Genome-wide identification of resistance genes and transcriptome regulation in yeast to accommodate ammonium toxicity

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Abstract

Background: Ammonium is an important raw material for biomolecules and life activities, and the toxicity of ammonium is also an important ecological and agricultural issue. Ammonium toxicity in yeast has only recently been discovered, and information on its mechanism is limited. In recent years, environmental pollution caused by nitrogen-containing wastewater has been increasing. In addition, the use of yeast in bioreactors to produce nitrogen-containing compounds has been developed. Therefore, research on resistance mechanisms that allow yeast to grow under conditions of high concentrations of ammonium has become more and more important.

Results: To further understand the resistance mechanism of yeast to grow under high concentration of ammonium, we used NH4Cl to screen a yeast non-essential gene-deletion library. We identified 61 NH4Cl-sensitive deletion mutants from approximately 4200 mutants in the library, then 34 of them were confirmed by drop test analysis. Enrichment analysis of these 34 genes showed that biosynthesis metabolism, mitophagy, MAPK signaling, and other pathways may play important roles in NH4Cl resistance. Transcriptome analysis under NH4Cl stress revealed 451 significantly upregulated genes and 835 significantly downregulated genes. The genes are mainly enriched in: nitrogen compound metabolic process, cell wall, MAPK signaling pathway, mitophagy, and glycine, serine and threonine metabolism.

Conclusions: Our results present a broad view of biological pathways involved in the response to NH4Cl stress, and thereby advance our understanding of the resistance genes and cellular transcriptional regulation under high concentration of ammonium.

Keywords: Saccharomyces cerevisiae, Ammonium, NH4Cl, Genome-wide screen, Transcriptome

Background

Ammonium is a building block for amino acids, nucleic acids, polysaccharides, and other important cellular structural components in all organisms, and at the same time is also an important raw material for the synthesis of secondary metabolites such as alkaloids and polyamines [1]. Ammonium is a paradoxical nutrient ion. First, ammonium is the key nitrogen source for all life forms, including bacteria, fungi, protists, plants, and animals. Whereas the ammonium used by plants mainly comes from natural nitrogen fixation, in heterotrophic cells,
it is mainly derived from cellular metabolites \([2, 3]\).

Second, high concentrations of ammonium are toxic to organisms \([4–6]\). The downstream molecular events of \(\text{NH}_4^+\) as a nutrient component have been extensively studied; it is mainly related to biosynthesis, carbon metabolism, energy metabolism, primary nitrogen metabolism, plant hormones, cell wall stability, and signaling pathways \([7]\). However, the mechanism underlying ammonium toxicity, and how to inhibit it, are areas of intense current research \([8–10]\).

In plants, excessive \(\text{NH}_4^+\) can inhibit photosynthesis, block the growth of plant roots and leaves \([11]\), and promote the accumulation of reactive oxygen species \([12]\). When ammonium is the primary nitrogen source, it usually significantly inhibits plant growth, manifesting as a phenotype of severely inhibited root growth and chlorosis, which is a well-known manifestation of ammonium salt toxicity \([13]\). For vertebrates, high concentrations of ammonium may displace \(\text{K}^+\) and depolarize neurons, leading to the activation of NMDA-type glutamate receptor, which leads to excessive \(\text{Ca}^{2+}\) influx and subsequent cell death in the central nervous system, ultimately leading to convulsions, coma, and even death \([6]\). In addition, ammonium salts are also important environmental pollutants in the ecosystem. Excessive use of ammonium fertilizer will not only cause soil acidification, resulting in the loss of soil nutrients and the aggravation of the harm of heavy metal pollution, but also lead to secondary salinization of the soil, resulting in large-scale reductions in crop yield and deterioration of quality \([14]\).

