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ORIGINAL ARTICLE

Heterologous expression and characterization of the hydrophobin HFBI in *Pichia pastoris* and evaluation of its contribution to the food industry

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Abstract The class II hydrophobin HFBI from *Trichoderma reesei* was heterologously expressed by *Pichia pastoris* using pPIC9 vector under the control of the promoter AOX1. The recombinant HFBI (rHFBI) was purified by ultrafiltration and reverse-phase high performance liquid chromatography. Tricine-SDS-PAGE and Western blotting demonstrated that rHFBI with the expected molecular weight of 7.5 kDa was secreted into the culture medium. X-ray photoelectron spectroscopy and water contact angle measurements indicated that rHFBI could lead to the conversion of the wettability of the hydrophobic siliconized glass and hydrophilic mica surfaces relying on the self-assembly membrane on hydrophobic/hydrophilic interfaces. It was demonstrated that rHFBI had the ability to stabilize oil droplets, which was far excess of the class I hydrophobin HGFI heterologously expressed in *P. pastoris* (rHGFI) and the typical food emulsifier sodium caseinate. In gushing experiments, it was shown that rHFBI was a strong gushing inducer in beer, whereas rHGFI did not display any signs of gushing. This provided the potential of rHFBI to be used as a novel emulsifying agent and a predictor of gushing risk.

Keywords Hydrophobin · Self-assembly · Heterologous expression · Emulsion · Gushing

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Introduction

Hydrophobins are a class of small proteins secreted by filamentous fungi, which show an extremely high surface activity (Wösten 2001; Linder 2009). All hydrophobins possess eight conserved cysteine residues that form four intramolecular disulfide bonds (Kwan et al. 2008). They are divided into two classes, I and II, depending on differences in physical properties and hydropathy profiles (Wessels 1994). The membranes formed by class II hydrophobins easily dissolve in aqueous solution (2% sodium dodecylsulfate and 60% ethanol), while those of class I hydrophobins are highly insoluble which can only be dissolved in trifluoroacetic acid and formic acid (Wessels 1996).

The characteristic property of hydrophobins is that they self-assemble spontaneously at hydrophobic–hydrophilic interfaces (e.g., water/air and water/oil) into amphipathic membrane and convert the surface from hydrophilic to hydrophobic and vice versa (Linder et al. 2005; Sunde et al. 2008; Basheva et al. 2011). Based on the remarkable surface activities and self-assembly abilities, hydrophobins are considered as interesting candidates for potential use in many applications, including biomaterials coatings (Qin et al. 2007; Asakawa et al. 2009; Hou et al. 2009), separation technologies (Linder et al. 2001; Lahtinen et al. 2008), biosensors and electrodes (Wang et al. 2010b), the proteinaceous glue and cosmetics productions (Misra et al. 2006). Especially, it has been shown that hydrophobins can stabilize foams and emulsions, which is beneficial for intriguing application in the food industry (Cox et al. 2009; Blijdenstein et al. 2010). For example, they have been proposed to use as novel foam-stabilizing ingredients in a commercial low fat food system (Tchuenbou-Magaia et al. 2009), and as reliable indicator for beer gushing (Sarlin et al. 2005).

In the food industry, oil-in-water (O/W) emulsions are widely used and often stabilized by emulsifiers or proteins. These proteins and emulsifiers have the ability of stabilizing the oil-water interface to improve the stability and produce desirable properties of oil-in-water emulsions. Moreover, there is a drive to replace synthetic emulsifiers currently used in food products (Garti 1999). These proteins have the ability of stabilizing the oil–water interface to improve the stability and produce desirable properties of oil-in-water emulsions. Recently, it has been demonstrated that one of the most efficient approaches to long-term stabilization of emulsions and foams is through the use of surface-active hydrophobins that greatly modify the oil–water interfaces by forming stable interfacial amphiphilic layers (Askolin et al. 2006; Lumsdon et al. 2005; Murray 2007). Hydrophobins provide the real potential to be used as a novel emulsifying agent in commercial food products.

Although the stabilization of foam can be used in many food applications, it can also cause problems. Gushing (i.e. excessive foaming when opening a bottle) is provoked by surface-active molecules (Sarlin et al. 2007). It negatively affects the appearance of beer and causes great economic losses to breweries and maltsters. Hydrophobins have been shown to be gushing inducing factors (Lutterschmid et al. 2010; Stübner et al. 2010). Experimental evidence has found that the effective approach to avoid gushing was estimating the amount of hydrophobins in malt or barley (Sarlin et al. 2005).

