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Research Letter **Open Access**

Cloning and Characterization of *PutSTE24* Gene from *Puccinellia tenuifolra* Which Expressed in Response to Abiotic Stresses

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Abstract The *Puccinellia tenuifolra* cDNA library was expressed in yeast (*Saccharomyces cerevisiae*) and screened on agar plates containing toxic concentrations of aluminum. Ninteen cDNAs were isolated that enhanced the aluminum tolerance of yeast. One cDNA, named *PutSTE24*, has a ORF of 1 275 bp, encoding a predicted protein containing 424 amino acid, and has a high similarity of 77% with STE24 in *Arabidopsis thilinana*. *PutSTE24* and *AtSTE24* were transformed into yeast cells separately and were treated with $AlCl_3$, salt, drought, low pH and oxidation and metal ions stresses. Results revealed that these two recombinant yeast cells showed similarly and grew better in $AlCl_3$, salt and oxidation stresses than control cells, but no obvious difference in low pH and drought stresses. Additionally, on the responsive to the metal ions, these two genes have obvious resistance to the stresses of K^+, Mg^{2+} and Cu^{2+}, are somewhat resistant to Fe^{3+}, Cd^{2+}, and have no obvious responsive relationship with Ca^{2+}, Mn^{2+} and Ba^{2+}, but to the metal ions of Co^{2+}, Ni^{2+} and Zn^{2+}, these two recombinant yeast cells are sensitive, growing worse than the control cells, especially the Zn^{2+}. It is basically confirmed the gene *STE24* is related to metal stresses, which has no report in the previous studies.

Keywords *Puccinellia tenuifolra*; *PutSTE24* gene; Yeast; Stresses

Background

Aluminum is a non-toxic element in the earth's crust at normal pH values. But in the acid soils, at the low pH values (pH<5.5), Al^{3+} is solublilized from aluminosilicate clay minerals and is toxic to crop plants (Kochian et al., 2004). Toxic aluminum can disrupt a series of cellular processes, such as nutrient acquisition, cell wall loosening, nuclear division, cytoskeleton stability, cytoplasmic Ca^{2+} homeostasis, hormone transport and signal transduction(Matsumoto, 2000). Previous studies showed that aluminum-activated root malate or citrate exudation from plasma membrane or vacuolar membrane played an important role in plant Al^{3+} tolerance (Hoekenga et al., 2006). For instance, genes *AtALMT1* and *TaALMT1* discovered in *Arabidopsis thilinana* and wheat (*Triticum aestivum*), that encode aluminum-dependant malate tranporters, are the most important way to Al^{3+} tolerance (Kobayashi et al., 2007). Besides these, there are some genes or enzymes else existing in plants, including *ZmMATE*, *OsSTARA*1/2, *AtSTOP1*, *AtBCB* (Arabidopsis blue copper-binding protein), *parB* (tobacco glutathione S-transferase) and catalase et al. (Satoshi et al., 2007).

The modern studies focus on the Al^{3+} toxicity in acid soils, but aluminum can be also toxic in alkali circumstance, existing in complicated ionic ways. In this study, *Puccinellia tenuifolra*, a typical plant in alkali soils, was use to construct its full length cDNA library expressed in yeast, screened the Al^{3+} related genes with $AlCl_3$ in the medium. *PutSTE24*, showed a high similarity to *AtSTE24* (77%), was screened out.

The CAAX protease STE24, first identified in a genetic screen in yeast for mutants defective in the production of a biologically active a-mating pheromone, is a prenylation-dependent protease catalising

a kind of eukaryotic proteins' posttranslational modifications essential to their targeting (Apolloni et al., 2000). These proteins end by the residues recombination CAAX, named as CAAX proteins, and their post-translational modifications usually include the following three sequential, enzymatic steps. First, the proteins are prenylated by one of two prenyl-transferases named geranylgeranyltransferase Ⅰ or farnesyltransferase (Galichet and Gruissem, 2003), which happens in cytoplasm. In yeast and animal cells, prenylation is followed by proteolytic removal of the last three amino acids of the protein (AAX) by either of the two endoproteases, RCE1 and STE24 (AFC1) (Boyartchuk et al., 1997; Young et al., 2001), which is thought to take place on the cytoplasmic surface of the endoplasmic reticulum (ER) (Schmidt et al., 1998). Finally, the exposed isoprenyl-cysteine is methylated by and prenyl-dependent carbo-xylmethyltransferase (PCM) (Clarke, 1992; Romano et al., 1998).

