Research Article

Yeast Cell Surface Display of Bacterial Chitinase as a New Approach for Biocontrol of Phytopathogenic Fungi

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Abstract

Bacterial chitinase (EC 3.2.1.14) is a potential biological pesticide, but its biochemical properties for agricultural usage need to be improved. We adopted a new approach for using chitinase as a biocontrol application by immobilization of chitinase onto a yeast cell surface. To display the enzyme on the surface of *Saccharomyces cerevisiae*, a bacterial chitinase gene of *Bacillus circulans* No.4.1, was fused with the 3' half terminus of α-agglutinin and the glycosylphosphatidylinositol (GPI) anchored signal. The yeast surface display with chitinase hydrolyzed *p*-nitrophenyl-diacetyl-chitobiose up to 3- to 4-fold higher than that without chitinase on the cell surface and the enzyme activity was active for up to 20 hr. Our results demonstrated that chitinase was successfully displayed on the yeast cell surface in an active form with an antifungal activity against phytopathogenic fungi, especially *Fusarium oxysporum*. This new approach may provide an evident for a potential biocontrol against phytopathogenic fungi.

***Key Words*:** Antifungal activity; Biocontrol; *Fusarium oxysporum*; Immobilized chitinase; Plant pathogen; Yeast surface display

Introduction

Biocontrol or biological control is one of impact tools for defending with agricultural pests. Chemical pesticides exhibit deleterious effects on the environment, which have become a global problem. In addition, a rapid development of drug resistance to these synthetic pesticides continues to spread globally (Chastagner and Riley, 1990). Biocontrol and microbial control using beneficial microorganisms or their metabolites are, therefore, a potential alternative approach to pest, disease and weed control. Soil-borne phytopathogens are among the most difficult to control because root organs are much more difficult to reach than aerial organs (Alabouvette et al., 2009). *Fusarium oxysporum* is a representative soil-borne fungi and a commercially

important pathogen which causes fusarium wilt in high economic value crops worldwide (Michielse and Rep, 2009).

Chitinase (EC 3.2.1.14) is a widespread chitin-hydrolyzing enzyme, which takes place in various organisms: prokaryotes and eukaryotes, with a variety of biofunctions, including defence and pathogenesis (Felse and Panda, 1999; Flach et al., 1992; Kabir et al., 2006). It mainly digests chitin, which is a major cell wall component of various organisms, including fungi and insects. Chitin—a β1,4-linked polymer of N-acetyl-D-glucosamine—is a major cell wall component of most phytopathogenic fungi as well as insect shells, but it does not exist in plants, vertebrates and prokaryotes. Chitinase, therefore, has an antifungal activity by destroying the fungal cell wall. Chitinase-producing microorganisms have been reported as a biocontrol for various phytopathogenic fungi (Chen et al., 2004; Chernin et al., 1997; Freeman et al., 2004; Kobayashi et al., 2002; Kowsari et al., 2013). *Bacillus circulans*—one of the candidates for chitinase-producing microorganisms, has been proven to be a reliable biocontrol agent of diamond moth larvae (Siwayaprahm et al., 2006; Wiwat et al., 1996). Chitinase, therefore, is a promising biocontrol substance; an alternative method to chemical pesticides which are known to cause many problems, including drug resistance and environmental contamination.

Conventional biocontrol methods utilizing purified chitinase or chitinase-producing microorganisms themselves do not permit a high efficacy under field condition when compete with chemical pesticides (Brunner et al., 2005). The success of a biocontrol requires not only the selection of a microorganism strain as a source of biocontrol agent, but also the properties of its ecological fitness and the improvement of the agent production, formulation, and an application process (Alabouvette et al., 2009). Chitinase yeast-surface display may be the potential solution for achieving sufficient production and formulation of chitinase. It was employed in order to enhance the efficacy, stability and biocontrol capacity. We tested a yeast surface display system to deliver a whole cell catalyst and to be used as a biocontrol. The yeast surface display system was developed using cell-surface engineering (Murai et al., 1997; Ueda and Tanaka, 2001), which allowed a display of active enzymes, at least 10^5 – 10^6 molecules on the surface of one yeast cell, in terms of fusion enzyme being linked to C-terminal half α agglutinin (Shibasaki et al., 2001). The yeast surface display was achieved by co-expression of a recombinant target protein with an anchor protein, which leaded to display the target protein onto the cell wall surface of *S. cerevisiae*. Various anchor sequences for displaying proteins on the surface of yeast are in practical use. In the current study, the glycosylphosphatidylinositol (GPI) family—the 3' half terminal of α agglutinin—was fused with chitinase.