The harm of ammonium to animals and plants has long been known, and extensively studied; however, the discovery of ammonium toxicity in \(Saccharomyces cerevisiae\) is relatively recent \([15]\). The reason for this is that yeast cells have extremely high tolerance to ammonium under normal culture conditions \([16]\). In \(Saccharomyces cerevisiae\), ammonium uptake is facilitated by ammonium permeases (Mep1, Mep2, and Mep3), which transport \(\text{NH}_4^+\) ions and conduct ATP-dependent export of protons to maintain intracellular charge balance and pH. This process can put a great burden on the energy metabolism of cells, especially when there is an excess of ammonium salts outside the cells \([17]\). With growing interest in the biosynthesis of nitrogen-containing compounds using yeast bioreactors, the mechanisms of ammonia resistance in yeast cells have also received increased attention \([18]\).

Therefore, it is particularly important to fully understand the mechanism of action of ammonium metabolism in yeast cells and to find and discover resistance genes and pathways. This could aid in the development of ways to increase cellular ammonia resistance while reducing intracellular energy expenditure.

To gain a comprehensive and in-depth understanding of the causes and mechanisms underlying ammonium toxicity in yeast cells, we performed experiments using two genome-wide strategies. First, we used the yeast gene deletion library (SGA collection) to screen \(\text{NH}_4\text{Cl}\) resistance-related mutants. Next, we analyzed transcriptome changes in yeast cells at concentrations of \(\text{NH}_4\text{Cl}\) that significantly inhibited growth. Finally, the present study reveals the key genes and cellular pathways underlying resistance to \(\text{NH}_4\text{Cl}\) in the yeast genome, which provides an experimental basis for further cultivating high ammonium resistant strains and designing schemes to reduce ammonium toxicity.

**Results**

**Genome-wide screen for \(\text{NH}_4\text{Cl}\)-sensitive mutants**

To understand the specific mechanism of yeast under \(\text{NH}_4\text{Cl}\) stress, we used the SGA-V2 library to screen for mutants that are sensitive to \(\text{NH}_4\text{Cl}\). First, we diluted the control strain \(\text{his}^\Delta\) in plates with a gradient of concentrations of \(\text{NH}_4\text{Cl}\). The growth of the strain began to be inhibited under a concentration of 500 mM \(\text{NH}_4\text{Cl}\) (Fig. S1). This is consistent with previous reports that yeast have high \(\text{NH}_4\text{Cl}\) tolerance \([15]\). After determining the concentration range of \(\text{NH}_4\text{Cl}\) inhibition on yeast, one plate (SGA-V2–3) from the library was randomly selected for a preliminary experiment. The \(\text{NH}_4\text{Cl}\) concentration was chosen with reference to our previous criteria \([19]\); that is, the colony size of the control strain was reduced by nearly half, and some mutants showed significant growth inhibition. This criterion was met when the \(\text{NH}_4\text{Cl}\) concentration reached 800 mM (Fig. 1), so we chose 800 mM \(\text{NH}_4\text{Cl}\) as the treatment concentration for the genome-wide screen. About 4200 genes in the yeast gene deletion collection were screened. Based on the criteria described in the method, a total of 61 mutants were considered sensitive (Table S1).

**Drop test verifies high-throughput screening results**

To verify the reliability of the high-throughput screening results, we performed drop test experiments on each of the 61 deletion strains. Among these deletion strains, 34 remained sensitive to \(\text{NH}_4\text{Cl}\) in the drop test experiments (Fig. 2). Growth of these 34 deletion strains was inhibited, compared with control strains, in the presence of 800 mM \(\text{NH}_4\text{Cl}\). Information on these 34 mutants, together with their quantitative fitness scores in genome-wide screening, are summarized in Table 1. Based on the results of the drop test, we classified the degree of \(\text{NH}_4\text{Cl}\) inhibition of the mutants. Colony growth only in the first column was defined as “serious” inhibition, colony growth only in the first two columns was defined as “obvious” inhibition, “moderate” referred to colony growth in
Fig. 1 Images of colony growth on plates with or without 800 mM NH₄Cl. A Phenotypes of SGA control strain and the mutants in our screen, and each mutant has four adjacent clones. B The cell area of his3Δ, tps2Δ, and rvs167Δ under NH₄Cl showed reductions of 52, 75 and 67%, respectively, compared with the control.

