Absence of Yps7p, a putative glycosylphosphatidylinositol-linked aspartyl protease in Pichia pastoris, results in aberrant cell wall composition and increased osmotic stress resistance

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Abstract

Recently, studies performed on Saccharomyces cerevisiae and Candida albicans have confirmed the importance of fungal glycosylphosphatidylinositol (GPI)-anchored aspartyl proteases (yapsins) for cell-wall integrity. Genome sequence annotation of Pichia pastoris also revealed seven putative GPI-anchored aspartyl protease genes. The five yapsin genes assigned as YPS1, YPS2, YPS3, YPS7 and MKC7 in P. pastoris were disrupted. Among these putative GPI-linked aspartyl proteases, disruption of PpYPS7 gene confers the Ppyps7D mutant cell increased resistance to cell wall perturbing reagents congo red, calcofluor white (CW) and sodium dodecyl sulfate. Quantitative analysis of cell wall components shows lower content of chitin and increased amounts of β-1,3-glucan. Further staining of the cell with CW demonstrates that disruption of PpYPS7 gene causes a reduction of the chitin content in lateral cell wall. Consistently, transmission electron micrographs show that the inner layer of mutant cell wall, mainly composed of chitin and β-1,3-glucan, is much thicker than that in parental strain GS115. Additionally, Ppyps7D mutant also exhibits increased osmotic resistance compared with parental strain GS115. This could be due to the dramatically elevated intracellular glycerol level in Ppyps7D mutant. These results suggest that PpYPS7 is involved in cell wall integrity and response to osmotic stress.

Introduction

The cell wall is an essential component for fungal cell, providing a rigid structure to maintain cell shape. The organization of the fungal cell wall has been mainly characterized in the yeast Saccharomyces cerevisiae (Klis et al., 2006; Lesage & Bussey, 2006; Latge, 2007). The yeast cell wall is a bilayered structure, with an electron-transparent inner layer and an electron-dense outer layer (Osumi, 1998). The inner part is composed of a network of β-1,3-glucan with some branches linked to β-1,6-glucan and chitin, and the outer part of the cell wall is composed mainly of mannoproteins (Lesage & Bussey, 2006). Most proteins in the cell wall of ascomycetous yeasts are glycosylphosphatidylinositol-anchored proteins (GPI-modified proteins), which are covalently linked to β-1,6-glucan through a remnant of their GPI anchor (Moreno-Ruiz et al., 2009). GPI-modified proteins consist of three major regions: an N-terminal sequence for translocation into the endoplasmic reticulum, a mature domain, and a C-terminal hydrophobic region for anchorage to the endoplasmic reticulum membrane. A so-called x site is localized nine or 10 amino acids upstream of this C-terminal sequence, where the protein is cleaved prior to its ligation to a pre-assembled GPI anchor (Thomas et al., 1990). It was reported that a large number of the estimated 60 GPI-modified proteins predicted from the S. cerevisiae genome sequence served enzymatic functions required for the biosynthesis and the continuous shape adaptations of the cell wall (Pittet & Conzelmann, 2007).
The first GPI-anchored aspartyl protease (known as yapsins) member ScYps1p was discovered in *S. cerevisiae* more than a decade ago during a search for alternative yeast enzymes capable of cleaving the paired-basic residues flanking the α-MF leader peptide (Egel-Mitani *et al.*, 1990). ScYps1p was later proved to have a preference for cleaving substrates C-terminally to mono- and paired basic residues (Azaryan *et al.*, 1993). Over the years, several members of this gene family, first in *S. cerevisiae* and then in other fungi, have been isolated (Krysan *et al.*, 2005; Kaur *et al.*, 2007). However, the exact roles these genes played in the fungal cell were still not clear. Only recently was it confirmed that yapsins were a gene family required for cell wall integrity in *S. cerevisiae* (Krysan *et al.*, 2005). Gagnon-Arsenault and colleagues thoroughly reviewed the unique domain organization, cell surface localization, substrate specificity of fungal yapsins and their gene expression pattern in periods of cell-wall synthesis and remodelling (Gagnon-Arsenault *et al.*, 2006).