Although the yeast cell surface display has already been reported in various applications (Kuroda et al., 2009; Matsumoto et al., 2002; Sato et al., 2002; Tanaka et al., 2012; Ueda and Tanaka, 2000a; Zou et al., 2002), the approach for biocontrol is a novel approach for enhancing the efficacy of antifungal activity. In our study, in order to improve the performance of chitinase activity, the yeast surface display was performed using yeast cell surface engineering. The chitinase yeast surface display was characterized by its localization and also chitinolytic activity against either synthetic or natural chitin. The results suggested that this new approach of chitinase would be a potential alternative for biocontrol of phytopathogenic fungi.

Materials and Methods

Strains and media

Two strains of *S. cerevisiae* were used in this

experiment. The wild type MT8-1 (*MATa, ade, his3, leu2, trp1, ura3*) (Tajima et al., 1985) and a low-protease-producing strain CB018 (*MATa, Δpep4::HIS3, prb1::hisG, prc1::hisG, ade2-1, can1-100, his3-11, 15, leu2-3,112, trp1-1, ira3-1*), provided by Dr. Tomohiro Akashi, Nagoya University. Transformed yeast was cultured in synthesis dextrose (SD) medium [0.67% w/v yeast nitrogen base without amino acid, 2% glucose and appropriate amino acid supplements]. Recombinant DNA manipulation was performed in DH5α Escherichia coli [F-, *ψ80dlac*ZΔM15, Δ*(lacZYA-argF)U169*, *deoR*, *recA1*, *endA1*, *hsdR17(rK- mK+), phoA, supE44, λ-, thi-1, gyrA96, relA1*] and grown in 2xYT in the presence of 100 μg mL^{-1} ampicillin sodium.

Phytopathogenic fungi, *Fusarium solani* (Mart.) Sacc. (#DOAC2003), *F. oxysporum* (#DOAC1699), *F. moniliforme* Sheldon (#DOAC1877), *Rhizoctonia solani* (#DOAC1406), from the Crop Protection Research and Development Bureau, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand, were used as representatives for antifungal activity assay. DOAC represents the Department of Agriculture Culture Collection.

Plasmid construction

The chitinase gene of *B.circulans* No.4.1: GenBank: AF154827.1 (Siwayaprahm 2006; Wiwat et al. 1999) was modified by adding 6-repeated histidine codons at N-terminal of chitinase. Four plasmids were constructed in a multicopy pERS vector (Okochi et al. 2007) (Fig. 1): (1) pERS: Chi-AF (chitinase with an anchored fragment in the pERS plasmid) : the PCR fragment of 6xhis-tag chitinase without a stop codon was amplified with the primers ChiSacII-*Eco*RI-F0 (5' GTTTCTGC CGCGGAATTCATTAAAGAGGAG 3') and ChiNonstop*Xho*I R2 (5' ATACTCGAGGGCTTTAA AATGACACG 3') then subcloned to pERS vector, containing the secretion signal and 3` half of the α-agglutinin and Glycosylphosphatidylinositol (GPI) anchored sequence (Ueda and Tanaka 2000a). (2) pERS: EGFP-Chi-AF (chitinase with EGFP with anchored fragment): EGFP (a gift from Dr. Tomohiro Akashi, Nagoya University, Japan) (Fig. 1(a)) was amplified by PCR using the primers yEGFP/*Sac*II F (5' GCCGCGGATGTCTAAAGGTGAAGAATT 3') and yEGFP/*Sac*II R (5' TTCCGCGGCTTTGT ACAATTCATCCATAC 3') then subcloned into pERS:Chi-AF vector at the 5` end of the 6xhis-tag chitinase. (3) pERS:Chi (chitinase without anchored fragment) (Fig.1(b)): the PCR fragment was amplified with the primers ChiPstI 1400 F3 (5' GAAATTACACGCTGCTCCTG 3') and *Kpn*I/stop R (5'- CGGTACCtcaAATGACACGACATATTCC GT 3') and subcloned into pERS vector. It therefore had an absent anchor fragment; that is, a fragment of the 3` half of the α-agglutinin and GPI anchored sequence. (4) pERS:EGFP-Chi (chitinase with EGFP without anchored fragment): EGFP was amplified by PCR then subcloned into pERS:Chi vector at the 5' end of the 6xhis-tag chitinase.