In the present study, we successfully expressed recombinant class II hydrophobin HFBI in *P. pastoris* and explored the potential for rHFBI as emulsifying agent in food products and investigated its role in gushing induction.

Materials and methods

Strains, vectors and reagents

Escherichia coli strain DH5α, *P. pastoris* strain GS115 ($His^{-}Mut^{+}$), the plasmid pPIC9 were purchased from Invitrogen (Beijing, China). The recombinant class I hydrophobin HGFI (rHGFI) was preserved by our laboratory (Wang et al. 2010a). All restriction enzymes, Taq DNA polymerase, T4 DNA ligase and pMD19-T vector were obtained from TaKaRa (Dalian, China). DNA fragment Purification Kit, DNA markers and protein markers were supplied by TaKaRa (Dalian, China). All primers were synthesized by Takara (Dalian, China). Other chemicals were purchased from TaKaRa or Sigma.

Vector construction

The genome cDNA of *T. reesei* strain (VTT D-98692) was generated by reverse transcription of total RNA with random hexamer primers. According to the *T. reesei* hydrophobin HFBI sequence in Genbank (accession no. Z68124.1), a pair of PCR primers, *Xho*I-*hfbI*-F (5′-CG CTCGAGAAAAGAAGCAACGGCAACGGCAATG-3′) and *Eco*RI-*hfbI*-R (5′-CGGAATTCAAGCACCGACGGC GGTCTG-3′) were designed. In addition, the KEX2 protease recognition sequence was positioned behind the *Xho*I site of *Xho*I-*hfbI*-F. The HFBI coding sequence was amplified by PCR from the *T. reesei* cDNA library using primers *Xho*I-*hfbI*-F and *Eco*RI-*hfbI*-R. The expected PCR product was then inserted into pMD19-T vector to give pMD19-T-*hfbI* and the correct gene insertion was confirmed by DNA sequencing.

The *hfbI* gene in pMD19-T was then subcloned into the pPIC9 vector using both *Xho*I and *Eco*RI sites, yielding a recombinant plasmid pPIC9-*hfbI*. In pPIC9-*hfbI*, the *hfbI* gene was under the control of alcohol oxidase 1 promoter and in-frame behind the α-factor secretion signal to ensure secretion of rHFBI. The pPIC9-*hfbI* was transformed into *E. coli strain* DH5α and the plasmids from positive clones were confirmed by double-enzyme cleavage and sequencing.

Transformation of *P. pastoris* cells

The plasmid pPIC9-*hfbI* was linearized with *Stu*I, and transformed into competent *P. pastoris* GS115 His^{-} cells by electroporation using the Bio-Rad gene pulser apparatus (25 μF, 200 Ω, 2.0 kV). Transformants were plated on MD agar medium to screen for the His^{+} clones. Fifty His^{+} clones were picked and subsequently replicated onto MD and MM plates to determine the Mut^{+} phenotypes. Colony PCR was performed using 5′AOX1 (5′-GACTGGTTC CAATTGACAAGC-3′) and 3′AOX1 (5′-GCAAATGGC ATTCTGACATCC-3′) to confirm the integration of *hfbI* into the *P. pastoris* genome. Ten positive clones were selected for future work.

Fermentation and purification of *P. pastoris* expressed HFBI

The selected recombinant strains ($His^{+}Mut^{+}$) integrated with pPIC9-*hfbI* were incubated in 25 mL BMG medium at 30°C and 250 rpm until the culture reached an OD_{600} of 6. The cells were then harvested by centrifugation and resuspended in 10 mL BMM medium and cultured at 28°C and 250 rpm. Methanol was added to a final concentration of 0.5% (v/v) every 24 h to maintain the induction. The supernatants were gathered after 96 h of induction for purification and analysis of the recombinant protein. The resulting supernatants were analyzed by Tricine-SDS-PAGE (Schägger and von Jagow 1987). The clone that expressed the highest level of rHFBI was chosen by

scanning the intensity of the bands for large-scale production.

