




# High temperature induced disruption of the cell wall integrity and structure in *Pleurotus ostreatus* mycelia

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## Abstract

Fungal cells are surrounded by a tight cell wall to protect them from harmful environmental conditions and to resist lysis. The synthesis and assembly determine the shape, structure, and integrity of the cell wall during the process of mycelial growth and development. High temperature is an important abiotic stress, which affects the synthesis and assembly of cell walls. In the present study, the chitin and  $\beta$ -1,3-glucan concentrations in the cell wall of *Pleurotus ostreatus* mycelia were changed after high-temperature treatment. Significantly higher chitin and  $\beta$ -1,3-glucan concentrations were detected at 36 °C than those incubated at 28 °C. With the increased temperature, many aberrant chitin deposition patches occurred, and the distribution of chitin in the cell wall was uneven. Moreover, high temperature disrupts the cell wall integrity, and *P. ostreatus* mycelia became hypersensitive to cell wall-perturbing agents at 36 °C. The cell wall structure tended to shrink or distorted after high temperature. The cell walls were observed to be thicker and looser by using transmission electron microscopy. High temperature can decrease the mannose content in the cell wall and increase the relative cell wall porosity. According to infrared absorption spectrum, high temperature broke or decreased the glycosidic linkages. Finally, *P. ostreatus* mycelial cell wall was easily degraded by lysing enzymes after high-temperature treatment. In other words, the cell wall destruction caused by high temperature may be a breakthrough for *P. ostreatus* to be easily infected by *Trichoderma*.

**Keywords** *Pleurotus ostreatus* · Cell wall · Integrity · Structure · *Trichoderma*

## Introduction

*Pleurotus ostreatus* is a widely cultivated mushroom in China. It is produced mainly by traditional methods, which is cultivated in plastic greenhouse and is easily affected by external environment. Interestingly, if ventilation and cooling are untimely given, green mold disease may breakout in large areas when mycelia are subjected to high temperature during spawn running period. Green mold disease, which severely inhibits mycelial growth and the yield of *P. ostreatus*, is a severe disease of this mushroom (Komon-Zelazowska et al. 2007; Kredics et al. 2009). However, little is known about the reason

why high temperature-treated *P. ostreatus* mycelia are easily infected by *Trichoderma*.

Fungal cell wall is an important dynamic structure that can adapt to changes in the external environment to satisfy the needs for growth and development. In terms of composition, the fungal cell wall is mainly composed of glucans, chitin, mannans, and glycoproteins (Mendoza 1992; Oka et al. 2015). Although there are many important changes in the composition of the cell wall in different species, the framework of the cell wall in basidiomycetes and ascomycetes is similar. Structurally, the fungal cell wall mainly contains an electron-transparent inner layer network, which is constructed mainly of  $\beta$ -1,3-glucan chains linked with  $\beta$ -1,6 glycosidic linkages; the chitin inside the network branches to  $\beta$ -1,3-glucan chains with  $\beta$ -1,4 glycosidic linkages and  $\beta$ -1,6-glucan chains at the outside of network (Klis et al. 2006). The electron-dense outer layer of the fungal cell wall is a glycosylated mannoprotein layer (Osuni 1998). Proper assembly of polysaccharide chains is responsible for the strength and elasticity of the cell wall (Rees et al. 1982; Smits et al. 1999). The main difference between basidiomycetes and ascomycetous

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yeasts is that the former has a multi-layer cell wall that alternately appears in two regions with the electron-dense layer and the electron-transparent layer (Depree et al. 1993). With the strength and elasticity of the cell wall, there is an effective barrier to protect fungal cells (Levin 2011). When fungal cells suffer from environmental stresses, they initiate a series of metabolic pathways to maintain cell shape and cell wall integrity (CWI) (Klis et al. 2002). The CWI pathway is a principal signal pathway to maintain the stability of cell wall in response to cell wall stress (Levin 2005).

To resist the adverse environment stresses, CWI signaling pathway will be activated to synthesize the main cell wall components. These components are only assembled properly to form a complete cell wall and consequently alleviate cell damage (Cabib et al. 2001). High temperature is an important abiotic stress that can activate the expression of many genes involved in confirming the CWI (Jung and Levin 1999; Verna et al. 1997). When the CWI is destroyed, fungal cell will be sensitive to cell wall-perturbing agents, such as Calcofluor White (CFW) (Liu et al. 2004), sodium dodecyl sulfate (SDS) (Bickle et al. 1998), and Congo Red (CR) (Garcia et al. 2015). In the process of infecting other fungal mycelia, *Trichoderma* secretes cell wall-degrading enzymes (CWDEs, Geraldine et al. 2013). Incomplete cell walls will decrease the ability to resist CWDEs (Cortes et al. 2004).

A variety of *Trichoderma* species can cause *Pleurotus* green mold disease. Many previous studies have identified the pathogenic *Trichoderma* species which cause *Pleurotus* green mold disease (Błaszczuk et al. 2013; Kredics et al. 2009). Nevertheless, the role of high temperature in the outbreak of green mold disease is still poorly understood. In our previous study, we found that high temperature reduces the resistance of *P. ostreatus* mycelia to *Trichoderma asperellum* (Qiu et al. 2017). After *P. ostreatus* mycelium was treated with high temperature, it is easily infected by *T. asperellum*. The aim of this study is to detect the effects of high temperature on the CWI and structure of *P. ostreatus* mycelia and to explain the relationship between cell wall disruption and *Trichoderma* infection.

## Materials and methods

### Strain, media, and culture condition

*P. ostreatus* P89 (CCMSSC 00389) was provided by the China Center for Mushroom Spawn Standards and Control. For pure culture of *P. ostreatus*, a piece of P89 disc (a diameter of 5 mm punch) from solid medium was inoculated on potato dextrose agar (PDA; Difco-Becton Dickinson, Sparks, MD) and incubated at 28 °C for 7 days. For P89 mycelium collection, ten P89 mycelial discs (5 mm) were inoculated into 250 mL Erlenmeyer flasks containing 100 mL of potato

dextrose broth (PDB; Difco-Becton Dickinson, Sparks, MD) and incubated at 28 °C, 150 rpm for 5 days. Subsequently, the flasks were transferred into different rotary shakers (ZQLY-180F, Shanghai Zhichu Instrument Company Limited, Shanghai, China) with different temperatures (28, 32, 36, and 40 °C) for 2 days. The effects of different temperatures on *P. ostreatus* mycelia cultured with submerged cultivation were observed, and the temperature of the mycelia in submerged culture was more homogeneous.

