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ORIGINAL RESEARCH

Production of a transglutaminase from *Zea mays* in *Escherichia coli* and its impact on yoghurt properties

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A gene from Zea mays *coding a TGase was expressed in* E. coli *and identified by Western blotting. Under optimal expression conditions, the production and specific activity of refolded TGZ were 1.41 mg/L and 0.34 U/mg. The activated TGZ was employed to cross-link milk proteins. The yoghurt treated with TGZ showed a lower syneresis, higher apparent viscosity and texture than untreated yoghurt. The properties of TGZ-treated sample were better than those of MTG-treated samples, so TGZ may have potential as an additive in yoghurt manufacture.*

Keywords *Zea mays* transglutaminase, Protein expression, Optimisation, Enzymatic cross-linking effect.

INTRODUCTION

Transglutaminase (TGase, EC 2.3.2.13) is an important enzyme that can catalyse the acyl-transfer reaction between γ-carboxyamide groups of glutamine residues and ε-amino groups of lysines or other primary amines (Lorand and Graham 2003). This enzyme introduces either intra- or intermolecular cross-links in proteins, peptides and various primary amines, leading to the improvement of thermal stability, solubility, water-holding capacity and nutritional value of proteins (Yokoyama *et al.* 2004; Dube *et al.* 2007).

TGase was first detected in the human brain by Waelsch in 1957 and extracted from guinea pig liver by Fork *et al.* in 1973 (Folk and Chung 1973; Beninati *et al.* 2009). Subsequently, TGases from human blood and tissue, and fish have been reported (Yokoyama *et al.* 2000). Eukaryotic TGases are calcium-dependent and composed of several subunits (Marx *et al.* 2008). The study showed that animal TGase possess a catalytic triad comprising three amino acids (histidine, cysteine and aspartate), and the catalytic reaction proceeds through an intermediate linked to cysteine (Hettasch and Greenberg 1994). In prokaryotes, Microbial TGases (MTGs) are mainly found in *Streptoverticillium* sp., *Streptomyces* sp., *Physarum polycephalum*, *Bacillus subtilis*, *Candida albicans* and other organisms (Ando *et al.* 1989; Lin *et al.* 2004). MTGs are calcium independent and consist of one subunit (Marx *et al.* 2007). At present, MTG produced by *Streptomyces mobaraensis* is commercially available and widely used in food industry (Sommer *et al.* 2011).

The research on structure and function in plant TGase is less developed than in microorganisms and animals. Plant TGase was first observed in pea seedlings in 1987 (Icekson and Apelbaum 1987) and subsequently found in *Medicago sativa*, *Helianthus tuberosus* and soybean (Margosiak *et al.* 1990; Duca *et al.* 1994; Kang and Cho 1996). Bernet *et al.* detected TGase activity in maize callus and chloroplasts in 1999 (Bernet *et al.* 1999), and further study showed that the activity of this enzyme was affected by light and 2, 4-dichlorophenoxyacetic acid (Villalobos *et al.* 2001). Subsequently, two cDNA sequences, *tgz15* and *tgz21* named according to the number of B-type repeats in the

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noncatalytic domain of the enzyme, were cloned into *E. coli*. The recombinant TGases were mainly presented as inclusion bodies and only activated by a complicated refolding process (Carvajal *et al.* 2006; Carvajal-Vallejos *et al.* 2007). Although subsequent studies found that nondenaturing detergent triton X-100 could solubilise inclusion bodies resulting in a relatively high production of active proteins, the activity of refolded proteins was low and only 0.1 U/mg (Carvajal *et al.* 2011).

Optimisation of the expression conditions can improve the expression level of heterologous proteins in *E. coli*. The selection of host, inductor concentration, induction time and temperature after induction are relevant for the level of protein expression (Jana and Deb 2005; Yang *et al.* 2012; Berlec and Štrukelj 2013). It has been shown that temperature shift strategy could improve the expression level of soluble protein, and higher solubility could be obtained using an induction temperature lower than 37 °C (Kataeva *et al.* 2005; Marx *et al.* 2007).