After 96-h induction, the expression supernatant containing rHFBI was collected. The supernatant was purified by ultrafiltration using a hollow fiber membrane module (Tianjin MOTIMO Membrane Technology Ltd., China) and then lyophilized. Further purification was accomplished using A Vydac C4 reversed-phase column (4.6 × 250 mm, GRACE, China) according to Yu's method (Yu et al. 2008). The resulting elutes of the targeted protein were analyzed by 16% Tricine-SDS-PAGE and Western blotting. Western blotting analysis was performed using HFBI specific polyclonal antibodies (Nakari-Setälä et al. 1996).

Water contact angle measurements

The surfaces of mica sheets and siliconized glass were coated with 20 μL of 0.02 mg/mL rHFBI solution and incubated at ambient temperature for 30 min. The surfaces were rinsed with water for three times and dried by nitrogen stream. The proteins secreted by the wild-type strain were used as protein references in equal mass concentration of rHFBI. Water contact angle (WCA) measurements were performed to investigate the wettability of the treated surfaces. 5 μL water droplet was dripped on the surface at room temperature with an optical contact angle meter (Dataphysics, Inc., OCA20). Triple individual measurements were carried out at different locations of the same surface.

X-ray photoelectron spectroscopy measurements

The modification of siliconized glass by self-assembled rHFBI was examined on an XPS apparatus (Kratos Axis Ultra DLD) employing a monochromated Al-Ka X-ray source (hv = 1486.6 eV), hybrid (magnetic/electrostatic) optics and a multi-channel plate and delay line detector (DLD). All XPS spectra were recorded using an aperture slot of 300 × 700 microns, survey spectra were recorded with a pass energy of 160 eV, and high resolution spectra with a pass energy of 40 eV.

Emulsions microstructure and stability analysis

The emulsifying capacities of rHFBI and rHGFI were investigated and compared with the typical food emulsifier sodium caseinate.

Before emulsion, the solutions of rHFBI (100 μg/mL), rHGFI (110 μg/mL) and sodium caseinate (300 μg/mL) were prepared by adding the required amount of the proteins to distilled water and then gently stirred at room temperature until complete dispersal. 8% (v/v) soy oil (food grade) was added to 5 mL of the prepared solutions followed by vortexing for 30 s and sonication for 30 min at ambient temperature. The stabilities of the obtained emulsions were observed visually and with an optical microscope.

Gushing experiments

The gushing test was performed according to the method of Stübner et al. (2010) to determine the gushing potentials of rHFBI and rHGFI.

German Beck's beer and Tsingtao Chinese beer in 500 mL bottles were used for gushing detections. The beer bottles were chilled to 0°C in an ice bath. The freeze-dried rHFBI or rHGFI after ultrafiltration was added to beers at different concentrations. After opening, rHFBI or rHGFI was added and then bottles were tightly resealed with sterilized crown seals. The treated bottles were rotated at 28 rpm for 16 h at room temperature and then were kept still for 1 h prior to opening. The amount of gushing was tested by weighing bottles before and after opening. The proteins secreted by the wild-type strain were used as negative control and three untreated bottles without adding proteins were used to ensure the gushing tendency. All tests were carried out in triplicate.

Results and discussion

Expression and purification of heterologously expressed HFBI

The coding region of the *hfbI* gene was amplified from the cDNA of *T. reesei* using primers *Xho*I-*hfbI*-F and *Eco*RI-*hfbI*-R, and then cloned into pMD19-T to give rise to pMD19-T-*hfbI*. To obtain the secreted full-length HFBI without additional N-terminal amino acids, Lys-Arg sequence specifically recognized and cleaved by the KEX2 protease was added in front of the *hfbI* gene. DNA sequencing analysis confirmed that the sequence of the insert was fully in line with the GenBank data. Then the *hfbI* gene was cloned into the vector pPIC9 for heterologous expression of HFBI in *P. pastoris* in secreted form. The restriction enzyme digestion and DNA sequencing analysis confirmed that the recombinant plasmid was constructed correctly. The commercial pPIC9 vector containing MF α-factor prepro sequence could facilitate secretion of the expressed protein (Cregg et al. 1993; Cereghino and Cregg 2000).

The plasmid pPIC9-*hfbI* was linearized with *Stu*I in the *HIS4* region, which allowed the gene insertion event to occur between the *his4* locus in the chromosome and the *HIS4* gene on pPIC9-*hfbI* (Uehara et al. 2000). The

chromosomal *AOX1* gene was intact and Mut^+ recombinants were generated on MD plates. The transformants (His^+Mut^+) were selected from MM plates and were confirmed by PCR with 5′AOX1 primer and 3′AOX1 primer (data not shown). Ten positive clones were induced by 0.5% methanol at 28°C for 96 h. The fourth clone with the highest intensity of the target band was picked up by Tricine-SDS-PAGE analysis of the culture supernatants and used for fermentation and purification (Fig. 1a).