### Determination of chitin and $\beta$ -1,3-D-glucan contents in *P. ostreatus* mycelia

Two days after treatment under different temperatures (28, 32, 36, and 40 °C), mycelia were collected by filter paper (Whatman,  $\Phi$ 11, GE Healthcare Ltd., New Jersey, USA), washed three times with NaOH (0.1 M), dried in a vacuum freeze dryer (Christ ALPHA 1-2 LD plus, Osterode, Germany), and ground into powder. The aniline blue assay was used to detect the effects of different temperatures on  $\beta$ -1,3-D-glucan concentrations in *P. ostreatus* P89 mycelia (Fortwendel et al. 2009). Fluorescence readings were measured in Thermo Scientific Microplate Reader (Scientific Fluoroskan Ascent FL, Waltham, MA, USA) at an excitation wavelength of 405 nm and emission wavelength of 460 nm. To normalize values, curdlan, a  $\beta$ -1,3-D-glucan analog (Sigma-Aldrich, St. Louis, MO, USA) was used to construct the standard curve. Values were calculated and obtained from the fluorescence units of curdlan in unit weight. Each sample was performed in triplicate.

Chitin concentration was measured according to a previously described method (Bulik et al. 2003). The standard curve was created with GlcNAc (Sigma-Aldrich, St. Louis, MO, USA). Absorbance was measured at 585 nm using the Thermo Scientific Microplate Reader (Scientific Fluoroskan Ascent FL, Waltham, MA, USA). Measurement was performed three times for each sample.

### Fluorescence staining and microscopy

To observe the particular location of chitin in the skeletal structures, fluorescent brightener (F3543, Sigma-Aldrich, St. Louis, MO, USA), a kind of CFW, was applied with some modifications (Song et al. 2010). P89 mycelial discs were grown on a thin layer of PDA on the microscope slides. After 3 days of incubation at 28 °C, mycelia were transferred to incubators with different temperatures (28, 32, 36, and 40 °C) and incubated for 2 days. For staining, mycelia were incubated with fluorescent brightener (10  $\mu$ g/mL) at 28 °C for 5 min. Finally, mycelial fluorescence was observed under laser scanning confocal microscopy (Zeiss LSM700, Jena, Germany).

## Sensitivity of *P. ostreatus* mycelia to cell wall-perturbing agents

To investigate the effects of high temperature on the CWI of P89, cell wall-perturbing agents (i.e., CFW, SDS, and CR) were applied. P89 mycelial discs (5 mm) were inoculated onto the center of PDA plates with or without different concentrations of CFW (400 µg/mL), SDS (0.005%), and CR (300 µg/mL). The plates were first incubated at 28 °C for 3 days, and the colony diameter was recorded (D1). Subsequently, the plates were transferred to incubators with temperatures of 28 and 36 °C. After 2 days of incubation, the colony diameter was recorded (D2). The relative inhibitory rate of cell wall-perturbing agents on mycelial growth at 36 °C was calculated as follows.

$$\text{The relative inhibitory rate} = \left(1 - \frac{D2-D1}{D1}\right) * 100\% \quad (1)$$

## Observation of cell wall under scanning electron microscope (SEM) and transmission electron microscopy (TEM)

To clearly observe the effects of temperature on cell wall structure, the SEM (Inspect S50, FEI, OR, USA) and TEM (Tecnai G2 Spirit, FEI, OR, USA) were used. Ten P89 mycelial discs (5 mm) were inoculated into 250 mL Erlenmeyer flasks containing 100 mL of PDB and incubated at 28 °C, 150 rpm for 5 days. Afterward, the flasks were transferred into rotary shakers at different temperatures (28 and 36 °C) for 2 days. Mycelia were collected by centrifugation (Sigma 3K30, Osterode, Germany) at 10,000 ×g for 10 min. Samples for SEM were prepared according to a previously described method (Staniszewska et al. 2013). Samples for TEM were also prepared according to a previously described method (Zhang et al. 2011).

## Effect of high temperature on cell wall porosity

Proper cross-linking of cell wall main components can establish a tight cell wall. Mannose in the outermost layer of fungal cell wall can determine the cell wall porosity. The mycelial culture method was the same as that mentioned previously. Mycelia were collected by centrifugation and washed thrice with distilled water. Relative cell wall porosity was detected as previously described (De Nobel et al. 1990). Mannose content was measured by HPLC (Akabane et al. 2014). P89 mycelia were dried in a vacuum freeze dryer and ground into powder. Standard mannose was purchased from Sigma-Aldrich (St. Louis, MO, USA).

## Infrared absorption spectrum of alkali-insoluble β-glucan

To investigate the effects of high temperature on the cross-linking between β-glucan units, alkali-insoluble β-glucan

was extracted according to the method of a previous study with some modifications (Gopal et al. 1984). Two days after treatment under different temperatures (28 and 36 °C), mycelia were collected by centrifugation at 10,000 ×g for 10 min and washed thrice with distilled water. Mycelia (0.1 g) were mixed with 50 mL NaOH (1 M) and then boiled at 100 °C for 1 h. After cooling to room temperature, samples were centrifuged at 6000 rpm for 15 min. Precipitate was suspended in NaOH (0.75 M) and maintained at 100 °C for 15 min. After cooling, the pH value was adjusted to 4.5 by HCl (1 M). Briefly, samples were centrifuged at 10,000 ×g for 5 min and washed with distilled water. Finally, samples were washed twice with absolute ethyl alcohol, dehydrated with ether, and dried at 37 °C. Samples and potassium bromide were ground evenly and scanned with infrared spectrometer (VERTEX70, Bruker, Ettlingen, Germany).

## Lysing enzyme sensitivity assays

The sensitivity of *P. ostreatus* mycelia to lysing enzymes (purchased from Guangdong Institute of Microbiology, Guangzhou, China) was also performed to evaluate for defects in the CWI. Lysing enzymes were a kind of cell wall-degrading enzymes which were produced by *Trichoderma*. Ten P89 mycelial discs (5 mm) were inoculated into 250 mL Erlenmeyer flasks containing 100 mL of PDB and incubated at 28 °C, 150 rpm for 5 days. Subsequently, these flasks were transferred into rotary shakers at different temperatures (28 and 36 °C). After 2 days of treatment, mycelia were collected by filter paper and washed thrice with sterile mannitol (0.6 M). Afterward, 0.2 g of mycelia was added into lysing enzymes (1.5% in mannitol (0.6 M)) and incubated at 30 °C for 2.5 h. The number of protoplasts was counted using a hemocytometer at 0.5 h intervals. Experiments were performed in triplicate.

## Statistical analysis

Data are presented as the mean and the standard deviation (SD). Analysis of variance (ANOVA) and Duncan's multiple range tests ( $p \leq 0.05$ ) were applied for data analysis. SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

## Results

### Cell wall main components were altered after high temperature

Temperature can affect the synthesis of main cell wall components. The concentrations of chitin and β-1,3-D-glucan in *P. ostreatus* P89 mycelial cell were measured. After high-

temperature treatment (32, 36, and 40 °C), the chitin and  $\beta$ -1,3-D-glucan concentrations in *P. ostreatus* mycelia were changed. Significantly ( $p \leq 0.05$ ) more chitin accumulated in the cell wall after high temperature (Fig. 1a). Chitin concentrations were remarkably increased by 148.5%–238.5% after high temperature compared to that of mycelia incubated at 28 °C. After P89 mycelia were treated with 32 and 36 °C for 2 days, the  $\beta$ -1,3-D-glucan concentrations were also significantly ( $p \leq 0.05$ ) increased compared to those incubated at 28 °C (Fig. 1b). However, the  $\beta$ -1,3-D-glucan concentration after treatment at 40 °C was significantly ( $p \leq 0.05$ ) lower than those in other treatments.