In the recent ten years, the protein prenylation in plant has been clarified specifically, and genes encoding the above enzymes have cloned in *Arabidopsis thilinana*. There has been some reports showed that over-expression of some genes is related to stress tolerance of plant. In Arabidopsis, loss-of-function mutations in the *ERA1* gene, encoding the β-subunit of PFT, *ggb1* gene, encoding the β−subunit of PGGTⅠ, or *plp* gene, which encode α−subunit of these two enzymes, cause an enhanced response to abscisic acid (ABA) in seed germination and stomatal closure assays (Cutler et al., 1996; Pei et al., 1998; Running et al., 2004; Johnson et al., 2005). The above two enzymes involved in negative regulation of signaling in guard cells. *AtSTE24*, an Arabidopsis homologue of the CAAX protease STE24, was cloned and expressed in *rce1*Δ *ste24*Δ mutant yeast to demonstrate functional complementation (Bracha et al., 2002). To date, there are few studies were reported on AtSTE24, and fewer reports introducing its relationship with stresses tolerance and responsion reaction with metal ions.

This paper reports on the cloning and characterization of *PutSTE24* and *AtSTE24*, indicating that STE24 is a protease related to Al^{3+} tolerance and other stresses in yeast.

1 Results and Analysis

1.1 Cloning and sequence analisys of *PutSTE24*

In the previous studies, full length cDNAs over-expressing library of *Puccinellia tenuifolra* was constructed in yeast (*Saccharomyces cerevisiae*). A clone was screened out from this yeast library with medium containing $AlCl_3$. By PCR using the specific primers described in materials and methods and sequencing, results showed that *PutSTE24* cDNA contained full length of 1 700 nucleotides and had a open reading frame (ORF) of 1 275 bp nucleotides encoding a predicted 424 amino acids (Figure 1). The predicted protein was calculated to have a molecular mass of 48.3 kD and pI of 6.84.

The Blast algorithm identified three proteins with higher similarity to PutSTE24 (Figure 2). They are AtSTE24 from *Arabidopsis thaliana* (At4g01320, 77% amino acid identity), CAAX prenyl protease 1 from *Zea mays* (100286144, 79% amino acid identity), and putative STE24 from *Ricinus communis* (8286673, 77% amino acid identity). Like AtSTE24, PutSTE24 possesses two conservative sequence motifs: HEXXH that is a signature of zinc metalloproteases and a C-terminal KKXX, the ER membrane retention signal (Figure 2).

1.2 Over-expressing of *PutSTE24* and *AtSTE24* respectively in yeast and Al^{3+} tolerance analysis

In this study, *PutSTE24* was screened out with $AlCl_3$ stress, therefore, to further analyze the responsive relationship of it and its homologue *AtSTE24* with Al^{3+} stress, yeast transformed lines were constructed. One was transformed with empty vector pAUR123 as a control. The two else transformants were over-expressed *PutSTE24* and *AtSTE24* respectively (Figure 3; Figure 4 and Figure 5). In the presence of different concentrations of $AlCl_3$, the growth of these transformants showed differently (Figure 3). The growth of these two transformants showed similarly. At 6 mmol/L of $AlCl_3$, they grew much better than the control yeast; but at 6.5 or 7 mmol/L of $AlCl_3$ stress, this growth advantage disappeared, and they seemed similar to the control, even worse. The results indicated over-expressing of *PutSTE24* and *AtSTE24* can alleviate Al^{3+} stress at a degree.