The purified rHFBI was obtained according to a two-step procedure which included ultrafiltration followed by analytical RP-HPLC separation. The rHFBI lyophilisate after ultrafiltration or RP-HPLC purification was analyzed on 16% Tricine-SDS-PAGE, respectively. A band of about 7.5 kDa coincided with the expected HFBI theoretical size was obtained, while the same band was absent in *P. pastoris* transformed with pPIC9 vector (Fig. 1b). Ultrafiltration was evaluated as an efficient and preferred method for purifying hydrophobin proteins that removed low molecular weight contaminants and simultaneously concentrated the supernatant (Ghosh 2001; Wan et al. 2002). Considering the self-assembly abilities of hydrophobins resulted in fouling of the membrane, which caused a low permeate flux and consequent processing difficulties (Isono and Nakajima 1999), the operation pressure was 0.06 MPa to minimize the loss of targeted protein. The results show that the rHFBI purification yield obtained from the two-step procedure was 92% and a stable production of rHFBI about 120 mg protein per liter was achieved.

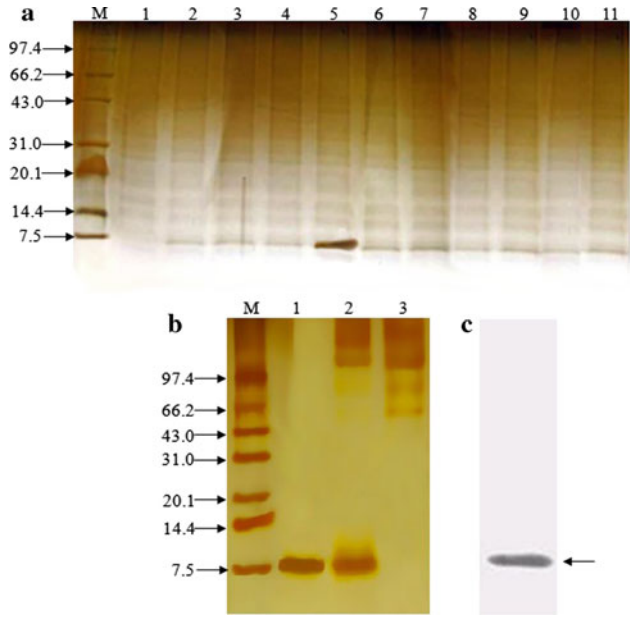

Fig. 1 **a** Silver-stained Tricine-SDS-PAGE analysis of expressed rHFBI in ten positive clones in *P. pastoris*. *Lane M* protein molecular weight marker, *lane 2* fermentation supernatant of *P. pastoris* transformed with pPIC9 vector, *lane 2–11* positive clones 1–10. **b** Silver-stained Tricine-SDS-PAGE analysis of the purified rHFBI expressed in *P. pastoris*. *Lane M* protein molecular weight marker, *lane 1* the RP-HPLC purified rHFBI, *lane 2* the rHFBI lyophilisate obtained after ultrafiltration, *lane 3* fermentation supernatant of *P. pastoris* transformed with pPIC9 vector. **c** Western blotting analysis of RP-HPLC purified rHFBI. The *arrow points* to the band of expected size

The final purified protein rHFBI was identified by Western blotting and mass spectrometry analysis. The result showed that rHFBI could specifically bind to anti-HFBI antibody (Fig. 1c). The molecular weight of rHFBI was very close to the theoretical value, which indicated that the target protein was correctly secreted to extracellular space without undesirable extra N-terminal amino acids.

Askolin et al. (2001) reported the homologous over-expression and purification of a hydrophobin, HFBI of *T. reesei*. The HFBI was over-produced at a high level in *T. reesei*. However, HFBI hydrophobin was purified from the cell walls of the fungus with a three-step method, which was tedious and time consuming. Furthermore, the final HFBI preparation was slightly yellowish. These problems needed to be overcome. *P. pastoris* is an ideal expression system for the production of recombinant hydrophobins with a two-step purification procedure (ultrafiltration-RP-HPLC) from the supernatant of the culture that was a simple and cost-effective approach for application in food industry.