The enzymatic properties of MTG have been extensively studied, and this enzyme is widely used in the food industry. Specific applications of MTG have been reported for the processing of dairy, meat, fish, bread, soybean and other foods (Yokoyama *et al.* 2004; Dube *et al.* 2007). Among the dairy proteins, casein is a favourable substrate for MTG mainly due to its highly accessible and flexible open-chain structure (Jaros *et al.* 2006, 2007). Whey proteins are less prone to catalysis by MTG mainly because the stability of their native globular structure inhibits the cross-linking reaction (Dejong and Koppelman 2002; Ozer *et al.* 2007). Earlier studies have shown that using MTG in yoghurt production increased the gel strength, stability and viscosity, while decreasing the syneresis due to increased water-holding capacity (Lorenzen *et al.* 2002; Farnsworth *et al.* 2006; Yüksel and Erdem 2010; Şanlı *et al.* 2011).

The research on plant TGase has been greatly delayed, and so far, only two plant TGases, AtPnglp of *Arabidopsis* and TGase of *Zea mays* (TGZ), have been heterologously expressed (Della Mea *et al.* 2004; Villalobos *et al.* 2004). Although there have been more studies on TGZ, these studies only focused on the heterologous expression, lacking property and applications research. The aim of this work was to produce active TGZ to study the effect of enzymatic cross-linking on the functional properties of yoghurt. In this study, TGZ was cloned and expressed in *E. coli*. The expression conditions of recombinant strain were also optimised. Finally, the active TGZ was employed to cross-link milk proteins, to provide experimental evidence for potential applications of TGZ in the food industry.

MATERIALS AND METHODS

Materials

Unless otherwise stated, all enzymes used in the experiment were purchased from Toyobo (Shanghai, China). Antibody specific for a His-tag was obtained from Tiangen (Beijing, China). AP-labelled Goat Anti-Mouse IgG and BCIP/NBT Alkaline Phosphatase Color Development Kit were purchased from Beyotime (Nantong, China). MTG derived from *S. mobaraensis* DSM40587 was extracted by our group and the specific activity reached 2.9 U/mg (Zhang *et al.* 2012). Bovine milk was obtained from Mengniu (Harbin, China). The protein and fat content of the milk were 3.0% and 3.7%. Probiotic yoghurt starter (Yo-Mix 161 LYO 375 DCU) was purchased from Danisco (Beijing, China). All other chemical reagents were of analytical grade. Deionised water was used throughout the experiments.

Bacterial strains, plasmids and growth conditions

ArcticExpress *E. coli*, *E. coli* BL21(DE3), *E. coli* BL21 (DE3)Rosetta and *E. coli* BL21(DE3)pLysS were purchased from Invitrogen and used as host strains for heterologous protein expression. *E. coli* DH5α was also obtained from Invitrogen and used for the propagation of plasmid. *E. coli* was grown in Luria-Bertani (LB) medium at 37 °C. Antibiotic ampicillin (Amp) was used for *E. coli* at 100 μg/mL.

Construction of a recombinant strain

The *tgz* and *ptgz* were amplified by PCR from the cloning plasmid pET28a-*tgz* and containing full length *tgz* were constructed by our laboratory (Qin *et al.* 2010). The primers AF and AR (Table 1) were used to amplify *tgz,* and primers PF and AR (Table 1) were used to amplify *ptgz* (*tgz* without predicted chloroplast import peptide). The PCR reaction was performed in a 50 μL final volume containing 0.4 μmol/L each primer, 1 × PCR buffer, 200 μmol/L each dNTP, 1 mmol/L Mg^{2+}, 1 U of KOD-Plus-DNA polymerase (Toyobo, Shanghai, China). Thermal cycling conditions were 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s,

Table 1 Oligonucleotide primers used in this study

Name	*Sequence (5′-3′)*	*Length (bp)*	T_m*(°C)*
AF	ACTGGATCCATGGCTCATCGTGGACATCT, a *Bam* HI site underlined	29	69
PF	ACTGGATCCATGCAAACTACAGAAGTGGA, a *Bam* HI site underlined	29	66.1
AR	GCAGTCGACTCACCATATTTGTCTGCTCAA, a *Sal* I site underlined	30	67.5

T_m, Melting temperature.

