]. In addition, mitochondrial complex I inhibition triggered a mitophagy-dependent ROS increase [52].

Overall, ethanol stress induced autophagy and increased the production of H$_2$O$_2$ and O$_2^-$ which were originated from the mtETC, although other sources of ROS are not excluded. Under ethanol stress, the high levels of H$_2$O$_2$ and O$_2^-$ markedly induced autophagy. Subsequently, the autophagy contributed to the elimination of H$_2$O$_2$ and O$_2^-$.

Ultimately, the autophagy assisted yeast in surviving ethanol stress during fermentation.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

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