WCA measurements

The changes of the wettability caused by rHFBI modification on siliconized glass and mica surfaces were tested by WCA measurements (Fig. 2) and all the WCA data were shown in Table 1. The WCA of the bare siliconized glass surface was 82.8 ± 2.1°. Yet with self-assembling of rHFBI on the surface, the WCA of modified siliconized glass surface was dramatically reduced to 36.1 ± 2.5° (close to that of native HFBI-coated siliconized glass). It was indicated that like the native HFBI the wettability of the siliconized glass surface had been greatly increased after rHFBI coating. On the mica surface, the rHFBI coating led to an increase in WCA from 7.3 ± 2.4° to 12.2 ± 2.2°, which showed the slightly decrease in wettability of the mica surface like native HFBI. There was almost no detectable change in wettability of the siliconized glass and mica surfaces coated with the proteins secreted by wild type strain *P. pastoris* GS115.

The ability of rHFBI to change the WCA of siliconized glass and mica surfaces after modification suggested that rHFBI preserved the surface activity of the native HFBI. A vital approach towards understanding how hydrophobins function associated with the crystallographic structure, which disclosed the structural element responsible for the function of this amphiphilic protein-the hydrophobic patch

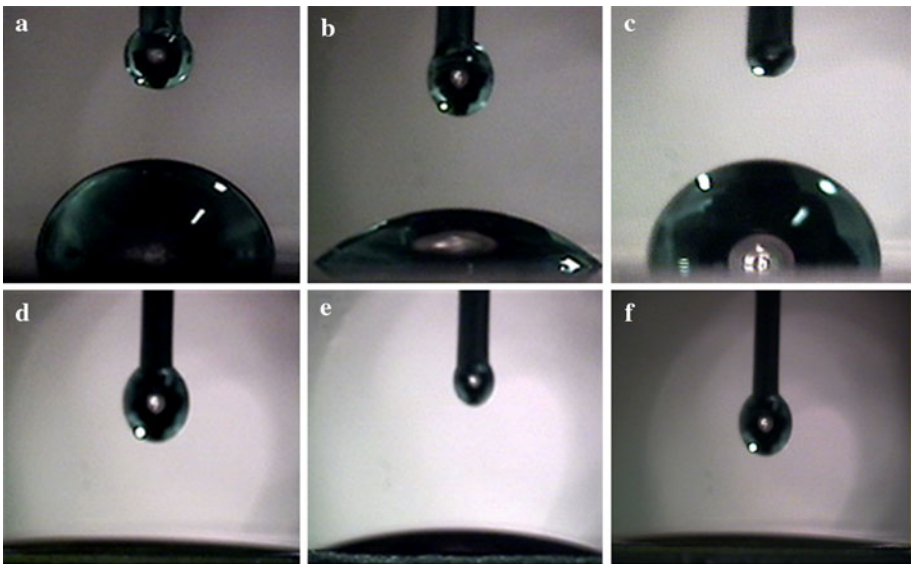

Fig. 2 Photographs of 5 μL water droplets on **a** a bare siliconized glass surface, **b** an rHFBI modified siliconized glass surface; **c** a protein references modified siliconized glass surface, **d** a bare mica surface, **e** an rHFBI modified mica surface, and **f** a protein references modified mica surface

Table 1 WCA on the bare, rHFBI and protein references modified siliconized glass and mica surfaces

Sample	Unmodified (°)	rHFBI modified (°)	Protein references modified (°)
Siliconized glass	82.8 ± 2.1	36.1 ± 2.5	81.9 ± 2.4
Mica	7.3 ± 2.4	12.2 ± 2.2	6.8 ± 2.6

(Hakanpää et al. 2004). The exposed patch consists of almost entirely of hydrophobic aliphatic side chains on the protein surface giving the molecule the character of an amphiphile. The hydrophobic patch was identified on the HFBI protein surface (Hakanpää et al. 2006).