Genome sequence annotation of the industrial host *Pichia pastoris* also revealed seven putative GPI-anchored aspartyl protease genes (De Schutter *et al.*, 2009). Until now, there have been no reports related to the function of GPI-anchored aspartyl proteases in *P. pastoris*. Research focused only on strain engineering via disruption of *PpYPS1* gene to reduce heterologous protein degradation (Werten & de Wolf, 2005; Yao *et al.*, 2009). Attempts to explore the possible application of the other putative GPI-anchored aspartyl protease gene null mutants to improve heterologous protein expression are also ongoing in our group. Unexpectedly, distinct phenotypes of the absence of one of the aspartyl protease genes, *PpYPS7*, were observed. Thus, in this paper we present the function analysis of loss of *PpYPS7* gene in *P. pastoris* and propose its possible role in cell wall integrity and osmotic stress response.

**Materials and methods**

**Plasmid, primers and strains**

The plasmid pPICZC, which was used to place the Cre gene under the control of the stringent AOX1 promoter, was kindly provided by Prof. Xin Yan. Primers used in this study are listed in Table 1. *Pichia pastoris* GS115 strain (Invitrogen), defective in histidine dehydrogenase gene (*his4*), was used as a parental strain for gene disruption and all the strains used in this study are listed in Table 2.

**Growth media and culture conditions**

The yeast cells used in this study were grown in the complex medium [YP: 1% (w/v) yeast extract; 2% (w/v) peptone] supplemented with 2% (w/v) glucose (YPD) at 30 °C. For the phenotype analysis, the yeast cells were grown in YPD or YP supplemented with 2% (w/v) methanol (YPM) or glycerol (YPG) instead of glucose. For the chitin content assay, the yeast cells were pre-cultured in YPD medium to the mid-exponential stage and further cultured in YPD or YP supplemented with 10 μg mL⁻¹ calcofluor white (CW) for 3 h. For intracellular glycerol determination, the yeast cells were pre-cultured in YPD medium to the mid-exponential stage. The cells were collected and resuspended in the same volume of fresh YPD supplemented with 1.0 M NaCl. Samples were collected at the time points indicated.

**Construction and verification of the *P. pastoris* yapsin gene disruption mutants**

A fusion/extension PCR-mediated gene replacement method (Fig. 1a) was adopted to disrupt the ORF sequence of *PpYPS7* gene using the selectable marker zeocin resistance gene (ZeoR) as described by Pan *et al.* (2011). Briefly, a pPICZC plasmid, in which the Cre gene was under the control of tightly regulated AOX1 promoter, was used as template to amplify the self-excizable *lox71*-Cre-ZeoR-*lox66* (CORE) cassette by PCR, with *lox71* and *lox66* sites introduced by primers Crep1 and Crep2, respectively (Fig. 1). The upstream (Up-arm) and downstream (Down-arm) homologous arms of the target gene were individually amplified from *S. cerevisiae* and then in *P. pastoris* GS115 genomic DNA by PCR using two sets of primers. The reverse primer of Up-arm fragment and the forward primer of Up-arm fragment each shared 30–40 common nucleotides with the CORE cassette. Then the CORE cassette and homologous arms were spliced together by fusion PCR to generate the gene disruption cassettes (homologous region-*lox71*-Cre-ZeoR-*lox66*-homologous region) according to a two-step procedure described by Shevchuk *et al.* (2004). The product of gene disruption cassettes was purified by gel extraction and then introduced into competent cells of the parental strain GS115 by the standard electroporation procedure. The zeocin-resistant (ZeoR) clones screened on YPDD (YPD with 100 μg mL⁻¹ zeocin) plate were randomly selected for further verification by genomic PCR analysis using primer Wp1 and primer Pc. After verification of the disrupted genotype, the ZeoR selective marker was subsequently removed by Cre-mediated recombination between *lox71* and *lox66* through a methanol induction step in YPM medium. The genotype of the resulting zeocin-sensitive (ZeoS) transformants were further identified by genomic PCR using primer Wp1 and Dp2, and the expression analysis of disrupted gene was verified by RT-PCR using *PpACT1* gene as an internal control (Fig. 1b).
Phenotypic analysis

Cells were grown overnight in liquid YPD medium at 30 °C. Then equal amounts of cells (approximately $5 \times 10^7$ cells) for each strain were diluted in a series of 10-fold magnitude (from $10^{-1}$ to $10^{-5}$ relative to the initial culture) in water. Aliquots ($5 \mu$L) from each dilution series were then applied onto the indicated media and cultured at 30 °C for 2–4 days.