Fig. 2 Spot test to verify screening results. Cells were started from OD₆₀₀ = 0.5 and then serially diluted ten times. The 5 μl diluent was spotted onto YPD containing 800 mM NH₄Cl. Plates were photographed after 48 h incubation at 30 °C.
involved in NH₄Cl cellular resistance involve not single, but multiple, pathways.

Since low concentrations of NH₄Cl have no obvious inhibitory effect on yeast growth, we used a high concentration of 800 mM NH₄Cl for high-throughput screening. This concentration resulted in an obvious inhibitory effect on wild-type yeast. To verify the sensitivity of the mutants we screened to NH₄Cl, we performed gradient NH₄Cl experiments on the 34 mutants. A concentration gradient of 0, 100, 300, and 500 mM NH₄Cl was set up for the drop test, in accordance with the previous serial dilution method. At a concentration of 100 mM, the growth of yme1Δ and ure2Δ was severely inhibited,
indicating that these two genes are essential for cellular resistance to NH₄Cl toxicity (Fig. 3). In addition, _rvs167Δ, slt2Δ, vps51Δ, gtr1Δ, ser1Δ, aim29Δ, erg2Δ, erg6Δ, gyp1Δ, and aim44Δ_ were significantly inhibited at a concentration of 300 mM NH₄Cl. At a concentration of 500 mM NH₄Cl, most of the 34 mutants showed growth.

Fig. 3 Spot test of the 34 selected deletion mutants under different concentrations of NH₄Cl. Each strain was serially diluted in a 10-fold gradient and 5 μl were spotted onto differently treated agar plates. Plates were incubated at 30 °C and photographed after 48 h.
inhibition, while the growth of the control strain was still relatively normal, indicating that the 34 genes obtained by high-throughput screening played different roles in the resistance of cells to \( \text{NH}_4 \text{Cl} \). Deletion of these genes makes cells sensitive to different degrees of \( \text{NH}_4 \text{Cl} \) stress. Previous studies have revealed a set of multidrug resistant genes whose deletions are associated with sensitivity to multiple compounds with diverse modes of action that are utilized by cells in a wide range of stress responses [20–22]. Among the 34 genes corresponding to sensitive deletion mutants, 13 were previously thought to contribute to general multidrug resistance, including \( \text{ERG}2, \text{ERG}6, \text{GTR}1, \text{PAT}1, \text{RVS}167, \text{SLA}1, \text{SLT}2, \text{SNF}1, \text{URE}2, \text{VPS}51, \text{VRP}1, \text{YKR}007W, \) and \( \text{YME}1 \). YME1 VRP1, and YKR007W VPS51, and YME1.

### Classification of genes related to \( \text{NH}_4 \text{Cl} \) resistance

To further understand the function of the screened genes, we conducted a bioinformatics analysis of these genes. First, to understand the localization of these genes, we analyzed and mapped all of the genes for the Saccharomyces genome database (https://www.yeastgenome.org/) (Fig. 4D). The genes for five of the 34 genes found that they were mainly enriched in categories related to Gtr1-Gtr2 GTPase complex, cytoskeleton, MAPK activity, TOR signaling, mitophagy, and metabolic pathways (Fig. 4B and C, Table S2). In addition, functional classification of the 34 genes resistance was performed according to the functional description of the Saccharomyces genome database (https://www.yeastgenome.org/) (Fig. 4D). The genes for five of the deletion mutants were unidentified in terms of function. Of the remaining 29, the genes could be divided into seven groups: lipid metabolism- and ammonia metabolism-related genes, post-translational modification, mitophagy, Gtr1-Gtr2 GTPase complex, DNA and RNA synthesis-related genes, and cell resistance- and endocytosis-related genes. Overall, this analysis revealed the complexity of the machinery involved in resistance to \( \text{NH}_4 \text{Cl} \) toxicity. \( \text{NH}_4 \text{Cl} \) may influence cells by affecting both growth and metabolism, and the integrity of these pathways plays an important role in cells’ resistance to ammonium toxicity.