60 °C for 30 s, 72 °C for 50 s and a final extension step of 68 °C for 5 min. The *tgz* and *ptgz* PCR products were purified using the Biospin Gel Extraction Kit (BioFlux, Hangzhou, China) following the instructions provided by the manufacturer. Then, the purified PCR products were digested with *Bam* HI and *Sal* I and cloned into the compatible sites (*Bam* HI and *Sal* I) of expression vector pCold-SUMO. Plasmid DNA was prepared and sequenced, at least twice in both directions, using an ABI PRISM 377 DNA sequencer (Perkin-Elmer Cetus Instruments, Norwalk, CT, USA). The recombinant plasmids, pCold-*tgz* and pCold-*ptgz*, were transformed into ArcticExpress *E. coli* for expression of the TGZ. The resulting recombinant strains were named ArcticExpress (pCold-*tgz*) and ArcticExpress (pCold-*ptgz*). ArcticExpress *E. coli* was also introduced with the empty plasmid pCold-SUMO to generate a control strain ArcticExpress (pCold-SUMO).

Small-scale expression of recombinant protein

Recombinant strains were grown in 5 mL LB medium (ampicillin, 100 μg/mL) for 16 h at 37 °C and shaken at 160 r/min. Two mL of culture were added into 100 mL LB medium containing ampicillin (100 μg/mL) and grown at 37 °C. When the value of OD_{600} reached approximately 0.6, cells were induced by adding IPTG to a final concentration of 0.2 mmol/L, followed by growth for up to 8 h at 15 °C. After induction, cells were harvested by centrifugation and then resuspended in equal volumes of 2 × sample buffer and boiled for 5 min.

The boiled samples were analysed by SDS-PAGE using 5% stacking gel and 12% separating gel. Electrophoresis was performed by a Mini-Protean II (Bio-Rad Laboratories Inc., Hercules, CA, USA), and gels were stained with Coomassie Brilliant Blue R-250 (Laemmli 1970). After SDS-PAGE, proteins were transferred into a nitrocellulose membrane. The membrane was thereafter incubated with antibody specific for a His-tag (1:2000, dilution) as primary antibody, followed by incubation with AP-conjugated goat anti-mouse (1:1000, dilution) as secondary antibody. Signals were visualised by treating the membranes with NBT/BCIP stock solution, and colour development was stopped with ddH_2O.

Optimisation of expression condition of TGZ

Recombinant plasmid pCold-*tgz* was transformed into different host strains, ArcticExpress *E. coli*, *E. coli* BL21(DE3), *E. coli* BL21(DE3)Rosettas and *E. coli* BL21(DE3)pLysS, for the expression of TGZ (Francis and Page 2010). The selected recombinant strain was induced according to the method mentioned above. The IPTG concentration, induction time and initial OD_{600} value before IPTG induction were optimised. Along with the recombinant strain induced by 0.2 mmol/L IPTG, cells were collected by centrifugation every 4 h until 24 h. The recombinant strain was induced by different IPTG concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mmol/L), and different values of OD_{600} (0.2, 0.4, 0.6, 0.8 and 1.0) were harvested at 8 h.

Purification of recombinant TGZ

The cultivated cells were harvested by centrifugation at 8000 × *g* at 4 °C for 10 min and resuspended in buffer A (20 mmol/L Tris-HCl, pH 8.2, 1 mmol/L $MgSO_4$) containing 0.05% lysozyme and 0.01% DNase. The harvested cells were disrupted by ultrasonication and centrifugated. The collected inclusion bodies were washed twice with buffer B (20 mmol/L Tris-HCl, pH 8.2, 0.2% Triton X-100) and solubilised by gentle agitation in buffer C (20 mmol/L Tris-HCl, pH 8.2, 8 mol/L urea). Then, the sample was applied to a 1 mL HisTrap™ HP (GE Healthcare, Uppsala, Sweden) column, and bound TGZ was eluted using a pH gradient (from 8.2 to 4.5). The refolding of inclusion bodies was performed according to the method described by Carvajal *et al.* (2011).

Enzyme assay

The activity of TGZ was assayed according to the colorimetric procedure using *N*-carboxybenzoyl-L-glutaminyl-glycine (N-CBZ-Gln-Gly) as a substrate (Grossowicz *et al.* 1950). A calibration curve was prepared using L-glutamic acid-γ-monohydroxamate (Sigma, Shanghai, China). One unit of TGase activity was defined as the amount of enzyme which catalysed the formation of 1.0 μmol L-glutamic acid-γ-monohydroxamate per minute at 37 °C. Protein concentrations were determined with a Bradford Protein Assay Kit (Beyotime, Shanghai, China) using bovine serum albumin as the standard.

