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The *FKS* family genes cause changes in cell wall morphology resulted in regulation of anti-autolytic ability in *Saccharomyces cerevisiae*

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ABSTRACT

The aim of this study was to discuss the functions of *FKS* family genes which encode β-1, 3-glucan synthase regarding the viability and autolysis of yeast strain. Loss of *FKS1* gene severely influences the viability and anti-autolytic ability of yeast. Mutation of *FKS1* and *FKS2* genes led to cell reconstruction, resulting in a sharp shrinkage of cell volume and decreased stress resistance, viability, and anti-autolytic ability. Deletion of *FKS3* gene did not clearly influence the synthesis of β-1, 3-glucan of yeast but increased the strain's stress resistance, viability, and anti-autolytic ability. It is suggested that *FKS3* would be the potential target for improving the stress resistance of yeast. The results revealed the relationship among *FKS* family genes and demonstrated their functions on yeast cell wall construction and anti-autolytic ability.

1. Introduction

Yeast is one of the most popular strains used in fermentation industry including food fermentation and biochemical fermentation. During fermentation, yeast encounters a lot of stresses from the culture environment including high gravity, high toxicity, high osmotic pressure, and high temperature (Gibson et al., 2007). Autolysis of yeast usually happens at later period of fermentation when yeast cells are exposed to harsh environment (Büttner et al., 2006). The autolysis process which is nonreversible initiates from the hydrolyzations of cellular components by intracellular enzymes. The cell wall of *Saccharomyces cerevisiae* is a precise structure that plays significant roles in the establishment and maintenance of cell morphology (Klis et al., 2006). Furthermore, yeast cell wall, functioning as the very first barrier of the cell, exhibits a highly organized dynamic network structure to resist the environment (Atilgan et al., 2015). It is also required to maintain the shape of cell, which is essential to the cell division and formation of a bud (Levin, 2005, 2011). The autolysis and stress-resistance performance of *S. cerevisiae* were reported to have close relationships with cell wall whose primary structural component is β-1,3-glucan (Inouhe et al., 1997; Cabib et al., 2001). It is reported that during yeast autolysis the intensity of the infrared spectra of β-1,3-glucan increased with the increase of yeast autolysate (Burattini et al., 2008; Cavagna et al., 2010). Furthermore, modification in cell wall β-1,3-glucan synthesis would activate the cell wall integrity (CWI) pathway, resulting in the remodeling of yeast cell wall (Orlean, 2012).

β-1,3-glucan is a chief structural polymer of yeast cell wall and is synthesized by β-1,3-glucan synthase. Previous reports showed that β-1,3-glucan synthase consists of at least two subunits: a putative catalytic subunit and a regulatory subunit (Cabib et al., 2001). The catalytic subunit is Fks1p, which is a membrane-localized protein (Douglas et al., 1995). Fks1p and its alternative protein Fks2p share 88% homology including the region of putative catalytic domain (Lesage et al., 2004). Disruption of *FKS1* gene caused a reduced *in vitro* β-1,3-glucan synthase activity and consequently reduced the level of β-1,3-glucan in cell wall (Inoue et al., 1996). And disruption of the *FKS2* gene did not obviously affect the β-1, 3-glucan synthase activity or β-1, 3-glucan contents in vegetable cells (Mazur et al., 1995). However, *FKS2* was found to be important in the process of sporulation (Ishihara et al., 2007). Meanwhile, *FKS1* and *FKS2* genes were reported to be close interacted and have overlapping functions (Mazur et al., 1995). Fks3p, another homologous protein of Fks1p, was discovered by homology searching and it shares 56% homology with Fks1p and Fks2p (Mazur et al., 1995). It is reported that *FKS3* gene was required for the ascospore wall formation via modulating glucan synthesis (Ishihara et al., 2007). And from the result of genome-wide responses analysis, *FKS3* was shown to have no interactions with *FKS2*. Besides the very important functions in *de novo* glucan synthesis, the *FKS* family genes were revealed to be related with the stress resistance and autolysis of *S. pastorianus* during beer fermentation (Wang et al., 2014; Xu et al., 2014a). These three

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Table 1
Strains and plasmids used in this work.