### High temperature changed the distribution of cell wall chitin

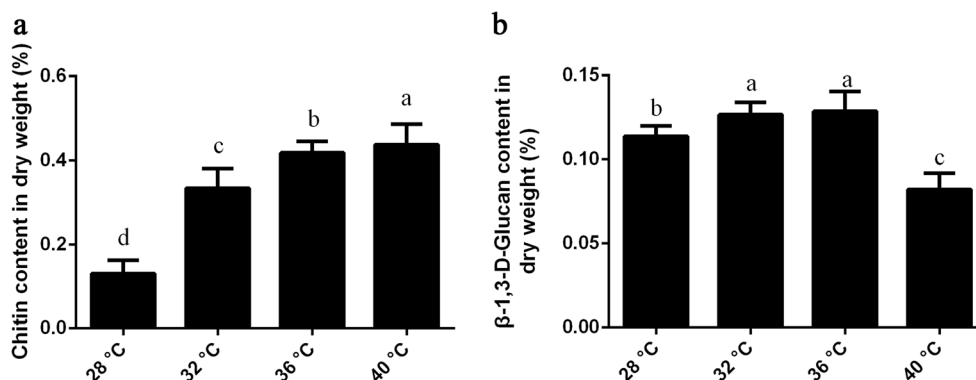
To further characterize the distribution of chitin in P89 mycelia, CFW was applied. CFW can bind to fungal cell wall chitin. Consequently, chitin distribution can be indicated by CFW fluorescence under laser scanning confocal microscopy. Chitin was evenly distributed throughout the mycelia incubated at 28 °C; the fluorescence at the septum and tips was slightly stronger than those in other parts (Fig. 2a). The fluorescence of mycelia treated with moderately high temperature (32 °C) was similar to that incubated at 28 °C. A slight difference was that few chitin deposition was observed in the cell wall (Fig. 2b). However, chitin distribution was abnormal in the cell wall after mycelia were treated with 36 °C. A considerable amount of aberrant chitin deposition was also observed, and extensive bright patches were unevenly distributed on the cell wall of *P. ostreatus* P89 mycelia (Fig. 2c). Additionally, the whole mycelia treated at 40 °C showed intense fluorescence (Fig. 2d). These results were consistent with the chitin concentrations in the cell wall after high-temperature treatment.

### High temperature enhanced the sensitivity of mycelia to cell wall-perturbing agents

Disruption of CWI can enhance the sensitivity of mycelia to cell wall-perturbing agents. The mycelial growth of P89 was inhibited under the condition of CFW, SDS, and CR at 28 °C (Fig. 3a). Moreover, high temperature (36 °C) can also inhibit mycelial growth (Fig. 3a). However, the mycelial growth of P89 was strongly inhibited in the plates with cell wall-perturbing agents at 36 °C. The sensitivity of P89 mycelia to these agents at 36 °C was also significantly enhanced according to the relative inhibitory rate (Fig. 3b). The increased sensitivity to cell wall-perturbing agents at 36 °C indicated that the cell wall integrity of P89 mycelia was defective. The above-mentioned results together suggest that high temperature can destroy the integrity of the cell wall.

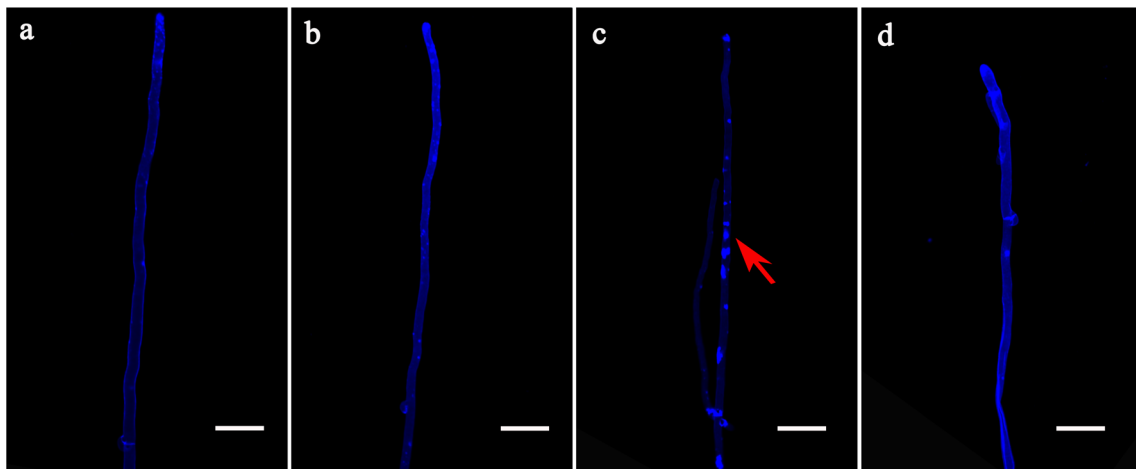
### High temperature altered the cell wall structure

The ultrastructural features of cell wall surface and structure can be clearly examined by using SEM and TEM. The mycelia incubated at 28 °C showed a smooth and homogeneous cell wall surface and intact cell wall structure (Fig. 4a, b). After P89 mycelia were treated with 36 °C for 2 days, the cell wall structure shrank or became distorted (Fig. 4c, d). Transmission electron micrographs of P89 mycelia were shown in Fig. 5. The cell wall structure of mycelia incubated at 28 °C was intact and tightly cross-linked (Fig. 5a). After mycelia were treated with 36 °C for 2 days, the cell wall thickness increased. This result was consistent with the changes in the concentrations of the main cell wall components. Nonetheless, the construction of electron-dense outer cell wall became loose (Fig. 5b). High temperature also caused the occurrence of plasmolysis (Fig. 5b–d). The cell membranes of some cells were also destroyed, which resulted in extravasation of cytoplasm (Fig. 5d).



**Fig. 1** Effect of high temperature on the concentrations of cell wall main components. **a** Chitin concentrations in mycelia treated with different temperatures. **b**  $\beta$ -1,3-D-Glucan concentrations in mycelia treated with

different temperatures. Data were analyzed by Duncan's ANOVA test. Error bars represent the standard deviation of three replicates. Different letters indicate significant differences between the lines ( $p \leq 0.05$ )



**Fig. 2** Effect of high temperature on chitin distribution (bar = 50  $\mu\text{m}$ ). The distribution of chitin on the cell wall at different temperatures: **a** (28  $^{\circ}\text{C}$ ), **b** (32  $^{\circ}\text{C}$ ), **c** (36  $^{\circ}\text{C}$ ), and **d** (40  $^{\circ}\text{C}$ ). Red arrowhead represents the location of chitin deposition

### High temperature increased the cell wall porosity of *P. ostreatus* mycelia

Relative cell wall porosity can be indicated by the mannose content (%) and relative DEAE-dextran sensitivity (Nishikawa et al. 2002). After high-temperature treatment, the mannose content in P89 mycelia significantly ( $p \leq 0.05$ ) decreased by 26% (Fig. 6). Furthermore, the relative DEAE-dextran sensitivity significantly ( $p \leq 0.05$ ) increased by 20% (Table 1). These results showed that cell wall porosity increased after high-temperature treatment.