Cross-linking effect of TGZ on yoghurt

The 100 mL milk samples were pasteurised at 95 °C for 5 min and inoculated with 0.04% probiotic yoghurt starter culture (YO-MIX™, Yoghurt Cultures, Yo-Mix 161 LYO 375 DCU, Danisco, Beijing, China) to produce the enzyme-free control yoghurt samples (C). The inoculated milk was also added into different concentrations of recombinant TGZ and MTG (0.25, 0.5, 1 U/g protein for milk) to produce the enzyme-induced yoghurt samples. Then, the milk was incubated at 42 °C until the pH reached 4.6, and the resulting yoghurt was placed at 4 °C. The syneresis, apparent viscosity and texture of yoghurt was analysed. Yoghurt syneresis index was measured using the method described by Farnsworth *et al.* (2006). Texture and apparent viscosity were carried out using the method of Zhang *et al.* (2012).

Statistical analysis

All results represented the average of three separate experiments and expressed as mean values ± standard deviation (SD). Statistical analyses were performed using the SPSS 14.0, and Duncan's post-test was used for one-way analysis of variance (ANOVA). Differences were considered significant when *P* values were less than 0.05.

RESULTS AND DISCUSSION

Expression of *tgz* in *E. coli*

TGase extraction from plant tissues was difficult, so an efficient method to produce plant TGase was established in this study. According to the previous report, the TGZ protein sequence possessed a chloroplast import peptide made up of the first 47 amino acids. In the chloroplast import peptide, a casein kinase II phosphorylation, a protein kinase C and three myristoylation sites existed (Villalobos *et al.* 2004). To study the effect of the import peptide for protein expression and transport, *tgz* and *ptgz* were respectively expressed in ArcticExpress *E. coli*. After induction, total cellular proteins were separated by SDS-PAGE and identified by Western blotting. Compared with the negative control (cell fractions from ArcticExpress (pCold-SUMO) and cell fractions from recombinant ArcticExpress *E. coli* without induction), bands of approximately 74 and 67 kDa were detected in cell fractions from IPTG-induced ArcticExpress (pCold-*tgz*) and ArcticExpress (pCold-*ptgz*), respectively (Fig. 1a). The molecular weights were identical to the calculated molecular mass of TGZ and PTGZ. The results of Western blotting further showed that the proteins expressed by recombinant ArcticExpress *E. coli* were the TGZ (74 kDa) and PTGZ (67 kDa) (Fig. 1b), but the recombinant proteins were mainly presented as inclusion bodies. These results indicated that *tgz* and *ptgz* successfully expressed in the ArcticExpress *E. coli*. But comparison with the recombinant strain ArcticExpress (pCold-*tgz*), the expression level of recombinant strain ArcticExpress (pCold-*ptgz*) did not significantly improve. So this import peptide did not affect the expression level and the solubility of recombinant protein, and this result has also been reported by Carvajal-Vallejos *et al.* (2007).

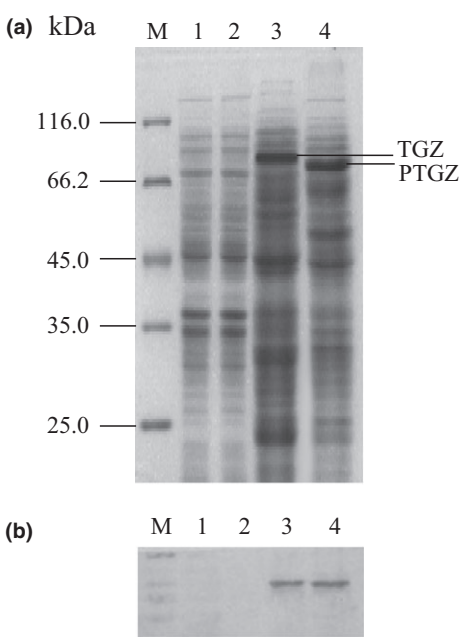

Figure 1 Expression and identification of recombinant proteins. (a) Expression of TGZ in ArcticExpress *E. coli* detected by 12% SDS-PAGE. (b) Western blotting analysis of the recombinant proteins. Lane M, protein marker; lane 1, cell fractions from ArcticExpress (pCold-SUMO); lane 2, cell fractions from recombinant ArcticExpress *E. coli* induced without 0.2 mmol/L IPTG; lane 3, cell fractions from ArcticExpress (pCold-*tgz*) induced by 0.2 mmol/L IPTG; lane 4, cell fractions from ArcticExpress (pCold-*ptgz*) induced by 0.2 mmol/L IPTG.