Measurement of chitin content

Total cellular chitin was measured as described by Bulawa et al. (1986) and modified by Imai et al. (2005). In brief, washed cells (~50 mg wet cells) were resuspended in 500 μL of 6% KOH and incubated at 80 °C for 90 min. After cooling at room temperature, 50 μL of glacial acetic acid was added. Insoluble material was washed twice with water and resuspended in 250 μL of 50 mM sodium phosphate (pH 6.3). A 2-mg aliquot of *Streptomyces griseus* chitinase (Sigma) was added and the tubes were then incubated at 25 °C with gentle agitation for 2 h. After enzyme digestion, the tubes were centrifuged at 15 000 g for 5 min at room temperature, and 250 μL of supernatant was transferred to a fresh tube to which 1 mg of *Helix pomatia* β-glucuronidase (Sigma) was added. Tubes were incubated at 37 °C for 2 h with gentle agitation. N-acetylglucosamine content was measured according to Reissig et al. (1955). The cell wall chitin content was normalized by cell optical density and expressed as mg GlcNAc per $10^7$ cells.

Table 2. *Pichia pastoris* strains used in this study

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<th>Strain</th>
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Table 2. *Pichia pastoris* strains used in this study

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<td>Y1up2</td>
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Phenotypic analysis

Cells were grown overnight in liquid YPD medium at 30 °C. Then equal amounts of cells (approximately $5 \times 10^7$ cells) for each strain were diluted in a series of 10-fold magnitude (from $10^{-1}$ to $10^{-5}$ relative to the initial culture) in water. Aliquots (5 μL) from each dilution series were then applied onto the indicated media and cultured at 30 °C for 2–4 days.
Quantitative β-1,3-glucan measurement

The amount of β-1,3-glucan per cell was measured using aniline blue as described previously (Sekiya-Kawasaki et al., 2002) with modification. In brief, cells were grown to OD$_{600}$ 1.0 and 5.0 $\times 10^6$ cells were harvested. The cells were washed twice with TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and resuspended in 250 μL of TE. Then 6 N NaOH was added to the cells to a final concentration of 1 N. The tube was incubated at 80 °C for 30 min followed by the addition of 1.05 mL of AB mix [0.03% aniline blue (Shanghai Sangon), 0.18 N HCl, and 0.49 N glycine/NaOH, pH 9.5]. The tube was vortexed briefly, then incubated at 50 °C for 30 min. Fluorescence of β-1,3-glucan was quantified using a spectrofluorometer (Thermo Scientific Fluoroskan Ascent FL) with an excitation wavelength of 386 nm, and emission wavelength at 460 nm.

Intracellular glycerol determination

The intracellular glycerol was determined enzymatically by a commercial glycerol determination kit (Chaoyan, Shanghai) following the manufacturer’s specifications. Briefly, the cells (1.5 mL) were recovered by 5 min of centrifugation at 13 000 g, resuspended in the same volume of boiling water and heat-treated at 95 °C for 10 min in tubes. After cooling, the cells were collected by centrifugation, and the supernatant was assayed for its glycerol concentration. The number of cells was calculated by the value of OD$_{600}$ (1 OD$_{600}$ = 5 $\times 10^7$ cells).
and intracellular glycerol was expressed as μM glycerol per 5 × 10^7 cells.

**Fluorescence microscopy**

CW staining was performed according to Pringle (1991) and Imai *et al.* (2005). Briefly, log-phase cells grown at 30 °C were fixed with 3.7% paraformaldehyde and stained with 0.1 mg mL^-1^ CW for 1 h. Cells were washed three times with water and mounted on a slide. Images were obtained using a fluorescent microscope (Nikon).

**Transmission electron microscopy (TEM)**

Yeast cell samples were fixed in 2.5% (w/v) glutaraldehyde in phosphate buffer (pH 7.4) overnight at 4 °C. Thereafter, the samples were rinsed thoroughly with the same buffer three to six times and post-fixed with 1% (w/v) osmium tetroxide for 2 h at 4 °C. Then the samples were dehydrated in a graded ethanol series (30, 50, 70 and 90%, v/v) and in 1 : 1 mixture of 90% ethanol and 90% acetone, and finally in acetone three times. The samples were embedded in Epon812 and polymerized at 70 and 90%, v/v) osmium tetroxide for 2 h at 4 °C. Then the samples were cut with a diamond knife and collected on 200-mesh copper grids. After contrast with uranyl acetate and lead citrate, the grids were examined with a JEM-2100 transmission electron microscope.