Strains and plasmids	Genotype	Sources
E. coli Top10	F–*mcr*AΦ80*lacZ*ΔM15Δ(*mrr-hsd*RMS-*mcr*BC) Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G	GENEWIZ
S. cerevisiae w303	MATa ade2-1 ura3-1 his3-11 trp1-1 leu2-3 leu2-112 can1-100(ATCC)	Stored in authors' lab
fks1-QT	w303 *fks1*△::*G418*	Stored in authors' lab
fks2-QT	w303 *fks2*△::*G418*	Stored in authors' lab
fks3-QT	w303 *fks3*△::*G418*	Stored in authors' lab
fks1,2-QT	*fks1*-QT *fks2*△::*URA3*	This work
fks1,3-QT	*fks1*-QT *fks3*△::*HIS3*	This work
fks2,3-QT	*fks2*-QT *fks3*△::*HIS3*	This work
fks1,2,3-QT	*fks1,3-QT fks2*△::*URA3*	This work
pUC57-His	Cloning vector, amp^r, *his3*	GENEWIZ
pUC57-Ura	Cloning vector, amp^r *ura3*	GENEWIZ

genes together regulate the cell wall β-1, 3-glucan synthesis as well as the stress tolerance of *S. cerevisiae*, and to our knowledge, there is little report about the functions of *FKS* gene family on cell wall morphology and the anti-autolytic ability of yeast.

In this study, *FKS* family genes were sequentially knocked out in *S. cerevisiae* w303. β-Glucan synthesis of yeast was significantly regulated upon the deletion of different genes. And variations were found in cell morphology and stress resistance. Furthermore, the anti-autolytic ability and ethanol production of mutant strains changed a lot due to the deletion of *FKS* family genes.

2. Materials and method

2.1. Strains and plasmids

The strains and plasmids used in this study were listed in Table 1. *Saccharomyces cerevisiae* w303 was used as the wild-type strain. Strains *fks1*-QT, *fks2*-QT, *fks3*-QT were constructed in previous studies (Li, 2015). *Escherichia coli* Top10 was cultivated in LB medium [1% (w/v) tryptone, 1% (w/v) NaCl, and 0.5% (w/v) yeast extract] at 37 °C containing ampicillin (100 μg/mL) when necessary. Yeasts were grown in YPD medium [2% (w/v) peptone, 2% (w/v) glucose, and 1% (w/v) yeast extract] at 28 °C. Recombinant strains were selected in YPD or synthetic dropout medium [0.67% (w/v) yeast nitrogen without amino acids, 0.2% (w/v) dropout mix, and 2% (w/v) glucose] with 200 μg/mL G418 when necessary. 1.5% agar was added to produce solid medium.

2.2. Construction of double and triple mutants

Single mutant strains *fks1*-QT, *fks2*-QT, and *fks3*-QT were constructed in previous study by knocking out *FKS1*, *FKS2*, and *FKS3* genes in w303 strain with G418 selection marker (Li, 2015). Three types of media were used for mutant strain screening. Medium A consisted of Yeast Nitrogen Base (YNB) medium supplemented with amino acids except uracil, medium B consisted of YNB medium supplemented with amino acids except histidine. Medium C consisted of YNB medium supplemented with amino acids except histidine and uracil. In this study, *FKS2* gene in *fks1*-QT strain was disrupted by replacing the *FKS2* gene with a *URA3* cassette via homologous recombination, resulting in *fks1,2*-QT. The *fks1, 2*-QT strain was selected in medium A with 200 μg/mL G418. *HIS3* cassette was used to replace *FKS3* gene in *fks1*-QT, *fks2*-QT and *fks1,2*-QT strains, and mutant strains were selected in medium B (*fks1*,3-QT, *fks2,3*-QT) and medium C (*fks1,2,3*-QT), respectively. All mutants were confirmed by colony PCR and sequencing validation. The primers used in this study were listed in Table 2.