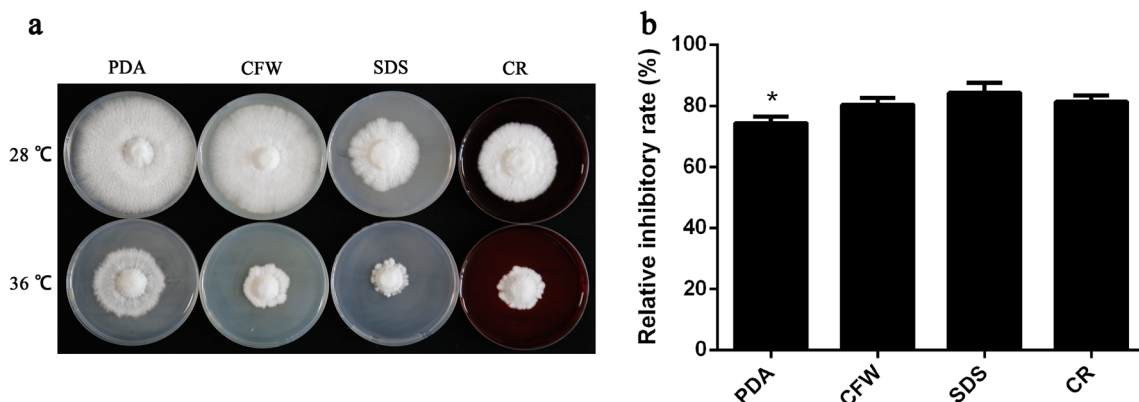
### High temperature destroyed the glycosidic linkage in cell walls

Infrared absorption spectrum is an efficient method to study cell wall components and their cross-links (Abidi

et al. 2014). The alkali-insoluble  $\beta$ -glucan collected from mycelia treated with 28 and 36  $^{\circ}\text{C}$  showed similar absorption bands (Fig. 7). The  $\beta$ -glycosidic linkage showed an absorption band at 893  $\text{cm}^{-1}$ . The bands at 1374  $\text{cm}^{-1}$  were characteristic of C-H variable-angle vibration. Two absorption bands at 1030 and 1069  $\text{cm}^{-1}$  were for asymmetric vibration of C-O-C pyranose ring. However, the intensity of vibration bands at 893, 1030, 1069, and 1374  $\text{cm}^{-1}$  all showed descending trends. The linkage between  $\beta$ -glucan units and the structure of  $\beta$ -glucan were altered after high-temperature treatment.

### Mycelia were sensitive to lysing enzymes after high-temperature treatment

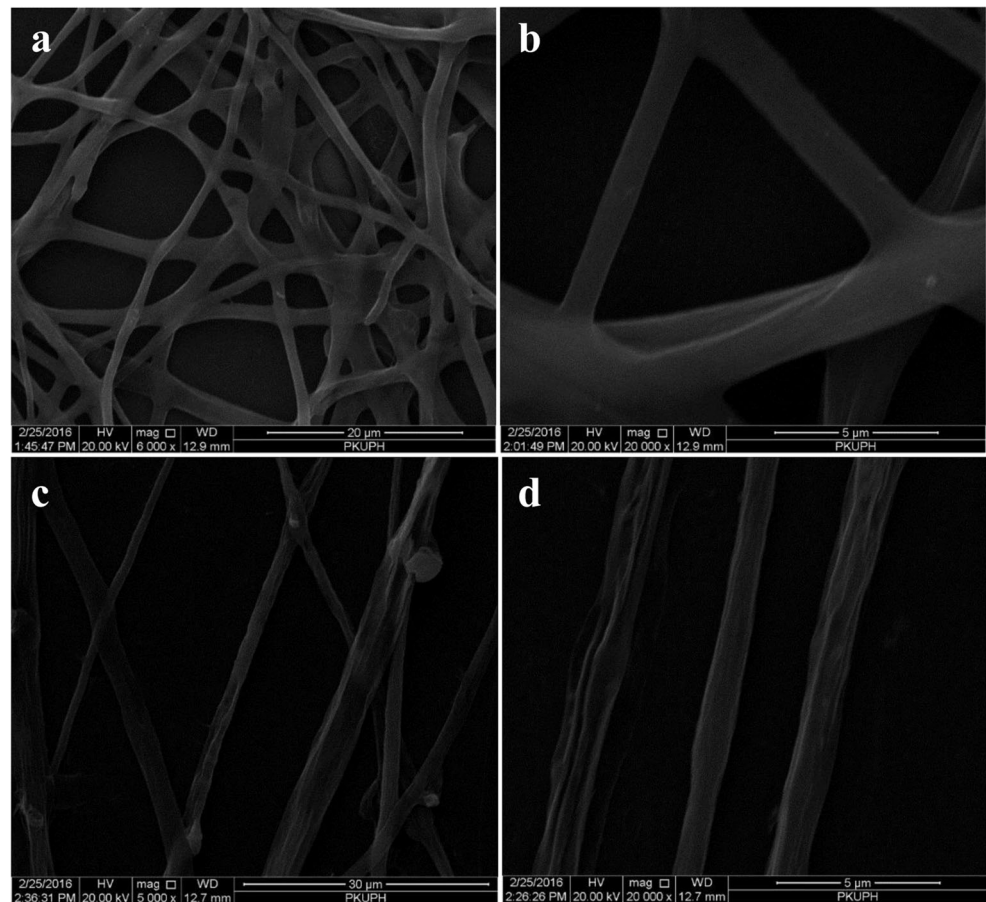
The number of released protoplast was quantified at 0.5 h intervals. After high-temperature treatment, the ability of P89 mycelia to resist the degradation of lysing



**Fig. 3** Sensitivity of cell wall-perturbing agents on the growth of P89 mycelia at 36  $^{\circ}\text{C}$ . **a** Sensitivity of mycelia grown on PDA plates with or without cell wall-perturbing agents. **b** The relative inhibitory rate of cell wall disrupting agents on mycelial growth at 36  $^{\circ}\text{C}$ . The relative

inhibitory rate was calculated according to Formula (1). Asterisks indicate a significant difference between the lines ( $p \leq 0.05$ ). Data were analyzed by Duncan's ANOVA test. Error bars represent the standard deviation of three replicates