Qualitative analysis of chitinase activity by using 4-methyl umbelliferyl diacetyglucosaminoglycan (4-MU $GlcNac_2$)

Chitinase activity was screened for a positive chitinase surface display clone by using 4-MU $GlcNac_2$ as a substrate with some modifications (O'Brien and Colwell, 1987; Thompson et al., 2001). Briefly, yeast cells were transfected with pERS:Chi-AF or pERS:Chi. They were then pre-cultivated in SD for 30 hr; after which 1/10 diluted cells were cultivated in YPD until OD_{600} reaches ~0.5. Cells were harvested by centrifuge at 1,500xg for 5 min and washed in cold PBS. The reaction was performed by adding 4-MU $GlcNac_2$ in PBS buffer pH 7.0 and incubated at 37°C for 1 hr. The supernatant was transferred to a 96-well black plate and after UV-254 nm excitation, the fluorescent intensity was measured with Fluorescent Scanner (Ettan™ DIGE Imager, GE healthcare life sciences, USA). The result revealed a fluorescent intensity of

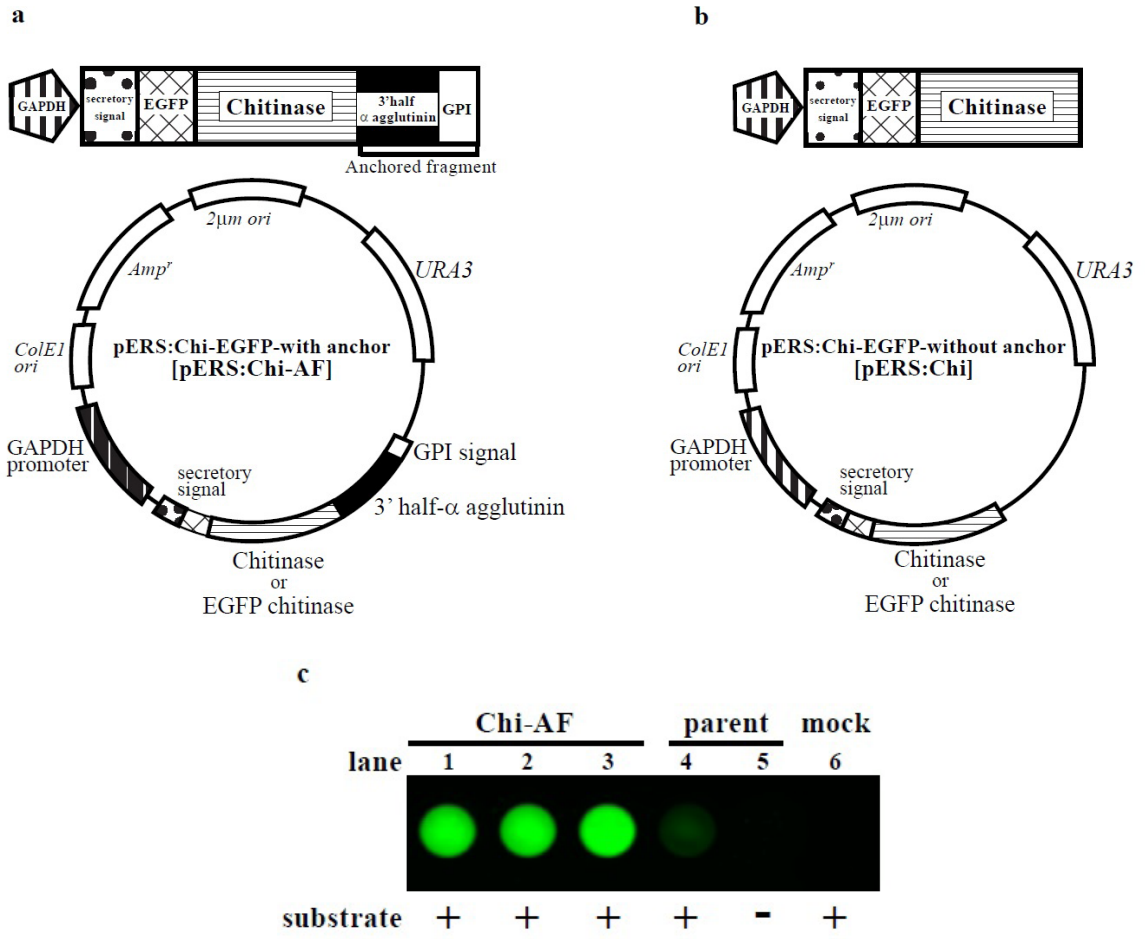

Figure 1 Schematic illustration of the EGFP-chitinase surface display of Saccharomyces cerevisiae and its expressed activity: (a) pERS:Chi-AF is the chitinase with anchored fragment (Chi-AF), 3' half α agglutinin and GPI anchoring signal, in pERS plasmid, (b) pERS:Chi is the chitinase without anchored fragment (Chi) in pERS plasmid, (c) chitinolytic activity of Chi-AF on surface of yeast without EGFP (lane 1-3), using 4-MU-GlcNac substrate, compared to parent cell (lane 4-5) and mock (lane 6). Reaction were performed by co-incubated engineered yeast cell with 4-MU GlcNac at 37 °C for 1 hr in 96-well plate and quantified by fluorescent spectrophotometer.

the tested clone (Chi-AF yeast), in relation to the substrate solution buffer with and without (mock) a MT8-1 parent strain.

Immunofluorescense of EGFP-chitinase on yeast surface displayed