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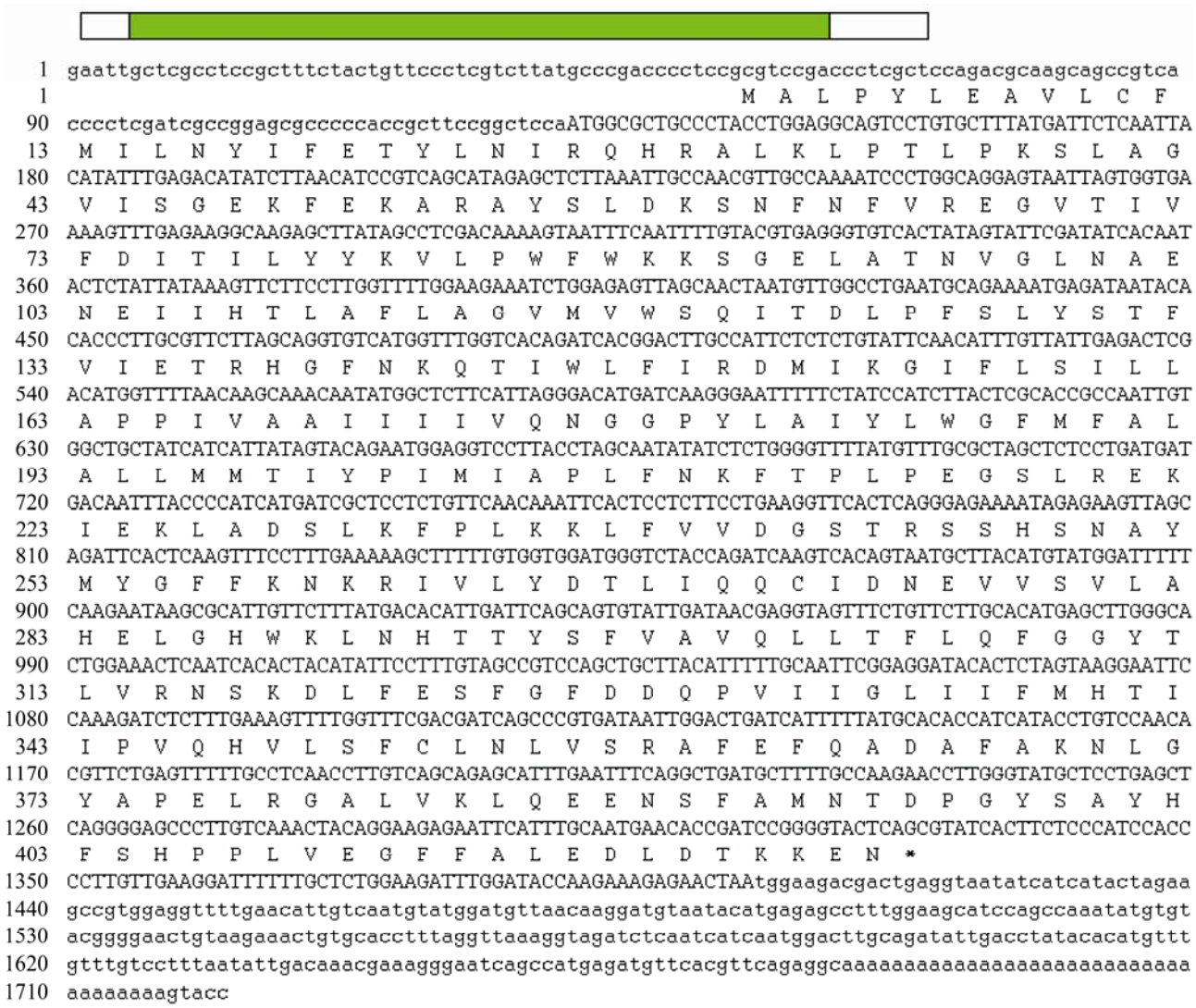

Figure 1 Nucleotide sequences and the encoded amino acid sequences of *PutSTE24*

Al^{3+} stress can also cause some other stresses at the same time, such as low pH and oxidation stresses, therefore, in this study, growth of these transformed yeast lines was observed in the conditions of pH 4.2, sorbitol, NaCl and H_2O_2 (Figure 4). The growth of the *PutSTE24* and *AtSTE24* transformants was the same as that of the control in the presence of low pH and sorbitol, but was better than that of the control on the media containing NaCl and H_2O_2. The results indicate that STE24 protease plays a role in response to salt and oxidation stresses and its role in Al^{3+} tolerance may be not specific.