### Transcriptome analysis in response to \( \text{NH}_4 \text{Cl} \) stress

In order to further understand the mechanism of \( \text{NH}_4 \text{Cl} \) toxicity on cells, we cultured wild-type strain BY4741 with different concentrations of \( \text{NH}_4 \text{Cl} \) to find suitable processing conditions for transcriptome sequencing (Fig. S2). Under the condition of 800 mM \( \text{NH}_4 \text{Cl} \) treatment, the cells grew for 8 hours and entered the logarithmic growth phase. At this time, the effect of \( \text{NH}_4 \text{Cl} \) on the cells was already reflected at the transcription level. Therefore, transcriptome sequencing analysis was performed using this treatment condition, and untreated samples grown at the same time were used as a control.

We analyzed the expression levels of 5966 genes in total, of which there were 451 significantly upregulated genes and 835 significantly downregulated genes. Then, we performed GO and KEGG analysis on the 451 significantly upregulated genes. The main enrichment pathways were cellular nitrogen compound metabolic process, cell wall, MAPK signaling pathway, oxidative phosphorylation, and TCA cycle (Fig. 5A and B). GO and KEGG analysis of 835 significantly downregulated genes found that these genes were mainly enriched in ubiquitin-mediated proteolysis, protein phosphorylation, lipid metabolic process, mitophagy, and glycine, serine, and threonine metabolism (Fig. 5C and D). It has been demonstrated that under stress, cells tend to optimize cellular resources for stress adaptation, leading to the massive expression of genes involved in stress adaptation accompanied by inhibition of expression of genes involved in proliferation and cell cycle progression [24].

To better compare our screening and transcriptome sequencing analysis results, we performed transcriptomic analysis of the expression of the 34 deleted genes. The significantly upregulated genes were \( \text{TPS}1, \text{IUT}1, \) and \( \text{GYP}1 \). The significantly downregulated genes were \( \text{SER}1, \text{SER}2, \text{ERG}6, \text{AIM}44, \) and \( \text{YOR}008C-A \). The genes for which expression levels were not significantly downregulated were \( \text{ECM}8, \text{SLA}1, \text{SGF}29, \text{PAT}1, \text{MNN}10, \text{RTT}103, \text{RVS}167, \text{DOS}2, \text{SWF}1, \text{GTR}2, \text{SLT}2, \text{STB}5, \text{MEH}1, \text{VPS}51, \text{AIM}29, \text{HOG}1, \text{VRP}1, \text{GTR}1, \text{ERG}2, \text{UBP}15, \text{URE}2, \text{PFA}4, \text{INP}53, \text{LCB}4, \text{YME}1, \) and \( \text{SNF}1 \) (Fig. 6A). Among the 34 genes screened, most showed no obvious expression changes in the transcriptome, a lack of such correlation was also previously reported [25].

Next, a correlation estimate was performed for all genes identified in both the mutant screening and transcriptomic analysis. As shown (Fig. 6B), the genes with
significantly upregulated expression and score $\leq -0.2$ were TPS1 and TPS2, and the genes with significantly downregulated expression and score $\leq -0.2$ were BNA1, ICS2, AIM44, TOM70, ERG6, BUG1, PUN1, SMI1, YOR008C-A, SER1, and SER2. The genes with significantly downregulated expression and score $\geq 0.2$ were GFD2, COQ10, MVP1, RKM3, and PRR1.