Immunofluorescense of chitinase on the cell surface was performed as previously reported (Kato et al. 2006). Cells were cultured and harvested as described above. Cells were then fixed in 3.7% formaldehyde in PBS. After washing with PBS, the cells were blocked in 10% FBS for 30 min followed by co-incubation with 1:100 diluted mouse antiGFP antibody (Roche) for 2 hr. Cells were washed three times in PBST (0.1% tween20 in PBS), then incubated in 1:1000 diluted AlexaFluor ®488 conjugated goat anti-mouse (Invitrogen, USA), then a secondary antibody was added and incubated for 1 hr. After washing in PBST, the cells were mounted on a slide and photographed under a fluorescence microscope.

Western blot analysis of EGFP-chitinase on yeast surface

The cell wall fraction was isolated from the strain CB018 harboring pERS:EGFP-Chi-AF and

pERS:EGFP-Chi, as previously reported (Yue et al., 2008) with minor modifications. Cells were collected (10 OD_{600}) and washed twice in a cold lysis buffer—50 mM Tris/HCl (pH 7.4) containing 5 mM EDTA and 1 mM PMSF. The cells were re-suspended in the lysis buffer containing glass beads (diameter 0.45 mm) at a ratio of 1:2:1 (cell : lysis buffer : glass beads) then vigorously homogenized by vortexing 10 times (1 min each) with 1-min cooling intervals on ice. After removing the glass beads by allowing the suspension to stand, the homogenate was centrifuged at 1,000×g, 4 °C for 10 min. The resulting pellet obtained was the cell wall fraction. The supernatant was centrifuged at 20,000×g, 4 °C for 15 min, and the resulting supernatant was used as a cell wall-free extract. Both fractions were analyzed by Western blotting by using 1:100 rabbit anti-GFP antibody (Sigma, USA) and 1:1000 Alexa Fluor®488 mouse anti-rabbit (Invitrogen, USA) as the primary and secondary antibody, respectively.