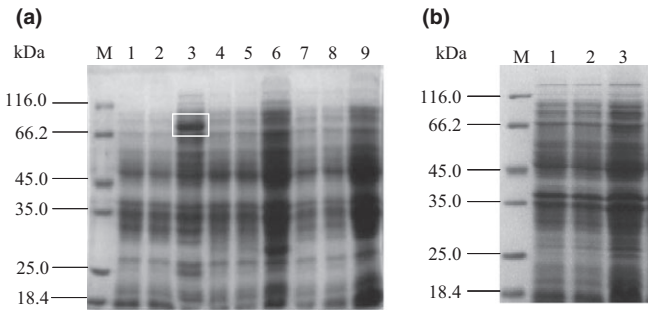

Figure 2 The expression of different host strains containing pCold-*tgz*. Recombinant strains were grown in LB medium (ampicillin, 100 μg/mL) and induced with 0.2 mmol/L IPTG. (a) Lane M, protein marker; lane 1–3, ArcticExpress *E. coli*; lane 4–6, *E. coli* BL21(DE3)pLysS; lane 7–9, *E. coli* BL21(DE3). Lane 1, 4 and 7, containing empty vector pCold-SUMO; Lane 2, 5 and 8, without induction; Lane 3, 6 and 9, containing pCold-*tgz*. (b) Lane M, protein marker; lane 1–3, *E. coli* BL21(DE3) Rosettas. Lane 1, containing empty vector pCold-SUMO; Lane 2, without induction; Lane 3, containing pCold-*tgz*. The recombinant protein was highlighted with pane.

Optimisation of expression condition and purification of recombinant TGZ

To overproduce the recombinant protein, host strain, IPTG concentration, induction time and initial OD_{600} value before

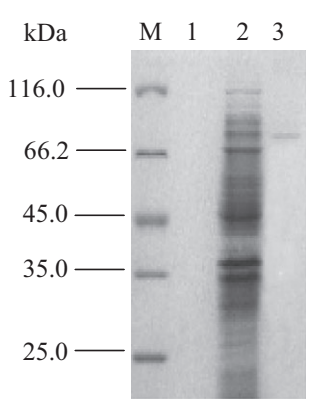

Figure 3 Purification of recombinant TGZ. Lane M, protein marker; lane 1, supernatant from ArcticExpress *E. coli* (pCold-*tgz*) induced by 0.2 mmol/L IPTG; lane 2, cell fractions from ArcticExpress *E. coli* (pCold-*tgz*) induced by 0.2 mmol/L IPTG; lane 3, the purified TGZ.

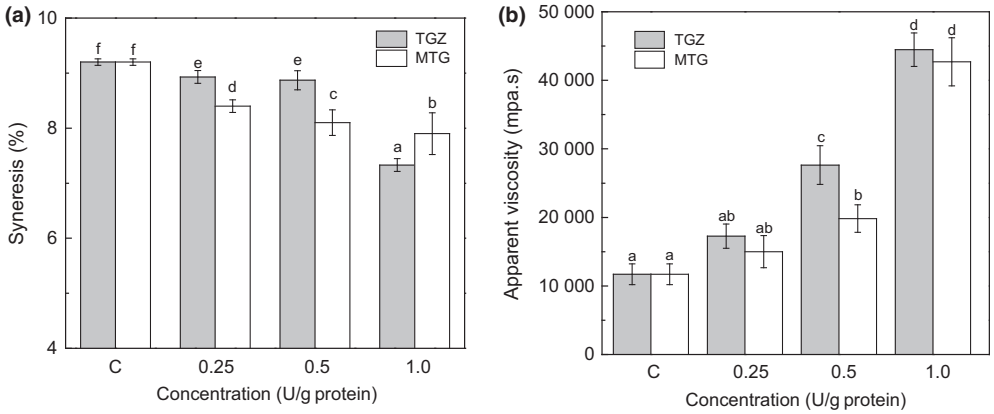

Figure 4 Cross-linking effect of recombinant TGZ and MTG on yoghurt. (a) Effect of TGZ and MTG on the syneresis of yoghurt. (b) Effect of TGZ and MTG on the apparent viscosity of yoghurt. C: cow's milk yoghurt without enzyme addition; 0.25–1.0: different concentrations of recombinant TGZ and MTG (0.25, 0.5 and 1.0 U/g). The bars with different letters are different ($P < 0.05$) based on the Duncan's post-test.