1.3 Responsion of *PutSTE24* and *AtSTE24* over-expressing cells to various of metal cations

To further discuss the responsive relationship of *STE24* with metal ions except Al^{3+}, serial dilutions were spotted onto solid yeast YPD medium supplemented without or with various of metal cations and the growth was monitored (Figure 5). As shown in Figure 5, the growth of the two *STE24* transformants was much better than that of the empty vector transformant on the media containing K^+, Mg^{2+} and Cu^{2+}; some better than the control with the Fe^{3+} and Cd^{2+}; and was almost the same as that of the control in the presence of Ca^{2+}, Mn^{2+} and Ba^{2+}. Interestingly, the

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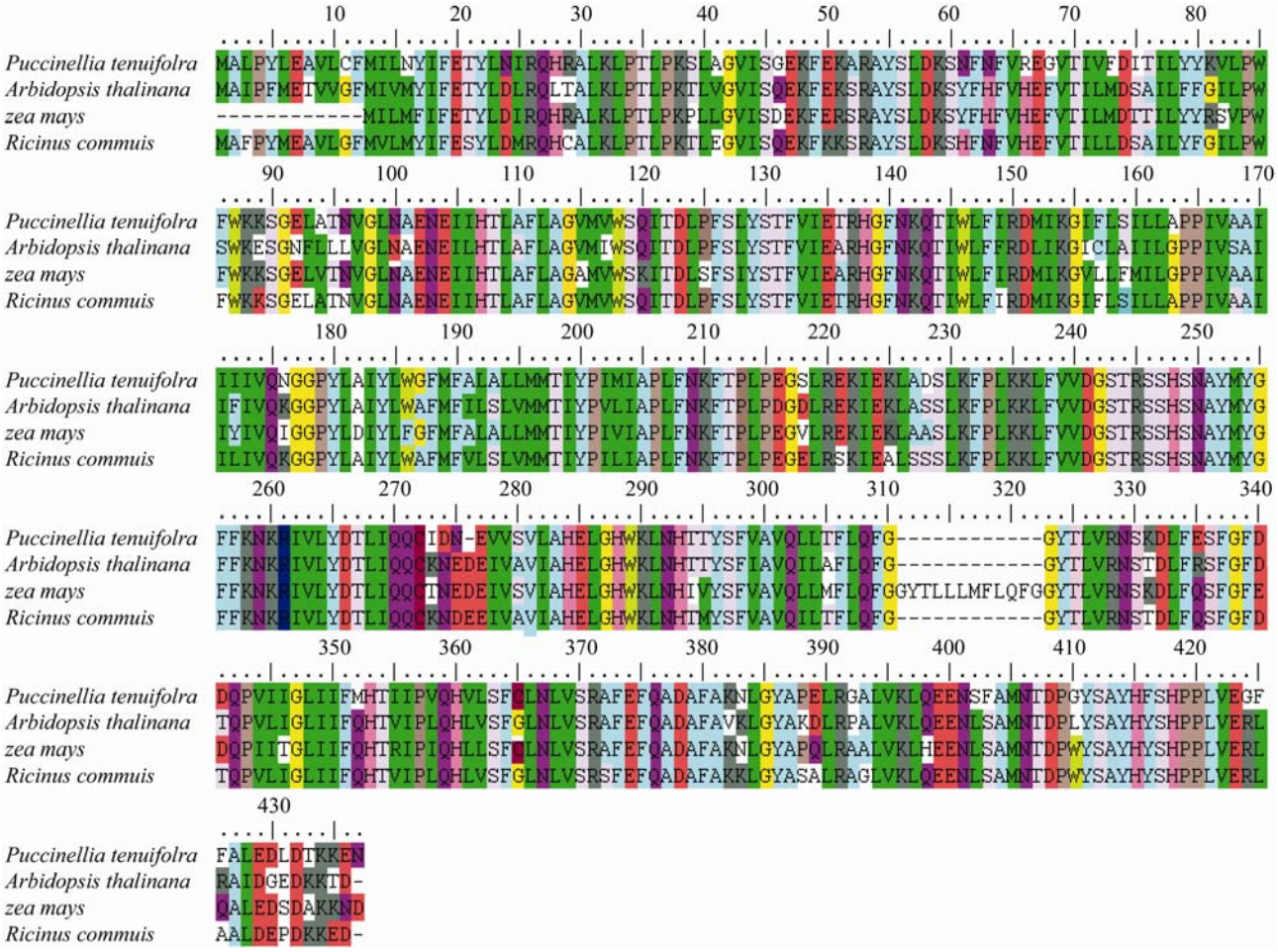