Table 2
Primers used in this work.

Primers	Sequences (5′–3′)
fura3-F	AAGGCATTATCCGCCAAGTA
fura3-R	ACCACATCATCCACGGTTCT
fks2-F	AGTGTATATATACTTCACACTTA
fks2-R	TCATCACTATATGAGATTCCACG
fhis3-F	CTAGTAAAGCGTATTACAAA
fhis3-R	ATGGCAACCGCAAGAGCCTT
fks3-F	CGCGCAATTAAAAGTCTTGG
fks3-R	CATTGTTGAGATTTAGCTGT

2.3. Growth performance assay and fermentation test

The mutants' resistances to different kinds of stresses were tested on YPD plate contain 1 mol/L NaCl, 10% ethanol, and 75 ng/mL micafungin, respectively (Dragosits et al., 2010). Yeast cells were cultured in 20 mL YPD at 28 °C for 20 h and harvested by centrifugation. Cells were washed twice with distilled water and suspended with 0.9% saline to $OD = 1.0$. Finally, a 10-fold serial dilution of this culture was made and 2.5 μL of each dilution was spotted onto appropriate solid medium and cultured at 28 °C for 2 days (Liu et al., 2010).

All of the strains were applied for ethanol fermentation tests to evaluate the fermentation performances of the mutants. 18° Brix wort was used as fermentation medium. Yeast cells were first cultured in YPD medium at 28 °C for 24 h, and then transferred to a 1-L flask with 600 mL 18 °Brix wort at 1% inoculum size. The fermentation was conducted for 3 days at 28 °C. And the ethanol yields were measured at the end of fermentation.

2.4. Transmission electron microscopy (TEM) analysis

Cell wall structure of yeast strains were analyzed with transmission electron microscopy (TEM). Yeast cells were cultured to stationary-phase in YPD medium at 28 °C and then harvested for TEM analysis. Yeast cells were fixed as described by Guan et al. (Guan et al., 2012). Cells were fixed with 2.5% (w/v) glutaraldehyde-PBS buffer (0.1 mol/L, pH 7.2) overnight at 4 °C, and then rinsed with the same buffer for six times and post-fixed with 1% (w/v) osmium tetroxide for 2 h at 4 °C. The samples were dehydrated with ethanol of increasing concentrations (30, 50, 70 and 90%, v/v) and finally in acetone for three times. They were then embedded in Epon812 and polymerized. Ultrathin sections of the samples were prepared with a diamond knife and examined with a QUANTA200F TEM (Martınez-Rodrıguez et al., 2001). The thickness of the cell walls were measured in cells where the cell wall structures were clearly observed, indicating that the cell wall was cut perpendicular to the cell surface (Wang et al., 2014). Cell walls of 100 cells which were unbudded from each strain were measured at 25,000 and 50,000 magnification per cell.

2.5. Scanning electron microscope (SEM) analysis

The extracellular structures of yeasts were measured with scanning electron microscope (SEM). Yeast cells were collected by centrifugation at 1500 r/min for 10 min after being treated at 28 °C in citrate buffer (pH 4.0) for 0 h and 60 h. Samples were fixed as previously described and finally dried and sprayed with gold crystal (Guan et al., 2012; Sun et al., 2015). The shapes and appearances of 100 cells were observed at 6000 magnifications (Martınez-Rodrıguez et al., 2001). The cells' shapes were observed and compared among different strains.

2.6. Analysis of the polysaccharides of different strains

The concentrations of polysaccharides in yeast cell wall were measured to examine the influences of *FKS* family genes' mutations on yeast