IPTG induction were optimised. The activity of the expressed TGZ was detrimental to the host strain, so ArcticExpress *E. coli*, *E. coli* BL21(DE3), *E. coli* BL21(DE3)Rosettas and *E. coli* BL21(DE3)pLysS were selected to express TGZ. These host strains could decrease basal expression of the target protein and could be used to express toxic proteins (Francis and Page 2010). The result showed that ArcticExpress *E. coli* had a higher expression level than the other host strains (Fig. 2), and the recombinant TGZ was mainly found in the inclusion bodies, as also reported by Carvajal-Vallejos *et al.* (2007) and Carvajal *et al.* (2006, 2011).

A temperature shift strategy with an induction temperature lower than the growth temperature could improve the expression level of soluble protein. Marx *et al.* (2007) reported that soluble MTG from *S. mobaraensis* could be expressed using a temperature shift strategy with an induction temperature of 24 °C, but the yield was low, only 4.5 mg/L. Yu *et al.* (2008) reported that MTG from *Streptomyces netropsis* was soluble when expressed under conditions comprising of growth at 37 °C and induction at 20 °C, with a maximum yield 180 mg/L. We cultured recombinant strain at 37 °C and induced at 15 °C according to the instruction of plasmid pCold-SUMO. The result indicated that the expression of soluble TGZ failed under the temperature shift strategy (data not shown) and recombinant protein was mainly expressed as inclusion bodies. For expression of soluble MTG, most attempts in *E. coli* have failed and a *Corynebacterium* expression system was established in recent years to secrete the soluble pro-MTG (Kikuchi *et al.* 2003; Itaya and Kikuchi 2008). The amount of pro-MTG was higher, approximately 900 mg/L (Date *et al.* 2004), and these results suggested that this was a useful method for efficient production of soluble MTG. In future work, the *Corynebacterium* expression system will be used to express soluble TGZ, but the advantages of fast cell growth, an inexpensive medium and the relatively simple gene manipulation of expression in *E. coli* would be lost using this gram-positive strain.

To obtain a large amount of TGZ, the expression conditions of the recombinant strain were optimised. In this work, three essential conditions for a higher yield of recombinant TGZ, including IPTG concentration, induction time and initial

Table 2 Texture analysis of cow's milk yoghurts cross-linked by different concentrations of recombinant TGZ and MTG

Cow milk yoghurt	*Firmness (g)*	*Consistency (gs)*	*Cohesiveness (g)*	*Index of viscosity (gs)*
C	192.94 ± 2.69^{a}	4642.43 ± 67.66^{a}	179.93 ± 1.59^{a}	352.12 ± 1.04^{a}
T0.25	281.67 ± 4.84^{c}	4904.66 ± 133.46^{bc}	278.47 ± 4.74^{c}	384.1 ± 2.89^{b}
T0.5	423.59 ± 5.02^{e}	5005.61 ± 85.98^{bc}	376.84 ± 4.41^{d}	433.39 ± 7.21^{c}
T1.0	528.92 ± 3.45^{f}	5112.17 ± 96.78^{c}	464.32 ± 5.79^{e}	441.53 ± 6.83^{c}
M0.25	198.31 ± 3.06^{a}	4784.52 ± 40.23^{ab}	218.80 ± 9.03^{b}	388.05 ± 19.09^{c}
M0.5	254.94 ± 8.26^{b}	4816.94 ± 91.18^{ab}	263.40 ± 14.33^{c}	418.34 ± 16.12^{bc}
M1.0	403.55 ± 8.33^{d}	5137.48 ± 143.59^{c}	364.61 ± 13.02^{d}	443.97 ± 29.53^{c}

C, cow milk yoghurt without enzyme addition; T0.25–T1.0, different concentrations of recombinant TGZ (0.25, 0.5 and 1.0 U/g); M0.25–M1.0, different concentrations of MTG (0.25, 0.5 and 1.0 U/g). All data were showed as mean values ± S.D ($n = 3$). Values followed by different letters in the same row are significantly different ($P < 0.05$).