Figure 2 Alignment of deduced amino acid sequences of *PutSTE24* with its homology in *Arabidopsis thaliana*, *Zea mays* and *Ricinus Communis*
Note: The upper box indicates an HEXXH Zn^{2+}-metalloprotease signature; The lower box indicates a KKXX ER membrane retention signal; Accession number of *Arabidopsis thaliana*: At4g01320, *Zea mays*: 100286144 and *Ricinus Communis*: 8286673

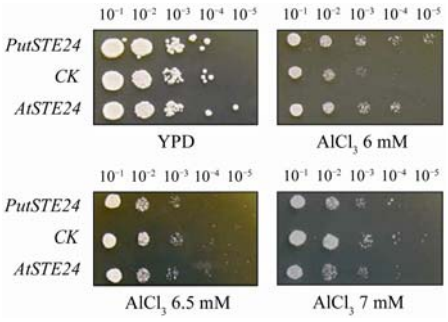

Figure 3 Growth assay of yeast expressing *PutSTE24* and *AtSTE24* in the stress of $AlCl_3$
Note: Yeast cells containing pAUR123, pAUR123−PutSTE24 and pAUR123−AtSTE24 were, respectively, incubated as described in materials and methods; Serial dilutions were spotted onto solid yeast YPD medium supplemented without or with additional $AlCl_3$ (6 mmol/L, 6.5 mmol/L and 7 mmol/L), growth were monitored for 3~6 d at 30℃

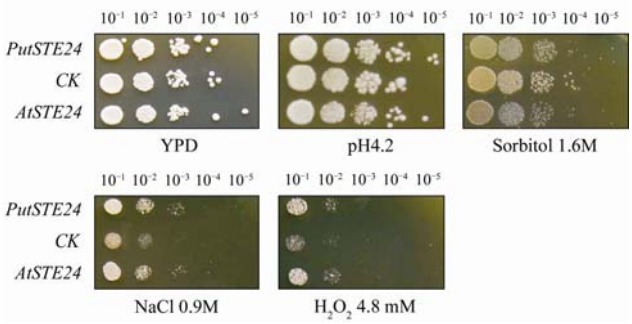

Figure 4 Growth assay of yeast expressing *PutSTE24* and *AtSTE24* in the different stresses
Note: Yeast cells containing pAUR123, pAUR123−PutSTE24 and pAUR123−AtSTE24 were, respectively, incubated as described in materials and methods; Serial dilutions were spotted onto solid yeast YPD medium supplemented without or with additional stresses, such as low pH (pH values 4.2), sorbitol 1.6 mol/L, NaCl 0.9 mol/L and H_2O_2 4.8 mmol/L, growth were monitored for 3~6 d at 30℃

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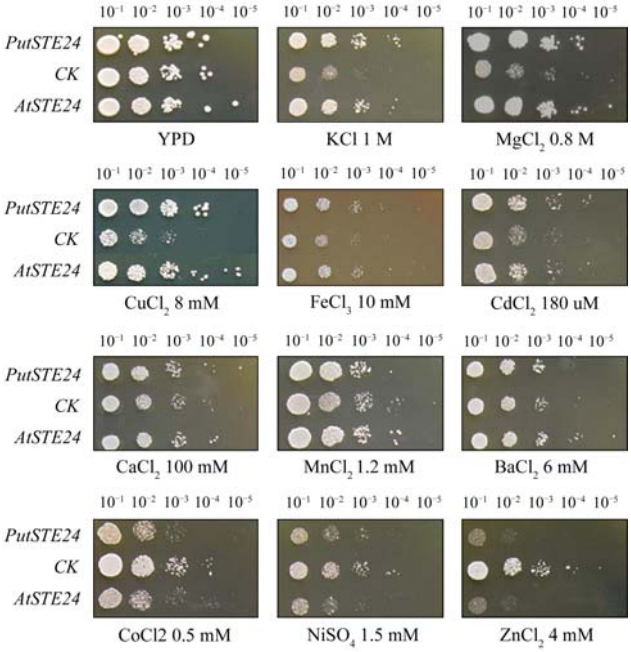

Figure 5 Growth assay of yeast expressing *PutSTE24* and *AtSTE24* in the stresses of various of metal ions