**Results**

**Bioinformatics of PpYPS7 gene**

According to the *P. pastoris* genomic sequence and Gene annotation Database of *P. pastoris* (http://www.pichiagenome.org/), *PpYps7p* was predicted to contain 582 amino acid residues with a molecular mass of 63 kDa. The PSORT programs predicted the presence of a cleavable signal peptide (residues 1–16) in *PpYps7p*. The PSORT programs and big-PI predictor (http://mendel.imp.univie.ac.at/gpi/gpi_server.html) also predicted the presence of an anchoring amino acid (the ω site, residues 555) and putative glycosylphosphatidylinositol (GPI) anchor attachment on its C-terminus, suggesting *PpYps7p* may be located at the plasma membrane or cell wall.

The *Ppyps7Δ* mutant is more resistant to cell wall perturbing reagents

To characterize whether there were any cell wall defects associated with loss of yapsin function, we examined the growth phenotype of the five yapsin gene disruption mutants in the presence of two cell wall-disturbing reagents, CW and congo red (CR) (Kaeberlein & Guarente, 2002). As the results show in Fig. 2a, all of the mutants except *Ppyps7A* mutant grew normally compared with the parental strain GS115 in the presence of CW or CR. Unexpectedly, *Ppyps7A* mutant showed increased resistance to CR and calcofluor white under the conditions tested (30 μg mL^-1^).

It has been reported that the growth of ScYPS7 (homolog in *S. cerevisiae*) null mutant was shown to be more sensitive to CR and CW (Krysan *et al.*, 2005). To further confirm the phenotype of *Ppyps7A* mutant, we determined the growth of *Ppyps7A* and parental strain GS115 on media containing 50 μg mL^-1^ CW or CR. We observed that the *Ppyps7A* mutant was consistently more resistant to these agents compared with the GS115 strain (Fig. 2b). In addition, *Ppyps7A* was also significantly more resistant to another cell wall-degrading reagent, sodium dodecyl sulfate (SDS). As methylotrophic yeast, *Ppyps7A* mutant grew normally on YPD and YPG plate as the parental strain GS115 did, and even somewhat better on a YPM plate. We also tested the effect of high temperature (37 °C) on growth of *Ppyps7A* mutant and found *Ppyps7A* mutant could grow normally at 37 °C on a YPD plate and YPD plate supplemented with 1 M sorbitol (Fig. 2).

**Disruption of *P. pastoris* YPS7 gene results in aberrant cell wall composition**

The increased resistance of *Ppyps7Δ* mutant to the tested cell wall-disturbing agents encouraged us to investigate the difference in cell wall composition in *Ppyps7Δ* mutant. The content of chitin in the cell wall of *Ppyps7Δ* mutant and in wild-type strain GS115 were measured. As shown in Fig. 3a, the chitin content in the cell wall of *Ppyps7Δ* mutant was slightly lower than that of strain GS115. We also determined the chitin content of the yeast cells after treatment with 10 μg mL^-1^ CW in the culture medium for 3 h, and found the chitin content in *Ppyps7Δ* mutant cell was significantly lower than that in GS115 cell. Then we compared the β-1,3-glucan level in the cell wall of *Ppyps7Δ* mutant and in wild-type strain GS115. The result showed that there was an increased amount (~129%) of β-1,3-glucan in *Ppyps7Δ* mutant compared with GS115 (Fig. 3b).

We further examined the chitin content in the cell wall by staining the cell with CW by microscopy. For the parental strain GS115, the fluorescent dye stained strongly the bud necks and scars, and less strongly but uniformly the lateral cell wall (Fig. 4a). However, while the bud necks and scars were still showing very...
strong fluorescence, the fluorescence stained to the lateral cell wall of Ppyps7Δ mutant reduced significantly (Fig. 4b).

**Ppyps7Δ mutant shows osmotic stress resistance**

Further analysis of growth phenotype showed that Ppyps7Δ mutant also exhibited increased osmotic resistance (0.8 M NaCl) compared with strain GS115 (Fig. 5). This improved growth of Ppyps7Δ mutant under 0.8 M NaCl did not seem to be sodium-specific, as a similar result was also observed when the mutant cells were treated with 0.8 M KCl. Mutant cells were able to grow on higher salt concentration (1.2 M KCl and 1.2 M NaCl) in which GS115 normally could not grow (Fig. 5).