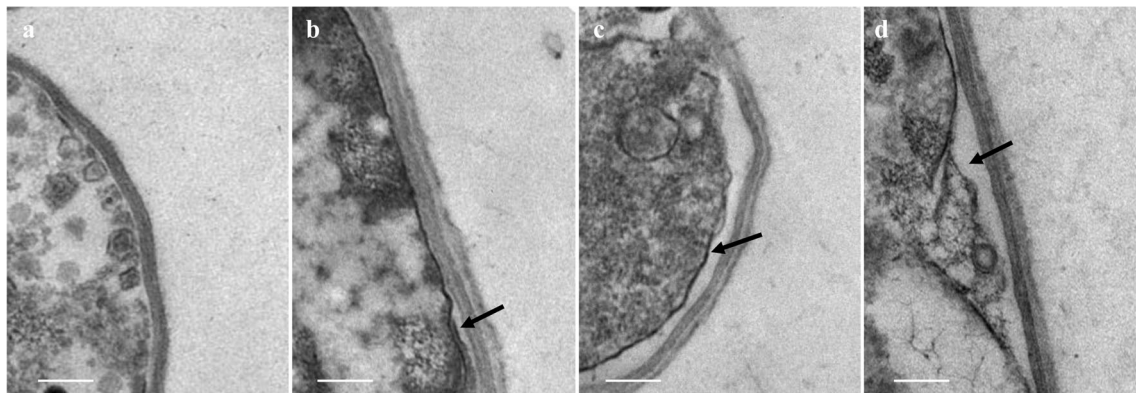
**Fig. 4** Scanning electron micrographs (SEMs) of P89 mycelia. **a, b** SEMs of P89 mycelia incubated at 28 °C. **c, d** SEMs of P89 mycelia incubated at 36 °C



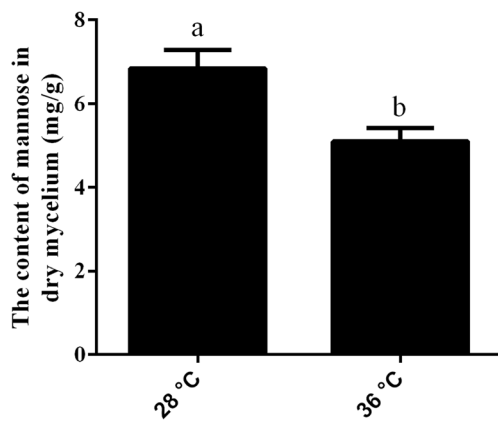
enzymes was decreased. Results showed that less protoplast was liberated from the mycelia which were grown at 28 °C than from the mycelia which were grown at 36 °C (Fig. 8). Significantly ( $p \leq 0.05$ ) high number of protoplast was released from mycelia in the unit time treatment for 0.5, 1, 1.5, and 2 h. Taken together, these results indicate that the cell wall is altered after high temperature.

## Discussion

Green mold disease occurs frequently during the cultivation of *P. ostreatus*. Cell wall is an important line of defense for the mycelium against a *Trichoderma* infection. This layer provides the cell with rigidity to prevent lysis and highly elastic network to resist adverse environment (Klis et al. 2006). The cell wall remodeling mechanism adapts to changes in the



**Fig. 5** Transmission electron micrographs (TEMs) of P89 mycelia (bar = 250 nm). **a** TEMs of mycelia incubated at 28 °C. **b–d** TEMs of mycelia incubated at 36 °C. Plasmolysis is indicated with arrowheads. The cell interior is located on the left side of the image



**Fig. 6** Mannose content in P89 mycelia. Data were analyzed by Duncan's ANOVA test. Error bars represent the standard deviation of three replicates. Different letters indicate significant differences between the lines ( $p \leq 0.05$ )

environment by synthesizing and assembling the main components.

The remodeling and synthesis of fungal cell wall are a complex process controlled by multiple genes and numerous biosynthetic pathways (de Groot et al. 2001). Severe environmental conditions may result in changes in the cell wall composition, disordered assembly, and structure destruction (Aguilar-Uscanga and Franco 2003). The content of chitin in mycelia of *P. ostreatus* P89 increased significantly ( $p \leq 0.05$ ) when mycelia were subjected to high temperature stress (Fig. 1a). The compensatory response of chitin synthesis to cell wall stress is essential. Previous study found that several metabolic precursors of chitin are up-regulated in response to cell wall stress (Bulik et al. 2003). Gfa1 catalyzes the production of glucosamine-6-phosphate, which is a rate-limiting step in the accumulation of UDP-GlcNAc for biosynthesis of chitin can be induced by several folds (Orlean 1997; Sobering et al. 2004). Ectopic expression of *GFA1* can also effectively increase the accumulation of chitin in lateral cell wall (Lagorce et al. 2002). Moreover,  $\beta$ -1,3-D-glucan concentrations increased by approximately 12% in P89 mycelia which were incubated at 32 and 36 °C. Moderately high temperature can induce the expression of *FKS2*, which is an alternative subunit of the glucan synthase complex and is responsible for the synthesis of  $\beta$ -1,3-D-glucan (Zhao et al. 1998). However, when incubated at 40 °C, P89 mycelia

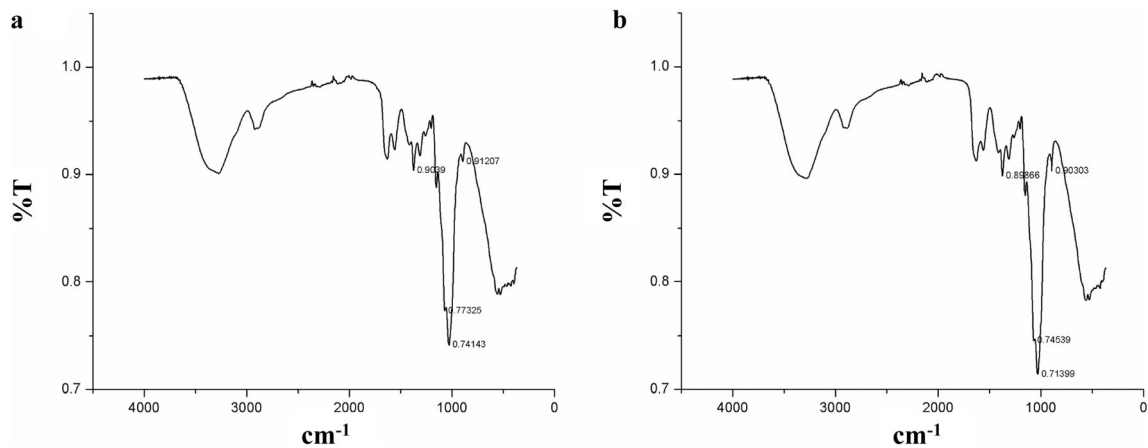
showed a significantly decreased  $\beta$ -1,3-D-glucan concentration in the cell wall. This result was similar to that in previous study, which demonstrated that excessive temperature could inhibit glucan synthase activity and increase chitin content (Ufano et al. 2004). These results indicated that high temperature can alter the concentrations of the main components in the cell wall of *P. ostreatus*.