**Discussion**

Compared with other organisms, the tolerance of yeast to ammonium is unimaginable, and the discovery of ammonium toxicity in yeast occurred significantly later than that of other stresses. It was not until the discovery of the relationship between $\text{NH}_4^+$ and $K^+$ that the major mystery of how yeast could...
accomplish this was solved [15]. However, more mechanisms, especially in high concentrations of ammonium under normal \(K^+\) culture conditions, have not been reported. We aimed to learn more about the mechanisms, through (1) screening of yeast deletion mutant libraries, to identify resistance genes, and (2) transcriptome sequence analysis, to identify transcriptional changes, under high-concentration ammonium treatment. In our results, we found many shared genes and pathways that were involved in resistance to multiple stresses, and also found many genes and pathways that were specific to ammonium resistance.

**Carbon and nitrogen metabolism maintains cellular ammonium resistance**

One of the ways in which cells respond to excess intracellular ammonium is to synthesize ammonium into amino acids and exclude the excess to the extracellular space [15, 16]. This process consumes a large amount of carbon skeleton, which is needed for processes such as glycolysis and the TCA cycle. This can be confirmed in the enrichment results of upregulated genes in our transcriptome analysis. Studies by others have come to similar conclusions. For example, In a genome-wide high-throughput screening study of multiple N-nitrosamine compounds using a library of yeast deletion mutants, Joseph Uche
Ogbede et al. found that ammonium sulfate caused growth defects in mutants of the arginine biosynthesis pathway [26]. Xueping Tian et al. discovered overall upregulation of starch synthesis and degradation pathways, as well as glycolysis and the TCA cycle, which could provide abundant carbon skeleton material for excess $\text{NH}_4^+$ assimilation and avoid carbon deficiency, a mechanism that might also be an important feature of the duckweed response to $\text{NH}_4^+$ toxicity [27]. The TCA cycle serves as a mitochondria-based hub for the final steps in carbon skeleton oxidative catabolism for carbohydrates, amino acids, and fatty acids [28]. $\text{NH}_4^+$ can be effectively detoxified into amino acids to maintain the availability of the carbon skeleton by utilizing the TCA cycle [29]. Chandran et al. discovered that upregulated genes are mainly enriched in metabolic processes for diverse amino acids as well as nitrogen compounds, by exploring the genomic responses in rice roots dealing with 0.5 mM (NH$_4$)$_2$SO$_4$ [30]. Rui Wang et al. found that a complex physiological and genetic regulatory network of processes including nitrogen metabolism, carbon metabolism, abiotic stress response, and secondary metabolism at the root and leaf levels was involved in NH$_4^+$ resistance in Myriophyllum aquaticum [31]. The same response has been observed in mudskippers under NH$_4^+$ stress. Xinxin You et al. discovered that reducing the catabolism of protein and amino acids could be an effective way to slow down internal ammonia accumulation [32]. It is reasonable that genes in various other cellular metabolic processes involving nitrogen could be downregulated. Similar to their results, our upregulated genes were also enriched in these pathways, which indicates that it is possible to improve tolerance to NH$_4^+$ toxicity in yeast by increasing the expression of the TCA cycle, glycolysis, starch and sucrose metabolism (Fig. 5B).

In the GO enrichment analysis of the 34 genes corresponding to sensitive deletion mutants, $URE2$, $GTR1$, and $GTR2$ were enriched in several pathways at the highest enrichment level. According to our experimental results, $ure2\Delta$ is one of two mutants showing significant growth inhibition at 100 mM NH$_4$Cl, and $URE2$ is not a multidrug resistant gene. The growth of both $gtr1\Delta$ and $gtr2\Delta$ was reduced by more than 40% compared with the wild type in the screening results. Ure2 is involved in the inhibition of nitrogen catabolism [33]. When an optimal nitrogen source is available, Ure2 acts as a transcriptional corepressor and downregulates the expression of many genes involved in nitrogen utilization. The Gtr1-Gtr2 GTPase complex is composed of Gtr1 and Gtr2, and is important for sensing the presence of amino acids in the medium by activating TORC1 [34]. Gtr1 is a subunit of the EGO complex, which is responsible for activating TORC1 in response to the utilization of amino acids. The combination of EGO complex and TOR can positively regulate microautophagy [35]. In contrast, $gtr1\Delta$ reduces the activity of TORC1, which increases the
expression level of nitrogen transporters and ammonium consumption but reduces amino acid consumption [36]. GTR2 encodes a Ras-like small GTPase that plays a role in regulating nutrition-responsive TORC1 kinase signal transduction, exocytosis sorting of endosomes, and epigenetic control of gene expression [37]. Deletion of GTR1 or GTR2 may render cells incapable of activating TORC1, resulting in cell toxicity due to the inability to degrade ammonia in vivo under high ammonia stress.