It was speculated that when rHFBI was coated on the siliconized glass surface, the hydrophobic patch exposed on its surface could face toward the hydrophobic substrate, and the hydrophilic side of the protein was exposed outward, resulting in the hydrophobic surface converting to hydrophilic. When coating the mica surface, the hydrophobic patch of rHFBI would expose to the outside, but the wettability of hydrophilic mica surfaces only slightly decreased. The protein structure may be related to the phenomena. The hydrophobic patch on the surface of rHFBI comprises only a small part of the total surface area and the major part is hydrophilic. In addition, it was speculated that the measurement parameters for WCA data including the hydrophobin amount, the incubation temperature and time might affect the changes in WCA after modification of mica surfaces with rHFBI (Askolin et al. 2006; Pedersen et al. 2011; Qin et al. 2007). Thus, the WCA data demonstrated that similar to the native HFBI, the rHFBI performed a unique amphiphile character that allowed them to self-assemble on siliconized glass and mica surfaces leading to the change of the wettability of rHFBI-coated solid substrates.

XPS measurements

XPS is an extensively used technique to detect the chemical composition of surfaces. The chemical compositions of the bare and rHFBI-coated siliconized glass surfaces were investigated by XPS measurements. Table 2 shows the relative elemental compositions of siliconized glass before and after modification with rHFBI. As shown in Fig. 3, the high-intensity of O 1 s and Si 2p signals and the low-intensity of C 1 s signal represented the typical XPS spectra of the bare siliconized glass surface. There was almost no N 1 s signal observed in the XPS spectra of siliconized glass. After rHFBI modification, a high intensity of N 1 s signal appeared and the intensity of Si 2p signal decreased. This could be ascribed to the rHFBI modification of the siliconized glass surface because the Si element only belongs to the siliconized glass surface, while the N element only exists in the amino groups in rHFBI.

Emulsifying properties of heterologously expressed HFBI

In this study, we examined the emulsifying properties of the class II hydrophobin rHFBI and class I hydrophobin rHGFI and compared them to sodium caseinate, which was commonly used as a food emulsifier.

The proteins in all sample solutions were prepared at the same molar concentrations. When soy oil (8% v/v) was emulsified in pure water, the hydrophobin and sodium caseinate solutions, respectively, white emulsions were formed. rHFBI and rHGFI clearly showed emulsifying activity and were not different from sodium caseinate in terms of stabilizing oil droplets after 3 h (Fig. 4a). However, the rHFBI emulsion had a higher content of small oil

Table 2 The relative elemental compositions of the bare and rHFBI-modified siliconized glass surfaces

Sample type	C 1 s (%)	N 1 s (%)	O 1 s (%)	Si 2p (%)
Bare siliconized glass	8.96	0.90	55.34	22.50
Siliconized glass + rHFBI	56.78	13.09	27.61	2.52

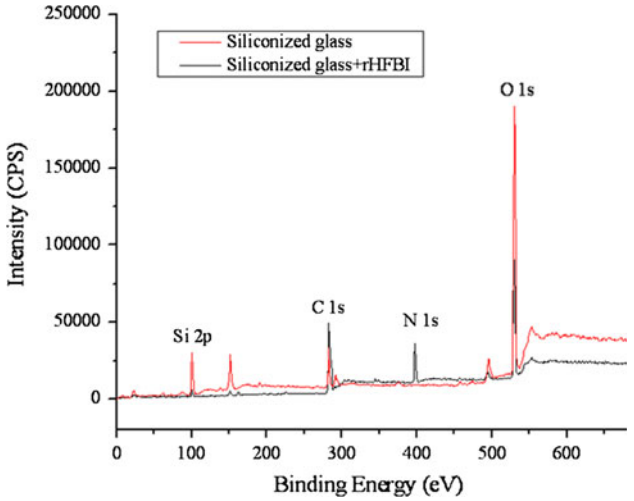

Fig. 3 XPS spectra of siliconized glass surfaces before and after rHFBI modification

droplets compared to pure water, the rHGFI and sodium caseinate emulsions (Fig. 4b). After 24 h, the pure water separated into two distinct phases with the upper oil-rich phase and the lower aqueous-rich phase. For the case of the rHGFI and sodium caseinate emulsions, there were some oil droplets floating on the top of the solutions and the diameter of oil droplets slightly increased. In contrast, soy oil emulsions stabilized by rHFBI were still fine. After 3 days, the rHFBI emulsion remained turbid and there were little changes in the amount and size of oil droplets, while rHGFI and sodium caseinate emulsions turned almost clear at the same stage and the diameter of oil droplets distinctly became larger (Fig. 4a, b).