During vegetative proliferation of fungal mycelia, the fungal cell wall is remodeled to establish and maintain cell shape. In P89 mycelia incubated at 28 °C, the chitin was evenly distributed on the cell wall and mostly distributed at the tips and septa (Fig. 2). The metabolism of mycelial tips and septa is relatively vigorous, the cell wall synthesis is fast, and the chitin content is high (Song et al. 2010). The present study revealed that abnormal distribution of cell wall chitin was found in the mycelia which were treated with 36 °C (Fig. 2). Many bright patches representing chitin deposition also appeared on the surface of cell wall. Although high temperature can promote the synthesis of chitin, the chitin assembly showed a defect, which resulted in chitin deposition. Abnormal chitin deposition is a characteristic of impaired CWI (Ufano et al. 2004). The signal pathway of CWI ensures the normal synthesis and assembly of cell wall components under external discomfort (Levin 2005). Although increased temperature can activate the CWI pathway to synthesize the main cell wall components, these components should be tightly assembled in a certain order to form a complete cell wall (Zhou et al. 2007). In the present study, P89 mycelia were hypersensitive to cell wall-perturbing agents at 36 °C. This result suggested that excessive heat will damage the CWI of P89 mycelia.

The formation of a tight cell wall is a complicated process that requires complex enzyme systems, component synthesis, and cross-linking between components (Garcia et al. 2015). Although high temperature led to the increase of major cell wall components content, the links among these components became less compact, and plasmolysis and extravasation of some cytoplasm were also found (Fig. 5b–d). These results indicated that 36 °C was a relatively high temperature for *P. ostreatus* P89, which can hardly alleviate the high temperature-induced damage with its self-compensatory mechanism in mycelia. Consequently, the CWI and structure were altered. Mannans are also the major cell wall components of fungi that can maintain cell morphology and cell wall porosity; decreased mannose will cause high cell wall porosity (Nishikawa et al. 2002; Shimma et al. 1997). After high-temperature treatment, the mannose content was remarkably decreased (Fig. 6), and the relative cell wall porosity was significantly increased (Table 1). These results demonstrated that cell wall porosity was increased after high temperature. The reduced mannose content causes a partial disorganization on the cell wall (Henry et al. 2016). With high cell wall porosity, the cell wall is highly sensitive to lyticase which can

**Table 1** Relative DEAE-dextran sensitivity under different temperatures

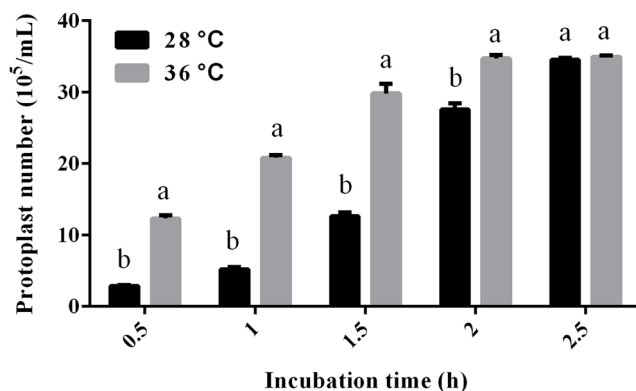
Treatment	Cell leakage (A260)		Relative DEAE-dextran sensitivity (%)
	DEAE-dextran	Poly-L-lysine	
28 °C	0.196	0.258	43.64 ± 2.19 b
36 °C	0.224	0.274	52.38 ± 2.41 a



**Fig. 7** Infrared absorption spectrum of  $\beta$ -glucan. **a** Infrared absorption spectrum of  $\beta$ -glucan in mycelia grown at 28 °C. **b** Infrared absorption spectrum of  $\beta$ -glucan in mycelia treated with incubated at 32 °C for 2 days

hydrolyze poly- $\beta$ -1,3-glucose on fungal cell wall (Ganeva et al. 2013). Moreover, several macromolecules can penetrate into the cell wall; consequently, the cell will be hypersensitive to antibiotics (Jigami and Odani 1999).

Rigid cell wall structure is closely related to the synthesis and assembly of  $\beta$ -glucan in the inner layer of the cell wall (Lesage and Bussey 2006).  $\beta$ -Glucan units are linked with  $\beta$ -1,3- and  $\beta$ -1,6-glycosidic linkages (Kanetsuna and Carbonell 1970). In the present study, the glycosidic linkage intensity was decreased after high-temperature treatment (Fig. 7). This result indicated that glycosidic linkages were broken down, or their amount in the cell wall glucan was decreased. Moreover, the C-H and C-O-C intensities were decreased, which suggested that  $\beta$ -glucan structure was also damaged. All these results resulted in loose  $\beta$ -glucan connection and damaged cell wall. Loose cell wall will reduce the resistance to lysing enzymes. During mycoparasitism, *Trichoderma* can secrete CWDEs to degrade fungal cell wall (Geraldine et al. 2013).



**Fig. 8** Lysing enzyme sensitivity assays for P89 mycelia. The released protoplast was quantified at 0.5 h intervals. Data were analyzed by Duncan's ANOVA test. Error bars represent the standard deviation of three replicates. Different letters indicate significant differences between the lines ( $p \leq 0.05$ )

In our study, P89 mycelia treated with incubated at 36 °C were easily degraded by lysing enzymes, and more protoplasts were released in unit time (Fig. 8). Our previous study found that *P. ostreatus* mycelia treated with high temperature can induce the activities of CWDEs (Qiu et al. 2017). In the present study, cell wall chitin and  $\beta$ -1,3-glucan contents were increased after treatment at 36 °C. In the presence of cell wall main components, CWDEs activities can be induced (Huang et al. 2011). On the basis of our results, we infer that high temperature causes the increase in chitin and  $\beta$ -1,3-glucan contents and their irregular assembly, thereby resulting in incomplete cell walls that can be easily degraded by lysing enzymes. Taken together, these results indicate that the disruption of the cell wall may reduce the resistance to *Trichoderma*.

This paper is the first to report about the effects of high temperature on the cell wall of *P. ostreatus* mycelia. In summary, the present results indicated that although high temperature can increase the main components of cell wall in *P. ostreatus*, the loose structure and cross-linking can negatively affect the maintenance of CWI and structure. Consequently, *P. ostreatus* mycelia will be easily degraded by lysing enzymes because of the disruption on CWI and structure, which may be the reason why high temperature-treated *P. ostreatus* mycelia will be easily infected by *Trichoderma*.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** The article does not contain any studies with human participants or animals performed by any of the authors.