Quantitative analysis of chitinase activity by using p-nitrophenyl N,N'-diacetyl-β-chitobioside (p-NP diacetyl chitobiose)

Chitinase activity was detected with some modifications to a previous methodology (Thompson et al., 2001). Briefly, cells were pre-cultured in SD followed by continuous culturing in YPD at 30 °C and harvested after 48 hr or when the OD_{600} reached 0.5. The reaction was performed by adding p-NP diacetyl chitobiose (Sigma, USA) in sodium phosphate buffer pH 6.5 and incubated at 37 °C until a pale yellow color was observed. To stop the reaction, 0.2 M Na_2CO_3 was added in equal volume at several time points, viz. 0, 5, 10, 20, and 30 hr. The supernatant was collected and the absorbance was measured at 405 nm with a plate reader (FLUOstar OPTIMA, BMG LABTECH, Germany). A buffer with substrate was used as a blank. The activity of chitinase on the yeast cell surface was calculated using the following formula.

$$\text{Acitivity (nM/10}^4\text{ cells)} = \frac{\text{Amount of } p\text{-NP (nM)}}{\text{number of yeast cells} \times 10^4}$$

where, the amount of p-NP was calculated from the standard curve for standard *p*-nitrophenol (Sigma, USA) $y = 0.002x + 0.015$, $R^2 = 0.998$.

Antifungal assay of EGFP-chitinase on yeast surface

The antifungal assay against the phytopathogenic fungi—*F. solani* (Mart.) Sacc., *F. oxysporum, F. moniliforme* Sheldon, *Rhizoctonia solani, Collectotrichum gloespoloides*—of chitinase-displayed yeast was determined. After 48-hr culturing of each strain of yeast (the parent strains being the control), yeast harboring pERS:EGFP-Chi-AF or pERS:EGFP-Chi were spread on 10-cm Potato Dextrose Agar (PDA) plates. The same amount of mycelia were inoculated at the center of each plate and cultured at 30 °C for 5 days. The radial growth rate of each colony on each plate was measured: the difference in growth between plates having EGFP-chitinase with and without a GPI signal strain was compared to the control.

Results

Display of bacterial chitinase on cell surface of Saccharomyces cerevisiae

Yeast cell surface display is a promising method for functionalizing protein to serve a wide variety of applications. This technique was first adopted to express chitinase—a chitin lysed enzyme—on the cell surface, as used in this study for antifungal biocontrol. One of the effective targets of antifungal biocontrol is to damage chitin on the cell wall; damaging cell wall integrity and inhibiting growth of the fungus, thus halting pathogenesis in the host. Bacterial chitinase from *B. circulans* was therefore manipulated to express on the outer surface of *S. cerevisiae* by fusion with an anchor fragment.

Molecular constructs of fusion chitinase with

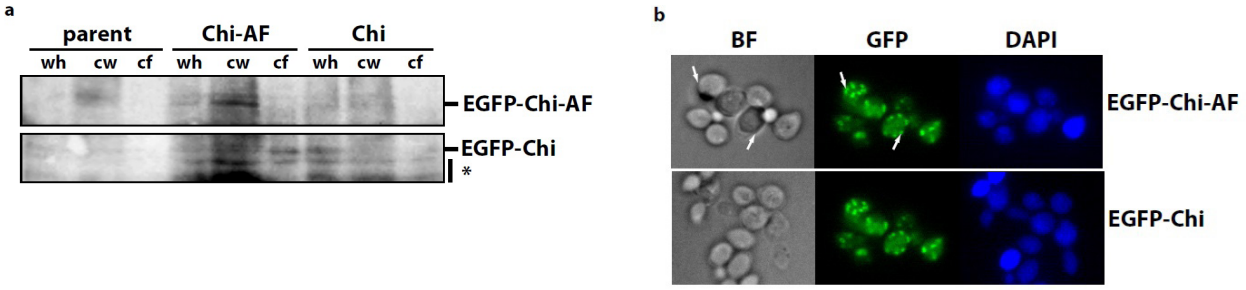

Figure 2 EGFP-chitinase with anchor protein displayed on cell surface of *Saccharomyces cerevisiae* :(a) Cell fractionation of CB018 yeast carried chitinase with anchored fragment (Chi-AF), or chitinase without anchored fragment (Chi), or parent yeast was analyzed by Western blotting using anti-EGFP antibody. wh: whole cell fraction, cw: cell wall fraction, cf: cell free fraction, EGFP-Chi-AF: fusion protein of EGFP chitinase with anchor (MW = 120 kDa), EGFP-Chi: fusion protein of EGFP chitinase without anchor (MW = 83 kDa), asterisk indicated possible degraded chitinase products. (b) Immunofluorescense staining cell of EGFP-chitinase (EGFP-Chi-AF) and EGFP-Chi on CBO18 cell surface by anti EGFP antibody (green) and DAPI (blue). Arrows indicated EGFP-chitinase displayed on yeast cell wall.