Osmotic stress is one of the causes of ammonium toxicity. TPS1 is one of the few genes in our screen for which deletion affects cell growth, but which is significantly upregulated in the transcriptome. TPS1 encodes trehalose 6-phosphate synthase and catalyzes the first step in trehalose biosynthesis. In Saccharomyces cerevisiae, trehalose is a major reserve carbohydrate involved in responses to thermal, osmotic, oxidative, and ethanol stresses [38]. Genes in the trehalose metabolic pathway, including TPS1, TPS2, and NTH1, were all upregulated. TPS2 is responsible for reaction catalysis of step 2 in trehalose synthesis. The NTH1 gene product might contribute to trehalose mobilization in S. cerevisiae under saline stress conditions [39]. NTH1, as well as trehalose biosynthesis genes, was upregulated under saline stress conditions [40]. Cells may be able to reduce the toxicity caused by NH₄Cl through saline stress by increasing the expression of these genes (TPS1, TPS2, and NTH1).

In addition, Hog1 is an important regulator of transcription in conditions of osmotic stress in yeast [41]. Fang Li et al. found that Hog1 and Slt2 were down-regulated under osmotic and cell wall stresses, respectively [42]. Hog1-mediated transcriptional control of ERG genes accounts for most of the downregulation of sterol levels upon osmotic stress [43]. In our transcriptome results, the expression of HOG1, SLT2, ERG2, and ERG6 were all downregulated, and erg2Δ and erg6Δ were also more sensitive than other mutants, showing severe growth inhibition at 300 mM NH₄Cl. These results suggest that NH₄Cl may also be toxic to cells through osmotic stress.

Mitophagy plays an important role in ammonium resistance. Among the 34 sensitive mutants, KEGG and functional classification were all enriched in the mitophagy pathway, including yme1Δ, slt2Δ, and hog1Δ, of which yme1Δ showed severe growth inhibition at 100 mM NH₄Cl, and slt2Δ was significantly inhibited at 300 mM NH₄Cl. The mitochondrial protease Yme1 plays an important role in the ability of cells lacking tafazzin function to maintain mitochondrial structural integrity, mitochondrial quality control, and mitochondrial autophagy [44]. Yme1 is an ATP-dependent protease located on the inner mitochondrial membrane that is required for the growth of yeast lacking a complete mitochondrial genome [45]. The processing of Atg32 by Yme1 is an important regulatory mechanism of mitophagy [46]. Slt2 and Hog1 are both required for mitophagy. Slt2 is a MAPK of the cell wall integrity pathway and is necessary for the degradation of mitochondria. Hog1, another member of the MAPK family, is a kinase that regulates and is regulated by Sch9p and is independent of the PKA and TOR pathways in response to stress [47]. Hog1 plays a role in Atg32 phosphorylation, and this is necessary for mitophagy [48, 49].