OD_{600} value before IPTG induction were studied. Although different IPTG concentrations did not affect the expression level of recombinant protein, higher IPTG concentration might be deleterious for the host cell (Takehana *et al.* 1994), so we chose a lower inducer concentration of 0.2 mmol/L as induction concentration. The results of induction time showed that there was an increased expression of recombinant TGZ along with time, but there was no significant increase after 16 h. Because the accumulation of recombinant protein might have an adverse effect on the cell, we should try to reduce the induction time. But, if the induction time is too short, the expression level of recombinant protein is also reduced (Jana and Deb 2005; Marx *et al.* 2007). So, 16 h was chosen for the induction time. When the recombinant strain was induced to an OD_{600} of 0.4, the amount of recombinant TGZ reached the maximum. This might be because the host strain reached a logarithmic growth phase at the OD_{600} of 0.4, which promoted the expression of recombinant TGZ. Therefore, an OD_{600} value of recombinant strain ArcticExpress (pCold-*tgz*) of 0.4, and IPTG concentration of 0.2 mmol/L and expression time of 16 h were judged to be the optimal conditions for the TGZ expression.

The recombinant strain ArcticExpress (pCold-*tgz*) was cultured using a shake flask for the purification. The expressed TGZ was solubilised in 8 mol/L urea and purified by HisTrap™ HP column as described in the Materials and methods. The TGZ was captured by Ni-NTA column and eluted by a pH gradient. The fractions containing purified TGZ were analysed by SDS-PAGE, and only a band of 74 kDa corresponding to TGZ was found (Fig. 3). To achieve higher active protein, TGZ was renatured using Triton X-100 (Carvajal *et al.* 2011). The production of purified TGZ was 1.41 mg/L, and the specific activity of refolded TGZ was 0.34 U/mg, which was onefold higher than the activity before optimisation.

Cross-linking effect of TGZ on yoghurt

The cross-linking reaction of TGase was used to modify the hydration, emulsifying and rheological properties of proteins (Lauber *et al.* 2000). To estimate the effect of TGZ on yoghurt, MTG was chosen for another control. Different concentrations of recombinant TGZ and MTG were added into the milk; syneresis, apparent viscosity and texture of yoghurt were analysed.

The results of syneresis indicated that syneresis of yoghurt treated with TGZ and MTG was both reduced compared with the enzyme-free control yoghurt samples (Fig. 4a). The result showed that a lower concentration of MTG had a significant effect on the yoghurt compared with recombinant TGZ. But, when TGZ was added to 1.0 U/g protein for cow's milk, the syneresis of TGZ-treated yoghurt sample was significantly reduced (Fig. 4a). The apparent viscosity of the milk yoghurt treated with recombinant TGZ and MTG was studied (Fig. 4b). The apparent viscosity of yoghurt treated with TGZ and MTG increased with the amount of enzyme added. However, a significant increase was observed in different samples at an enzyme level of 0.5 U/g protein. The apparent viscosity of the cow's milk with added recombinant TGZ at the level of 1.0 U/g protein was increased more than 3.75 times compared with the untreated sample. As shown in Fig. 4b, the effect catalysed by TGZ was higher than that treated with MTG. Texture analysis was an important way to evaluate the quality of the yoghurt. Ozer *et al.* (2007) and Bönisch *et al.* (2007) demonstrated that rheological properties could be improved by forming a denser and finer gel network. And Gauche *et al.* (2009) further indicated that these improvements might be caused by the formation of ε-(γ-glutamyl) lysine bonds.

The parameters including firmness, consistency, cohesiveness and index of viscosity of the different treatments were significantly higher compared to the enzyme-free control yoghurt samples (Table 2). Increasing the amount of TGZ and MTG was shown to improve yoghurt texture. A significant increasing was observed at 1.0 U/g protein.

CONCLUSION

In this paper, a method for producing TGZ in *E. coli* with a good yield was described. Although the majority of TGZ was found in the insoluble inclusion bodies, we were able to obtain active enzyme by refolding of urea-extracted inclusion bodies. In addition, the active recombinant TGZ was used to cross-link proteins in the yoghurt, and the properties of TGZ-treated yoghurt was improved compared with the negative control, and higher than that of the MTG-treated samples. This further indicated that plant TGase could potentially be used in the food industry in the future.

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