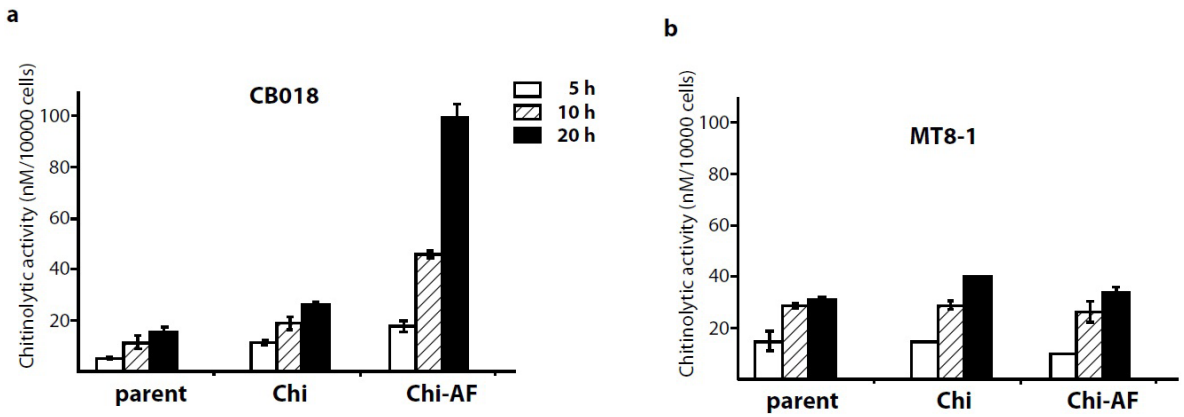

Figure 3 Chitinase on yeast surface promoted chytinolytic activity against *p*-Nitrophenyl chitobiose : Chitinolytic activity of yeast, CB018 (a) or MT8-1 (b), carried chitinase with (Chi-AF) or without (Chi) anchored fragment was determined using p-NP chitobiose at 37 °C for 5 hr (white), 10 hr (stripe) and 20 hr (black). p-nitrophenol, chitinolytic product, from at least 3-replicate of discrete experiments, was quantified and calculated with standard curve by spectrophotometer at wavelength 405 nm. Details of experiment were shown in "Materials and methods".

an anchor fragment (pERS:Chi-AF) were designed (Fig. 1(a)). The bacterial chitinase gene of *B. circulans* (Siwayaprahm, et al. 2006; Wiwat et al., 1999) is supposed to provide chitinolytic activity (Chen et al., 2004). The anchor fragment, 3' half of α-agglutinin and GPI, was fused to the C-terminus of chitinase because the chitinase active site is located on the N-terminus (Watanabe et al., 1994). A secretion signal was also fused at the N-terminus of the chitinase to facilitate transport and directing to the cell surface. The *GAPDH* promoter was inserted into the construction to maintain a high expression at all times and the EGFP was tagged on the N-terminus for aiding detection. As a control,