## References

- Abidi N, Cabrales L, Haigler CH (2014) Changes in the cell wall and cellulose content of developing cotton fibers investigated by FTIR spectroscopy. *Carbohydr Polym* 100:9–16. <https://doi.org/10.1016/j.carbpol.2013.01.074>
- Aguilar-Uscanga B, Francois JM (2003) A study of the yeast cell wall composition and structure in response to growth conditions and mode of cultivation. *Lett Appl Microbiol* 37(3):268–274. <https://doi.org/10.1046/j.1472-765X.2003.01394.x>
- Akabane M, Yamamoto A, Aizawa S-i, Taga A, Kodama S (2014) Simultaneous enantioseparation of monosaccharides derivatized with L-tryptophan by reversed phase HPLC. *Anal Sci* 30(7):739–743. <https://doi.org/10.2116/analsci.30.739>
- Bickle M, Delley PA, Schmidt A, Hall MN (1998) Cell wall integrity modulates RHO1 activity via the exchange factor ROM2. *EMBO J* 17(8):2235–2245. <https://doi.org/10.1093/emboj/17.8.2235>
- Blaszczyk L, Siwulski M, Sobieralski K, Fruzynska-Jozwiak D (2013) Diversity of *Trichoderma* spp. causing *Pleurotus* green mould diseases in Central Europe. *Folia Microbiol (Praha)* 58(4):325–333. <https://doi.org/10.1007/s12223-012-0214-6>
- Bulik DA, Olczak M, Lucero HA, Osmond BC, Robbins PW, Specht CA (2003) Chitin synthesis in *Saccharomyces cerevisiae* in response to supplementation of growth medium with glucosamine and cell wall stress. *Eukaryot Cell* 2(5):886–900. <https://doi.org/10.1128/ec.2.5.886-900.2003>
- Cabib E, Roh DH, Schmidt M, Crotti LB, Varma A (2001) The yeast cell wall and septum as paradigms of cell growth and morphogenesis. *J Biol Chem* 276(23):19679–19682. <https://doi.org/10.1074/jbc.R000031200>
- Cortes JC, Katoh-Fukui R, Moto K, Ribas JC, Ishiguro J (2004) *Schizosaccharomyces pombe* Pmr1p is essential for cell wall integrity and is required for polarized cell growth and cytokinesis. *Eukaryot Cell* 3(5):1124–1135. <https://doi.org/10.1128/EC.3.5.1124-1135.2004>
- de Groot PW, Ruiz C, Vázquez de Aldana CR, Duenas E, Cid VJ, Del Rey F, Rodríguez-Peña JM, Pérez P, Andel A, Caubín J, Arroyo J, García JC, Gil C, Molina M, García LJ, Nombela C, Klis FM (2001) A genomic approach for the identification and classification of genes involved in cell wall formation and its regulation in *Saccharomyces cerevisiae*. *Comp Funct Genomics* 2(3):124–142. <https://doi.org/10.1002/cfg.85>
- De Nobel JG, Klis FM, Munnik T, Priem J, Van Den Ende H (1990) An assay of relative cell wall porosity in *Saccharomyces cerevisiae*, *Kluyveromyces lactis* and *Schizosaccharomyces pombe*. *Yeast* 6(6):483–490. <https://doi.org/10.1002/yea.320060605>
- Depree J, Emerson GW, Sullivan PA (1993) The cell wall of the oleaginous yeast *Trichosporon cutaneum*. *J Gen Microbiol* 139(9):2123–2133
- Fortwendel JR, Juvvadi PR, Pinchai N, Perfect BZ, Alspaugh JA, Perfect JR, Steinbach WJ (2009) Differential effects of inhibiting chitin and 1,3- $\beta$ -D-glucan synthesis in ras and calcineurin mutants of *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 53(2):476–482. <https://doi.org/10.1128/AAC.01154-08>
- Ganeva V, Galutzov B, Teissie J (2013) Evidence that pulsed electric field treatment enhances the cell wall porosity of yeast cells. *Appl Biochem Biotech* 172(3):1540–1552. <https://doi.org/10.1007/s12010-013-0628-x>
- García R, Botet J, Rodríguez-Peña JM, Bermejo C, Ribas JC, Revuelta JL, Nombela C, Arroyo J (2015) Genomic profiling of fungal cell wall-interfering compounds: identification of a common gene signature. *BMC Genomics* 16:683. <https://doi.org/10.1186/s12864-015-1879-4>
- Geraldine AM, Lopes FAC, Carvalho DDC, Barbosa ET, Rodrigues AR, Brandão RS, Ulhoa CJ, Lobo Junior M (2013) Cell wall-degrading enzymes and parasitism of sclerotia are key factors on field biocontrol of white mold by *Trichoderma* spp. *Biol Control* 67(3):308–316. <https://doi.org/10.1016/j.biocontrol.2013.09.013>
- Gopal PK, Shepherd MG, Sullivan PA (1984) Analysis of wall glucans from yeast, hyphal and germ-tube forming cells of *Candida albicans*. *J Gen Microbiol* 130(12):3295–3301. <https://doi.org/10.1099/00221287-130-12-3295>
- Henry C, Fontaine T, Heddergott C, Robinet P, Aimanianda V, Beau R, Beauvais A, Mouyna I, Prevost M-C, Fekkar A, Zhao Y, Perlin D, Latgé J-P (2016) Biosynthesis of cell wall mannan in the conidium and the mycelium of *Aspergillus fumigatus*. *Cell Microbiol* 18(12):1881–1891. <https://doi.org/10.1111/cmi.12665>
- Huang X, Chen L, Ran W, Shen Q, Yang X (2011) *Trichoderma harzianum* strain SQR-T37 and its bio-organic fertilizer could control *Rhizoctonia solani* damping-off disease in cucumber seedlings mainly by the mycoparasitism. *Appl Microbiol Biotechnol* 91(3):741–755. <https://doi.org/10.1007/s00253-011-3259-6>
- Jigami Y, Odani T (1999) Mannosylphosphate transfer to yeast mannan. *Biochim Biophys Acta-Gen Subjects* 1426(2):335–345. [https://doi.org/10.1016/S0304-4165\(98\)00134-2](https://doi.org/10.1016/S0304-4165(98)00134-2)
- Jung US, Levin DE (1999) Genome-wide analysis of gene expression regulated by the yeast cell wall integrity signalling pathway. *Mol Microbiol* 34(5):1049–1057. <https://doi.org/10.1046/j.1365-2958.1999.01667.x>
- Kanetsuna F, Carbonell LM (1970) Cell wall glucans of the yeast and mycelial forms of *Paracoccidioides brasiliensis*. *J Bacteriol* 101(3):675–680
- Klis FM, Mol P, Hellingwerf K, Brul S (2002) Dynamics of cell wall structure in *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* 26(3):239–256. [https://doi.org/10.1016/s0168-6445\(02\)00087-6](https://doi.org/10.1016/s0168-6445(02)00087-6)
- Klis FM, Boorsma A, De Groot PW (2006) Cell wall construction in *Saccharomyces cerevisiae*. *Yeast* 23(3):185–202. <https://doi.org/10.1002/yea.1349>
- Komon-Zelazowska M, Bissett J, Zafari D, Hatvani L, Manczinger L, Woo S, Lorito M, Kredics L, Kubicek CP, Druzhinina IS (2007) Genetically closely related but phenotypically divergent *Trichoderma* species cause green mold disease in oyster mushroom farms worldwide. *Appl Environ Microbiol* 73(22):7415–7426. <https://doi.org/10.1128/AEM.01059-07>
- Kredics L, Kocsube S, Nagy L, Komon-Zelazowska M, Manczinger L, Sajben E, Nagy A, Vagvolgyi C, Kubicek CP, Druzhinina IS, Hatvani L (2009) Molecular identification of *Trichoderma* species associated with *Pleurotus ostreatus* and natural substrates of the oyster mushroom. *FEMS Microbiol Lett* 300(1):58–67. <https://doi.org/10.1111/j.1574-6968.2009.01765.x>
- Lagorce A, Le Berre-Anton V, Aguilar-Uscanga B, Martin-Yken H, Dagkessamanskaia A, François J (2002) Involvement of *GFA1*, which encodes glutamine-fructose-6-phosphate amidotransferase, in the activation of the chitin synthesis pathway in response to cell-wall defects in *Saccharomyces cerevisiae*. *Eur J Biochem* 269(6):1697–1707. <https://doi.org/10.1046/j.1432-1327.2002.02814.x>
- Lesage G, Bussey H (2006) Cell wall assembly in *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* 70(2):317–343. <https://doi.org/10.1128/MMBR.00038-05>
- Levin DE (2005) Cell wall integrity signaling in *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* 69(2):262–291. <https://doi.org/10.1128/MMBR.69.2.262-291.2005>
- Levin DE (2011) Regulation of cell wall biogenesis in *Saccharomyces cerevisiae*: the cell wall integrity signaling pathway. *Genetics* 189(4):1145–1175. <https://doi.org/10.1534/genetics.111.128264>
- Liu Y, Flanagan JJ, Barlowe C (2004) Sec22p export from the endoplasmic reticulum is independent of SNARE pairing. *J Biol Chem* 279(26):27225–27232. <https://doi.org/10.1074/jbc.M312122200>