The powerful emulsifying behavior of rHFBI could be attributed to the stable and highly elastic film formed at oil/water interface (Kisko et al. 2009). The elastic nature of the film could prevent interdroplet coalescence by dramatically reducing the oil/water surface tension (Cox et al. 2007). The results showed that rHFBI had the ability to stabilize oil droplets for longer time compared to rHGFI and sodium caseinate. The ability of rHFBI to stabilize emulsion may offer the potential to be utilized as novel emulsifying agent used in food applications.

Gushing potential of heterologously expressed HFBI

The ultrafiltrated, freeze-dried culture supernatant from *P. pastoris* strain GS115 harboring the class II hydrophobin rHFBI was added to German Beck's beer and Tsingtao Chinese beer in 500 mL bottles in order to evaluate its gushing inducing potential. In comparison, the class I hydrophobin rHGFI was also employed in beer for gushing experiments and the wild-type strain *P. pastoris* GS115 transformed with pPIC9 vector was used as negative control. Gushing volumes from the experiments were shown in Fig. 5.

Addition of 2, 4, 6 and 8 mg rHFBI lyophilisate in German Beck's beer resulted in a gushing volume of 92 ± 35, 141 ± 16, 211 ± 14 and 263 ± 9 mL per bottle, respectively. It was shown that rHFBI could induce gushing in beer and the gushing volumes constantly increased with the addition of amounts up to 8 mg. However, higher amounts of rHFBI lyophilisate did not led to further increase of the gushing volumes. When rHFBI lyophilisate was added to Tsingtao Chinese beer in the same amount, a gushing volume of 85 ± 12, 131 ± 18, 195 ± 25, 258 ± 4 mL was obtained, respectively. Yet the beers treated by rHGFI lyophilisate did not display any signs of gushing. Untreated beers and the beers added with the freeze-dried culture supernatant from negative control strain also did not show gushing.

It was speculated that the rHFBI self-assemble at the gas/water interfaces of carbon dioxide bubbles in beer which enabled it to stabilize bubbles by interaction with both CO_2-bubbles and liquid. This characteristic feature allowed the protein to overcome the energy barriers and caused the CO_2-bubbles aggregation and nucleation, which result in the microbubbles radius increasing and exceeding the critical diameter size and give rise to stronger growing bubbles. So when opening the bottle, the explosion of microbubbles occurred due to the difference in pressure between the inside and outside the bottle, which induced gushing.

Hydrophobins cause beer gushing which is due to their foaming ability (Linder et al. 2005). In previous study, class II hydrophobins have stronger tendency in foaming than class I and only class II hydrophobins induce gushing. However, class I proteins have more tendency towards adhering to surfaces than class II (Linder 2009). In this study, the class I hydrophobin rHGFI did not result in gushing in beer. This may be due to the variations of their amino acid sequences and different structures which lead to distinct properties. This result was consistent with the fact that class II hydrophobins seemed more favorable towards gushing and foaming than class I (Shokribousjein et al. 2011).

The gushing volumes obtained by adding the same amounts of rHFBI or rHGFI lyophilisate in German Beck's