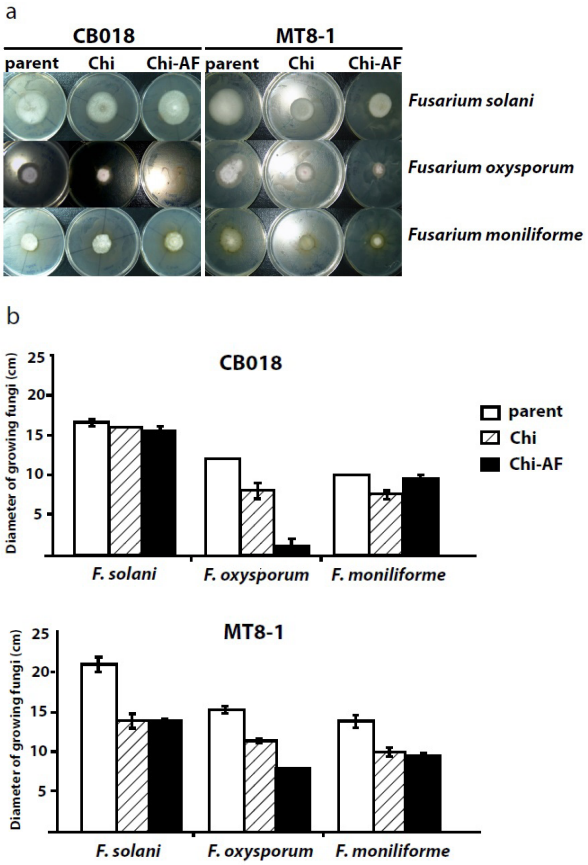

Figure 4 Antifungal activity is consequence of chitinase display on yeast cell surface : (a) Typical photos of antifungal assay are shown. (b) Antifungal activity of EGFP-chitinase with (Chi-AF, black bar) or without (Chi, stripe bar) anchored fragment in comparison to parent strain (white bar) of *Saccharomyces cerevisiae* strains CB018 or MT8-1 against plant pathogenic fungus; *Fusarium solani*, *F. oxysporum*, and *F. moniliforme*. Engineered yeasts were spread and co-cultured with each of plant pathogenic fungus at 30 °C for up to 5 days, and measured diameter of growth fungi from at least 3 replicates of discrete experiments.

the same chitinase expression plasmid—without the anchored fragment (pERS:Chi)—was constructed (Fig. 1(b)). In a preliminary experiment, several clones of each engineered yeast were isolated by transforming the plasmid of pERS:Chi-AF into the MT8-1 strain. Then preliminary chitinolytic activity with 4-Methyl umbelliferyl diacetylglucosaminoglycan (4-MU-$(GlcNac)_2$) was determined. All clones revealed chitinolytic activity up to 7-fold higher than the parent yeast (Fig. 1(c)), whereas no difference in growth was observed; implying that the expression of functional chitinase successfully displayed on the cell surface.

Characterization of chitinase expression on cell surface

To characterize and demonstrate that the anchored protein, viz. α agglutinin-GPI fusion protein, would lead to a recombinant chitinase on the yeast cell surface, both the localization and activity of chitinase were examined. The localization of displayed chitinase on the yeast cell surface was evaluated by both cell fractionation and intact-engineered yeast. Cell fractionation of EGFP-Chi-AF, EGFP-Chi, or the parent yeast cell was confirmed using Western blot with anti-EGFP antibody. The EGFP-Chi-AF and EGFP-Chi fusion proteins were supposed to be 120 and 83 kDa, respectively. The EGFP-Chi-AF fusion protein was detected at the expected size in only in the cell wall fraction of the EGFP-Chi-AF strain (Fig. 2(a)–top panel). By comparison, the EGFP-Chi and some degraded products were observed in both the EGFP-Chi-AF and EGFP-Chi yeast but not the parent yeast (Fig. 2(a)–bottom panel).

Corresponding to the Western blot experiment, immunofluorescence of chitinase of the intact EGFP-Chi-AF and EGFP-Chi yeast was also performed (Fig. 2(b)). Data confirmed that EGFP-Chi-AF was expressed and located at the cell surface, as a particularly strong green signal was observed for the EGFP-Chi-AF yeast as indicated by the arrow (Fig. 2(b)). A faint signal was detected for the EGFP-Chi yeast.

In order to investigate the function of yeast displayed-chitinase, chitinolytic activity of the engineered yeast was determined using

p-nitrophenylchitobiose as a substrate. The activity of EGFP-Chi in CB018—a low protease producing yeast strain—gradually increased over time, while activity of the EGFP-Chi-AF dramatically increased up to 20 h (Fig. 3(a)). Its activity was 5-fold higher than EGFP-Chi and the parent yeast strain, suggesting that chitinase on the cell surface (EGFP-Chi-AF) was active and stable (i.e., not losing its activity over time), against secreted chitinase (EGFP-Chi). The MT8-1 yeast strain, however, showed no significantly different activity among the three engineered-yeasts; viz. EGFP-Chi-AF, EGFP-Chi and the parent strain (Fig. 3(b)).