Note: Yeast cells were incubated as described in materials and methods; Serial dilutions were spotted onto solid yeast YPD medium supplemented with or without metal cations, including K^+ 1 mol/L, Mg^{2+} 0.8 mol/L, Cu^{2+} 8 mmol/L, Fe^{3+} 10 mmol/L, Cd^{2+} 180 μmol/L, Ca^{2+} 100 mmol/L, Mn^{2+} 1.2 mmol/L, Ba^{2+} 6 mmol/L, Co^{2+} 0.5 mmol/L, Ni^{2+} 1.5 mmol/L and Zn^{2+} 4 mmol/L, growth were monitored for 3~7 d at 30℃

growth of the two *STE24* transformants seemed hyper-sensitive in the presence of Co^{2+}, Ni^{2+} and Zn^{2+}. These results indicate that *STE24* is a gene related to some metal ions stresses besides Al^{3+}, which have not been reported previously. This responsive relationship is deduced to caused by the post-translation modification of some cations transporters under the action of STE24 protease.

2 Discussions

In this study, the growth of yeast transformed with *PutSTE24* and *AtSTE24* was assayed in the presence of various of abiotic stresses. We have got the conclusions that *STE24* is a gene related to some metal ion stresses, but the molecular mechanism involved in have not been clear.

3 Materials and methods

3.1 Materials

Yeast full-length cDNA library of Puccinellia tenuiflora (1 865 000 clones), cDNAs of *Arabidopsis thaliana*, *Escherichia coli* strain JM109, Yeast strain (*Saccharomyces cerevisiae*) *InVSCI*.

3.2 Cloning *PutSTE24* and *AtSTE24* from plant and sequence analysis

The ORF portion of Put *STE24* was amplified from the yeast expression library of *P. tenuiflora* with the primers F-F: 5'−GCAGCTGTAATACGACTCAC−3' and F-R: 5'−TTACATGATGCGGCCCTCTA−3'. The ORF portion of *AtSTE24* was amplified from the yeast expression library of *Arabidopsis thaliana* with the primers F-F: 5'−GGTCACTCTTTTCTCAGCCATG−3' and F-R: 5'−ACAAGAGACGAGTTAAGCGGAC−3'. Homologous comparison was obtained with other plants according to the amino acid sequence of the two genes.

3.3 Plasmids construction of pAUR123−PutSTE24 and pAUR123−AtSTE24 and yeast transformation

The modified form of *PutSTE24* was constructed: SgsI-PutSTE24−SfaAI. The forward primer (F-P: 5'−GCGATCGCGCACTGTAATACGACTCAC−3') was designed to add *SgsI* site and the reverse primer (R-P: 5'−CTCGAGTTACACAAAAAAGCTTG−3') was designed to add *SfaAI*.

The modified form of *AtSTE24* was constructed: SgsI-AtSTE24−SfaAI. The forward primer (F-P: 5'−GGTACCTTTTCTCAGCCATG−3') was designed to add SgsI site and the reverse primer (R-P: 5'−GGCG CGCCTCTAGATGCATGCTCGAG−3') was designed to add *SfaAI*.

All amplified fragments were cloned into the pAUR123 vector (Invitrogen) and the constructed vectors were introduced into yeast mutant *InVSCI* using the LiAc/PEG method. The yeast transformants were selected on medium supplied with Aureobasidin A.

3.4 Tolerance of *PutSTE24/AtSTE24* overexpressing cells to various stress

For growth response assay, the yeast transformants of pAUR123, pAUR123−PutSTE24 and pAUR123−AtSTE24, were cultured in liquid YPD medium until OD_{600}≈0.6 respectively, and diluted 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} fold with ddH_2O. Then, aliquots of each dilution were spotted onto solid yeast YPD medium

supplemented with different concentrations of $AlCl_3$, NaCl, H_2O_2, pH, sorbitol, KCl, $MgCl_2$, $FeCl_3$, $MnCl_2$, $ZnCl_2$, $CaCl_2$, $CuCl_2$, $CdCl_2$, $NiSO_4$, $BaCl_2$ and $CoCl_2$ as indicated. The yeast transformant of pAUR123 empty vector was used as a control, growth were monitored for 3~7 d at 30℃.

Authors' contributions

MHZ, XXZ and LYW designed and conducted this experiments; LHD, BS and TT participated the experiment design and data analysis; SKL is the person who takes charge of this project, including experiment design, data analysis, writing and modifying of the manuscript. All authors have read and approved the final manuscript.

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