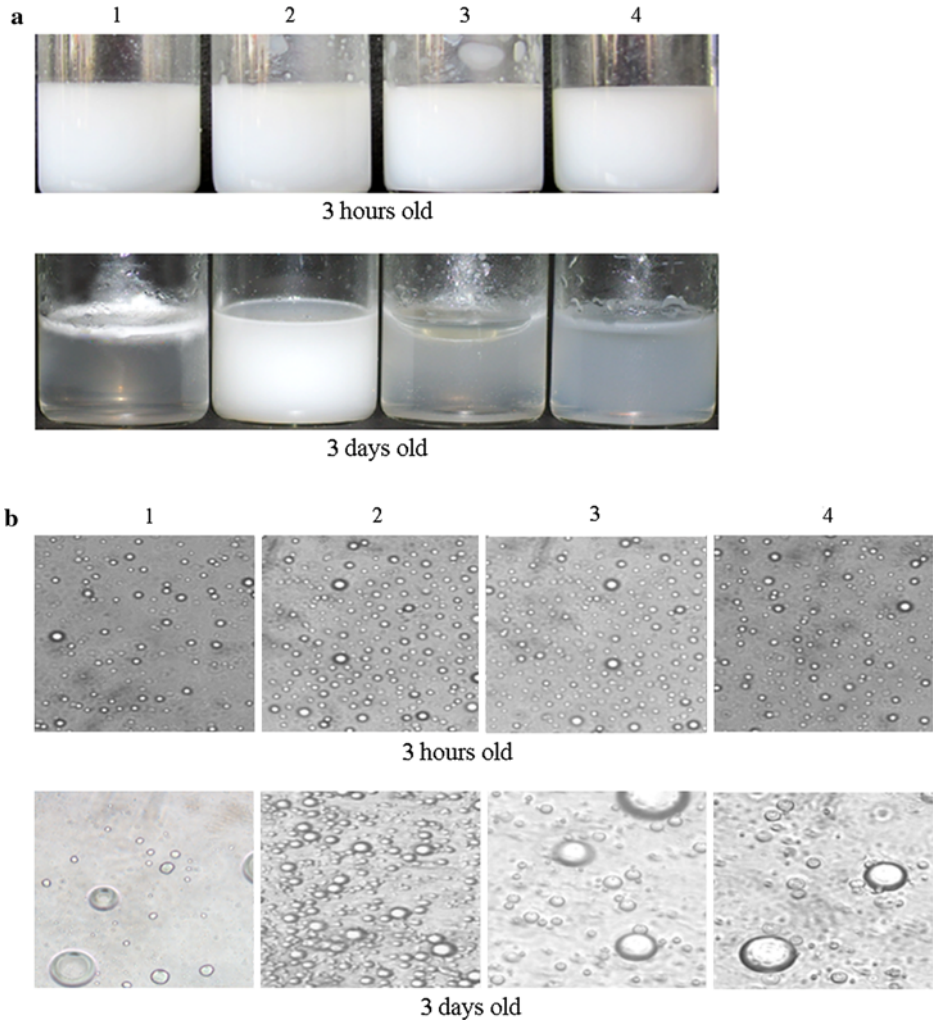

Fig. 4 The macroscopic solution images (**a**) and optical micrographs (**b**) of soy oil emulsions with the samples for 3 h and 3 days. *Numbers* show the following samples: *1* pure water, *2* rHFBI, *3* rHGFI, *4* sodium caseinate

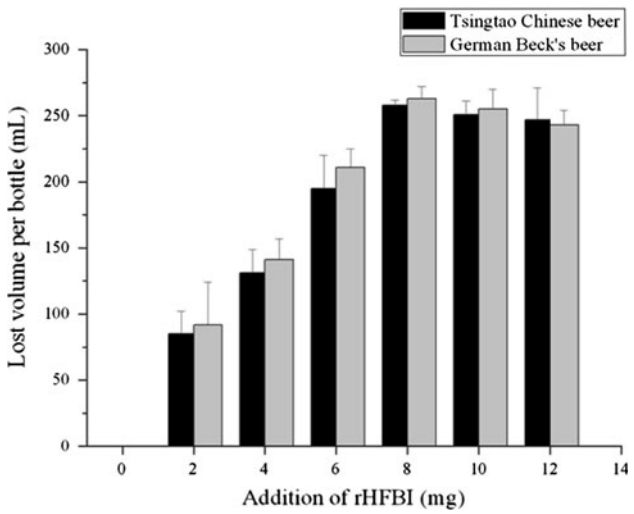

Fig. 5 Relationship between amount of added rHFBI (lyophilisate) and gushing volume in German Beck's beer and Tsingtao Chinese beer (0.5 L per bottle)

beer and Tsingtao Chinese beer were different. It was suggested that the composition of the beer and the process of beer production greatly influenced the gushing volumes, which also contributed to the high standard deviations of beer samples (Lutterschmid et al. 2011).

Conclusions

In this work, the class II hydrophobin HFBI derived from *T. reesei* was successfully expressed in methylotrophic yeast *P. pastoris* GS115. We have demonstrated that like the native HFBI, rHFBI could effectively self-assemble on the hydrophobic siliconized glass and hydrophilic mica surfaces, resulting in the wettability conversions by XPS and WCA measurements. It was also shown that rHFBI had the outstanding ability to stabilize oil droplets over a substantial period of time in excess of the class I hydrophobin rHGFI and food emulsifying agent sodium caseinate,

which was due to the highly ordered structures formed by rHFBI on the oil-water interfaces to prevent interdroplet coalescence. Furthermore, the results of the gushing tests clearly displayed that the rHFBI strongly induced gushing in beer. All the results suggested that in comparison with the class I hydrophobin rHGFI, the class II hydrophobin rHFBI had the better potential to be an emulsifying agent of food products and gushing inducer in beer. For a good understanding of the difference, further studies should be carried out.

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

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