Glycerol plays an essential role in the adaptation to osmotic stress in yeast (Tamas & Hohmann, 2003). To investigate whether the increased osmotic resistance of Ppyps7Δ mutant was correlated to an elevated intracellular glycerol level, we analyzed the intracellular glycerol levels of strain GS115 and Ppyps7Δ mutant cells after transfer to YPD medium containing 1.0 M NaCl. As demonstrated in Fig. 6, the intracellular glycerol concentration in the Ppyps7Δ cells was higher than that in GS115 cells in the initial time point of the assay, and became significantly much higher after 4 h ($P < 0.05$) following transfer to YPD medium containing 1.0 M NaCl.
**TEM analysis of Ppyps7Δ mutant cell**

There were significant differences in the TEM micrographs between the cell wall of GS115 and Ppyps7Δ mutant. As shown in Fig. 7, the inner layer of the cell wall, mainly composed of chitin and β-1,3-glucan, was much thicker in Ppyps7Δ mutant than that in GS115 cells. The electron density of the inner layer in the Ppyps7Δ mutant cell wall was also lower than that in GS115. These differences between GS115 and Ppyps7Δ mutant cells illustrated that disruption of PpYPS7 gene in *P. pastoris* altered the width of the inner layer of the cell wall composed of chitin and β-1,3-glucan.

**Discussion**

In this study, we have investigated the possible role putative GPI-linked aspartyl protease PpYps7p plays in the cell wall integrity in methylotrophic yeast *P. pastoris*. Other single disruption mutants such as Ppyps1Δ, Ppyps2Δ, Ppyps3Δ and Ppmkc7Δ constructed in this study showed normal growth phenotype in the presence of cell wall-disrupting agents compared with GS115. When PpYPS7 gene was disrupted, we observed Ppyps7Δ mutant showed abnormally increased resistance to the cell wall-perturbing agents CW, CR and SDS.

It has been proved that disruption of gene related to chitin synthesis or overexpression of gene repressing chitin synthesis, makes the cell more resistant to CW (Bulawa, 1992; Martin *et al.*, 1999; Trilla *et al.*, 1999). The primary binding targets for CR in yeast was proved to be chitin rather than glucan (Imai *et al.*, 2005). Consistently, the chitin content in the cell of Ppyps7Δ mutant was lower than that of strain GS115. Microscopical analysis of fluorescent stained chitin by CW also demonstrated that disruption of PpYPS7 gene resulted in a reduction of the chitin content in the lateral cell wall. Based on these research results, we propose that disruption of PpYPS7 gene may affect chitin synthesis in *P. pastoris*. CW preferentially binds to chitin and alters the assembly of its microfibrils in yeast (Elorza *et al.*, 1983), inhibiting the growth of yeast. CW treatment could also stimulate the chitin polymerization in vivo (Roncero & Duran, 1985). Accordingly, we investigated the chitin content in cell wall after treatment with CW (10 μg mL⁻¹) in the culture medium for 3 h. As expected, after treatment with CW, the chitin content in Ppyps7Δ mutant cells was significantly lower than that in GS115 cells. This significant repression of chitin polymerization in Ppyps7Δ mutant cells further suggested that the absence of Yps7p in *P. pastoris* reduced chitin content by repressing chitin synthesis and thereby conferred Ppyps7Δ mutant increased resistance to CR and CW. It is noteworthy that Ppyps7Δ was also significantly more resistant to another cell wall destabilizing reagent, SDS, indicating the cell wall of Ppyps7Δ may be strengthened. To verify our assumption, we observed the Ppyps7Δ mutant cell by electron microscopy. The result showed that the inner layer of the cell wall, mainly composed of chitin and β-1,3-glucan, was much thicker in Ppyps7Δ mutant. In addition, the β-1,3-glucan level determined in the cell wall of Ppyps7Δ mutant was higher than that of parental strain GS115. These results could be explained by a hypothesis that the absence of Yps7p in *P. pastoris* reduced chitin synthesis. Accompanied by lower chitin content, in turn, Ppyps7Δ mutant synthesized excessive glucan, which may serve as a compensatory response to ensure cell integrity (Ram *et al.*, 1998; Carotti *et al.*, 2002; Lesage *et al.*, 2005). Nevertheless, the exact mechanism of how PpYps7p affects chitin synthesis needs further study.