- Mendoza CG (1992) Cell wall structure and protoplast reversion in basidiomycetes. *World J Microbiol Biotechnol* 8(1):36–38. <https://doi.org/10.1007/BF02421486>
- Nishikawa A, Mendez B, Jigami Y, Dean N (2002) Identification of a *Candida glabrata* homologue of the *S. cerevisiae* *VRG4* gene, encoding the Golgi GDP-mannose transporter. *Yeast* 19(8):691–698. <https://doi.org/10.1002/yea.854>
- Oka T, Futagami T, Goto M (2015) Cell wall biosynthesis in filamentous fungi. In: Takagi H, Kitagaki H (eds) *Stress biology of yeasts and fungi: applications for industrial brewing and fermentation*. Springer Japan, Tokyo, pp 151–168
- Orlean P (1997) Biogenesis of yeast wall and surface components. In: Pringle JR, Broach JR, Jones EW (eds) *The molecular biology of the yeast Saccharomyces*, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 229–362
- Osumi M (1998) The ultrastructure of yeast: cell wall structure and formation. *Micron* 29(2–3):207–233. [https://doi.org/10.1016/s0968-4328\(97\)00072-3](https://doi.org/10.1016/s0968-4328(97)00072-3)
- Qiu Z, Wu X, Zhang J, Huang C (2017) High temperature enhances the ability of *Trichoderma asperellum* to infect *Pleurotus ostreatus* mycelia. *PLoS One* 12(10):e0187055. <https://doi.org/10.1371/journal.pone.0187055>
- Rees DA, Morris E, Thom D, Madden JK (1982) Shapes and interactions of carbohydrate chains. In: Aspinall GO (ed) *The polysaccharides I*. Academic Press, New York, pp 195–290
- Shimma Y, Nishikawa A, bin Kassim B, Eto A, Jigami Y (1997) A defect in GTP synthesis affects mannose outer chain elongation in *Saccharomyces cerevisiae*. *Mol Gen Genet* 256(5):469–480. <https://doi.org/10.1007/s004380050591>
- Smits GJ, Kapteyn JC, van den Ende H, Klis FM (1999) Cell wall dynamics in yeast. *Curr Opin Microbiol* 2(4):348–352. [https://doi.org/10.1016/s1369-5274\(99\)80061-7](https://doi.org/10.1016/s1369-5274(99)80061-7)
- Sobering AK, Watanabe R, Romeo MJ, Yan BC, Specht CA, Orlean P, Riezman H, Levin DE (2004) Yeast Ras regulates the complex that catalyzes the first step in GPI-anchor biosynthesis at the ER. *Cell* 117(5):637–648. <https://doi.org/10.1016/j.cell.2004.05.003>
- Song W, Dou X, Qi Z, Wang Q, Zhang X, Zhang H, Guo M, Dong S, Zhang Z, Wang P, Zheng X (2010) R-SNARE homolog MoSec22 is required for conidiogenesis, cell wall integrity, and pathogenesis of *Magnaporthe oryzae*. *PLoS One* 5(10):e13193. <https://doi.org/10.1371/journal.pone.0013193>
- Staniszewska M, Bondaryk M, Swoboda-Kopec E, Siennicka K, Sygitowicz G, Kurzatkowski W (2013) *Candida albicans* morphologies revealed by scanning electron microscopy analysis. *Braz J Microbiol* 44:813–821
- Ufano S, del Rey F, Vazquez de Aldana CR (2004) Swm1p, a subunit of the APC/cyclosome, is required to maintain cell wall integrity during growth at high temperature in *Saccharomyces cerevisiae*. *FEMS Microbiol Lett* 234(2):371–378. <https://doi.org/10.1016/j.femsle.2004.04.006>
- Verna J, Lodder A, Lee K, Vagts A, Ballester R (1997) A family of genes required for maintenance of cell wall integrity and for the stress response in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* 94(25):13804–13809. <https://doi.org/10.1073/pnas.94.25.13804>
- Zhang S, Xia Y, Keyhani NO (2011) Contribution of the *gas1* gene of the entomopathogenic fungus *Beauveria bassiana*, encoding a putative glycosylphosphatidylinositol-anchored  $\beta$ -1,3-glucanosyltransferase, to conidial thermotolerance and virulence. *Appl Environ Microbiol* 77(8):2676–2684. <https://doi.org/10.1128/AEM.02747-10>
- Zhao C, Jung US, Garrett-Engle P, Roe T, Cyert MS, Levin DE (1998) Temperature-induced expression of yeast *FKS2* is under the dual control of protein kinase C and calcineurin. *Mol Cell Biol* 18(2):1013–1022
- Zhou H, Hu H, Zhang L, Li R, Ouyang H, Ming J, Jin C (2007) O-Mannosyltransferase 1 in *Aspergillus fumigatus* (AfPmt1p) is crucial for cell wall integrity and conidium morphology, especially at an elevated temperature. *Eukaryot Cell* 6(12):2260–2268. <https://doi.org/10.1128/ec.00261-07>