Antifungal biocontrol activity of chitinase on yeast surface

Chitinase has demonstrated chitin-digestion activity as shown in the *in vitro* experiment. We applied this activity for use in an antifungal biocontrol approach against pathogenic plant fungi. The chitinase—in the form of EGFP-Chi-AF or EGFP-Chi—was expressed on two yeast strains (viz. CB018 and MT8-1). Each clone was examined for antifungal activity comparing to the parent strain using a co-culturing method with representative pathogenic fungi and measured for mycelium growth inhibition. Both the EGFP-Chi-AF and EGFP-Chi in the CB018 yeast effectively inhibited the growth of *F. oxysporum* and slightly inhibited the growth of *F. moniliforme* compared to the parent yeast strain. EGFP-Chi-AF dramatically inhibited the growth of *F. oxysporum*; while no inhibition against *F. solani* was observed (Fig. 4(a), (b)). As expected, EGFP-Chi also showed antifungal activity since the secreted chitinase was diffused and sufficient to inhibit growth of fungi on an agar plate.

When a different yeast strain, viz. MT8-1, was used for chitinase expression, the results were slightly different. EGFP-Chi-AF moderately inhibited all three pathogenic fungi, while EGFP-Chi was more effective than the parent strain, despite showing no effect on CB018 (Fig. 4(b)). Moreover, chitinase on the yeast surface also slightly affected the growth of *Rhizoctonia solani* and *Collectotrichum gloespoloides* (data not shown).

Discussion and Conclusion

Developing effective and consistent fungal biocontrols is challenging. One of the promising approach is chitinase—a chitin digested enzyme. Bacterial chitinase of *B. circulans* was successfully expressed onto the surface of yeast. The existence of active chitinase with an anchored protein (Chi-AF) on the surface of the yeast was verified and confirmed using Western blot analysis and immunofluorescence staining. The Chi-AF obtained in this study is a possessive and stable chitinase, which can actively digest synthetic chitin, p-nitrophenol-diacetylchitobiose, for up to 20 hr, which might be enough for biocontrol under field conditions. The advantage of immobilized chitinase on yeast not only improved efficacy but also extended the duration of action (Fig. 3). Chitins on fungal cell walls, moreover, resulted in growth inhibition of phytopathogenic fungi.

Biocontrol using chitinase has been reported for years (Wiwat et al., 1996; Kramer and Muthukrishnan, 1997; Wiwat et al., 2000; Chen et al., 2004; Lertcanawanichakul et al., 2004); however, there are some limitations in terms of activity and stability, which may require the preparation or usage of enzymes in some forms or others. In order to optimize the stability, activity and practical usage, modification of such enzymes is necessary. Yeast surface display chitinase in this study is the first use of such an approach. *S. cerevisiae* was a whole cell bioreactor, which means that one cell can produce and carry 10^5 molecules of active enzyme (Shibasaki et al., 2001). It is not only a whole cell bioreactor, which could produce enzymes easily and continuously, but also showed durability and

longevity of activity (i.e., of more than a day). The immobilized chitinase on the cell surface of the yeast facilitated stability of the enzyme in the environment (i.e., giving it a prolonged half life) (Shusta et al., 1999; Ueda and Tanaka, 2000b). The specific activity of chitinase on the cell surface showed its highest activity at 20 hr and still active for up to 30 hr; implying that when these cells were applied to field crops, this immobilized chitinase would have an extended half-life over a pure enzyme application.

The success of biocontrol depends not only on its killing effect, but also on its ecology fitness. *S. cerevisiae*, the host for immobilized chitinase, was controlled in terms of environmental contamination by using of an auxotroph marker in the plasmid; thus, this engineered yeast could produce enzyme continuously with limited proliferation. Moreover, yeast can provide chitinase itself beside of anchored bacterial chitinase from *B. subtilis* on the yeast surface. Chitinase from 2 species may facilitate inhibition of the growth of fungi.

The inhibition of phytopathogenic fungi, *Fusarium* spp., is a crucial issue when using this approach because *Fusarium* spp. is the main culprit in damaging cash crops. Similarly, this chitinase presenting on the surface of yeast showed a strong inhibition on *F. oxysporum*, a soil-borne phytopathogens, which causes wilting disease on cash crops. Chitinase-yeast surface display is therefore a potential alternative for biocontrol of phytopathogenic fungi.

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