Ppyps7Δ mutant also showed significantly increased osmotic resistance compared with parental strain GS115. The accumulation of glycerol in yeasts such as *S. cerevisiae*...
is a mechanism in cellular osmoregulation. To investigate whether the increased osmotic resistance of Ppyps7Δ mutant could be attributed to elevated intracellular glycerol level, we determined the intracellular glycerol level in Ppyps7Δ mutant. As expected, the intracellular glycerol concentration in the Ppyps7Δ cells became significantly higher than that in GS115 cells, suggesting that the elevated intracellular glycerol level is one of the factors contributing to osmotic resistance of Ppyps7Δ mutant.

Fig. 4. Fluorescent micrographs of chitin in the Ppyps7Δ mutant and parental strain GS115 cells by CW staining. The parental strain GS115 (a) and Ppyps7Δ mutant (b) cells were fixed in formaldehyde and observed by fluorescence microscope after staining with CW. Fluorescence images were obtained under a same capture conditions. (c, d) Corresponding bright-field images. 219 × 181 mm (120 × 120 DPI).

Fig. 5. Phenotypic analysis of Ppyps7Δ mutant and parental strain GS115 under osmotic stress. Aliquots (5 μL) of 10-fold serial dilutions of Ppyps7Δ mutant and parental strain GS115 cells were plated onto the designated media and cultured at 30 °C for 3 days. 212 × 231 mm (72 × 72 DPI).

Fig. 6. Intracellular glycerol level of Ppyps7Δ mutant and parental strain GS115 cells following transfer to YPD medium containing 1.0 M NaCl, respectively, for 0, 2, 4 and 8 h. Cells pre-cultured to mid-log phase in YPD medium were collected and subsequently transferred an equal volume of fresh YPD medium containing 1.0 M NaCl. Values are the mean of three independent experiments. The results were analyzed by Student’s t-test. *P < 0.05, **P < 0.01. 110 × 76 mm (300 × 300 DPI).
Although it is unlikely that the increase in the cell wall width of Ppyps7D mutant played a role in limiting the access of foreign toxic ion during osmotic stress created by salt (NaCl or KCl) treatment, the inner layer of the wall composed of β-1,3-glucan/chitin, could provide increased mechanical strength when the cell turgor pressure increased significantly during osmotic stress (Klis et al., 2002). However, it is still intriguing that the absence of Yps7p, a GPI-linked aspartyl protease, in P. pastoris conferred the Ppyps7D mutant increased osmotic resistance. As another aspartyl protease, Yps1p in S. cerevisiae, was reported to be involved in the Cdc42p-dependent MAPK pathway activation (Vadaie et al., 2008), we speculated that Yps7p might also be involved in osmotic stress response in addition to the cell wall integrity pathway in P. pastoris. Although the precise molecular mechanism is still unknown, a series of related reports (Alonso-Monge et al., 1999; Garcia-Rodriguez et al., 2000; Wojda et al., 2003; Levin, 2005) may provide some clues for uncovering the mechanism of Yps7p involved in osmotic resistance.

Primarily, the discovery of the GPI-anchored aspartyl protease gene was beneficial for the biotechnology industry. The deletion strains of these genes have been useful in the recovery of full-length recombinant proteins from yeast culture supernatants (Kerry-Williams et al., 1998; Yao et al., 2009; Cho et al., 2010; Ganatra et al., 2011). Thus, from a biotechnological aspect, PpYps7p is also potentially interesting as an engineering target for reduced degradation of heterologous protein during secretory expression. The Ppyps7A mutant, which is resistant to cell wall stress and osmotic stress, should have a significant advantage in industrial applications. This may represent another advantage of the PpYPS7 deletion mutant strain as an expression host strain.

In conclusion, the present work revealed Yps7p involvement in both increased resistance to the cell wall-perturbing agents and osmotic stress. Although we cannot yet attribute a molecular function to Yps7p, the pleiotropic phenotype of Ppyps7A mutant implied a possible role of Yps7p in connection with pathways for cell maintenance and osmotic stress response.

Acknowledgements

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References


![Fig. 7. TEM analysis of Ppyps7A mutant cell. Log-phase cells of Ppyps7A mutant (b, d, f) and parental strain GS115 (a, c, e) were fixed in 2.5% (w/v) glutaraldehyde in phosphate buffer (pH 7.4) overnight. (e, f) Glucan/chitin-rich inner layer and mannose-rich outer layer of the cell wall. Scale bars: (a, b) 1 μM, (c, d) 200 nm, (e, f) 100 nm. 705 × 470 mm (72 × 72 DPI).](https://academic.oup.com/femsyr/article-abstract/12/8/969/613583)


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