Mitochondria are important organelles that provide cellular energy and the carbon skeleton building blocks for the synthesis of macromolecular substances. Therefore, the removal of damaged mitochondria through mitophagy is essential for maintaining proper cell function [50]. When cells are exposed to ammonium, higher demands are placed on mitochondrial function. This is because, first, every time NH₄⁺ enters the cell, H⁺ is transported outside the cell, resulting in an increase in intracellular pH [51]. Ammonium uptake requires the consumption of large amounts of ATP for maintaining intracellular pH by using the plasma membrane bound H⁺-ATPase [17]. In addition, studies have found that ineffective transmembrane ammonium cycling in some species can also lead to energy consumption, because a large fraction of intracellular ammonium leaks out of the cell through the membrane. This ineffective transport causes great energy loss, resulting in adverse symptoms [52, 53]. Second, the assimilation of ammonium into amino acids requires a large amount of carbon skeleton, which leads to harmful effects due to insufficient carbon building blocks in the cell [52]. The high concentration of ammonium increases the synthesis rate of glutamic acid, alanine, and glycine, and accelerates the consumption of glucose and glutamine. The increase in the concentration of ammonium salt not only increases the energy requirements of the cells, but also affects the TCA cycle. These all place high demands on mitochondrial function, and the impairment of mitophagy leads to the sensitivity of cells to ammonium toxicity.

Conclusions
In this study, we analyzed the resistance mechanism that allow yeast to grow under high concentration of ammonium using yeast genome-wide screening. Our results showed that, out of nearly 4200 mutants, 34 mutants were identified and confirmed by drop test as being vulnerable to NH₄Cl. Furthermore, functional enrichment analysis indicated that these 34 genes were mainly
involved in lipid metabolism and ammonia metabolism, post-translational modification, mitophagy, Gtr1-Gtr2 GTPase complex, DNA and RNA synthesis-related genes, cell resistance, and endocytosis-related genes. Transcriptome analysis further supported the accuracy of our screening results and demonstrated that cells significantly upregulated carbon and nitrogen metabolism, TCA cycle and other stress adaptation pathways, and downregulated cell growth-related pathways under NH₄Cl stress. These results can provide us with a clearer understanding of the resistance mechanism that allow yeast to grow under NH₄Cl.

Materials and methods
Genome-wide screen to identify gene deletion mutants sensitive to NH₄Cl
The gene deletion library (SGA-V2) was kindly provided by Prof. Charlie Boone, University of Toronto, Canada [54, 55]. The library of non-essential haploid deletion strains containing about 4200 mutants was started from S. cerevisiae strain BY4741 (MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0). Each mutant was constructed by replacing the corresponding ORF with a KanMX cassette, and the strains in the library were arranged in 384 format. Mutant his3Δ::KanR in this library was designated as the control strain and added as a border around four edges of each plate [56]. The pinning steps for library handling were performed using a SINGER ROTOR HDA Robot (Singer Instruments, UK). The deletion library was cloned on YPD agar plates (with G418 added) and grown at 30°C for 2 days. The 384 strains in each plate were then transferred to a new agar plate, and each colony was repeated four times to finally form an array of 1536 colonies. The entire library in 1536 array was then cloned onto a solid plate with or without 800 mM NH₄Cl and grown at 30°C for 2 days. The growth status of the colonies was photographed by PhenoBooth (Singer Instruments, UK). Images were analyzed using SGAtools (http://sgatools.ccb.utoronto.ca/) to evaluate the growth of the colonies [57], and compare the growth of each mutant with or without NH₄Cl. First, images of plates with colonies were processed to give quantified colony sizes for the screen. Next, the colony sizes were normalized and filtered within plates, taking into account position effects and other confounding factors. Ratios of normalized colony sizes from NH₄Cl-treated and untreated mutants were used as a measure of sensitivity. A score $>0$ represented positive interaction, i.e., increased colony size, while a score $<0$ represented negative interaction, i.e., decreased colony size. According to previous studies [58], a score of $<−0.2$ generally indicates a strong effect, so we used scores $<−0.2$ and $P<0.05$ as thresholds to identify significantly affected mutants. Each experiment was repeated three times.

Culture conditions
Yeast strains were grown in YPD + G418 medium which contained 1% yeast extract, 2% peptone, 2% glucose, and 200 mg/mL G418. The 800 mM NH₄Cl medium consisted of 1% yeast extract, 2% peptone, 2% glucose, 200 mg/mL G418, and 800 mM NH₄Cl. All the strains which were mentioned in this article were cultivated in an incubator at 30°C.

Spot assays
For the drop test, we picked up the selected mutant from the SGA-V2 library and cultured it overnight in 3 mL YPD + G418 medium at 30°C. Then, the strains were diluted to $OD_{600}=0.1$, and cultured at 30°C until the mid-logarithmic stage. The culture was then continuously diluted tenfold in sterile water in 96-well plates and the dilutions were spotted on plates with or without NH₄Cl. Images were taken after culturing at 30°C for 2 days, and the growth of yeast colonies was observed. Shown are representative drop tests from three independent replicate assays.

Bioinformatics enrichment analysis and functional annotation
The GO Term Finder in the Saccharomyces Genome Database (https://www.yeastgenome.org/) was used to analyze the enrichment of GO terms in 34 genes [59]. Then, we used the gene-list analysis tool Metascape (http://metascape.org) with the background of genes corresponding to SGA-V2 library, to conduct KEGG pathway analysis, with a chosen $P$-value of less than 0.05 [60]. The functional annotation results were mapped and clustered using Cytoscape (version 3.8.0) [61].

Transcriptional RNA sequence analysis
Yeast cells treated with 0 mM and 800 mM NH₄Cl for 8 hours were used for transcriptome sequencing, and four sample replicates were set for each treatment. Total RNA was extracted using Trizol reagent (Thermofisher, 15,596,018), using the Bioanalyzer 2100 and RNA 6000 Nano LabChip Kits (Agilent, CA, USA, 5067–1511) to analyze the total RNA quantity and purity, the sequencing cDNA library was constructed using high-quality RNA with an RNA integrity number (RIN) $>7.0$. The library was sequenced using the Illumina Novaseq™ 6000 sequence platform. Cutadapt (https://cutadapt.readthedocs.io/en/stable/, version: cutadapt-1.9) was used to remove the Illumina adapter contamination and for trimming the reads and clipping...
the low-quality bases to get high-quality clean reads. Then, sequence quality was verified using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/, version: 0.11.9). After that, approximately 6 Gb of clean reads were produced [62]. Differentially expressed genes (DEGs) analysis was performed by DESeq2 software, between two different groups. The genes with the parameter of false discovery rate (FDR) below 0.05 and absolute fold change ≥1.5 were considered differentially expressed genes [63]. Then ClueGO in Cytoscape was used to perform the enrichment analysis of GO function and KEGG pathway for the significantly upregulated genes and the significantly downregulated genes.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-022-08742-y.

Additional file 1: Figure S1. Drop test of hi5α in plates with a gradient of concentrations of NH4Cl.

Additional file 2: Table S1. Data from three independent genome-wide screening experiments.

Additional file 3: Table S2. GO and KEGG-enriched gene list for the 34 NH4Cl-sensitive mutants.

Additional file 4: Figure S2. Growth curve of BY4741 at different concentrations of NH4Cl treatment.

Additional file 5: Table S3. Transcriptome sequencing data under 800 mM vs. 0 mM NH4Cl treatment.

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Authors’ contributions

B-DL, X-JJ, and X-LC designed the experiments. W-HF, X-LC, and T-TA performed the experiments. W-HF, X-LC, T-TA, H-HZ, JZ, and D-QL analyzed the data. W-HF and X-LC mapped all the Figs. X-LC and W-HF wrote the manuscript. X-JJ and B-DL revised the manuscript. All authors read, modified as needed, and approved the final manuscript.

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Availability of data and materials

All the sequencing data generated in this study have been deposited in the Sequence Read Archive (SRA) database under accession number PRJNA795291 (https://dataverse.northy.nhm.nih.gov/object/PRJNA795291?revi we=r3v8dgbe27ar7v1vbh4uscmmv).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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