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ORIGINAL ARTICLE

# Enhancing the stability of xylanase from *Cellulomonas fimi* by cell-surface display on *Escherichia coli*

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**Keywords**
anchor protein, cell-surface display, thermostability, xylanase.

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## Abstract

**Aims:** The cell-surface display of Cex, which encodes xylanase and exoglucanase from *Cellulomonas fimi*, was constructed on *Escherichia coli* using PgsA as the anchor protein. Characterization of the cell-surface display of Cex was performed.

**Methods and Results:** PgsA was fused to the N-terminus of Cex and six histidines were utilized as spacers between the targeting and anchor proteins. Successful cell-surface display of Cex was demonstrated by Western blot and immunofluorescence analyses on *E. coli* C41 (DE3). According to the time-course analysis, the xylanase activity of Cex was achieved at 49 U $g^{-1}$ dry cell weight after 12 h culture at 37°C. The optimal temperature and pH ranges of the cell-surface displayed protein with whole-cell were broader than the corresponding ranges of the purified form. Further determination of thermostability indicated that the half-life of cell-surface displayed Cex was 1·6 times longer than that of purified Cex at 60°C.

**Conclusions:** We have successfully developed the cell-surface display of xylanase on *E. coli*. The cell-surface display can enhance the stability of xylanase against changes in temperature and has the potential of becoming a whole-cell biocatalyst for industrial applications, such as biobleaching of paper and production of renewable energy.

**Significance and Impact of the Study:** The results demonstrated that the cell-surface display of xylanase embedded in the cell membrane is more stable than that of the purified enzyme. Thus, to improve the stability of heterologous proteins production, cell-surface display using the PgsA anchor protein as a tool can be considered in *E. coli*.

## Introduction

Xylan is a component of hemicelluloses and is the second most abundant polysaccharide. Xylan is composed of a backbone of $\beta$-1,4-linked-D-xylosyl residues with various side chains, depending on the different plant sources. Several enzymes are involved in the degradation of xylan, including endoxylanase, $\beta$-xylosidase, acetyl xylan esterase, $\alpha$-glucuronidase, $\alpha$-arabino furanosidase, feroryl and *p*-coumaroyl esterases (Beg *et al.* 2001). Endo-1,4-$\beta$-xylanase (EC 3.2.1.8) plays an important role in the cleavage of the internal linkages of the $\beta$-1,4-xylose backbone. The main applications of xylanase are in the paper and pulp industries as a bleaching agent used to replace toxic chlorine-containing chemicals (Bajpai 1999). Moreover, in the area of animal nutrition, the incorporation of xylanase into lignocellulosic feeds can result in reduced intestinal viscosity and enhanced efficiency of feed conversion (Murphy *et al.* 2009). Recently, depolymerization of xylan from wastes of agricultural and food industries wastes into xylooligosaccharides and xylose by xylanase has been used for the generation of renewable energy, such as ethanol and biogas (Chen *et al.* 2010; Weiss *et al.* 2010). Given that xylanase has substantial potential in various industrial applications, several studies have been carried out on alkaliphilic, thermophilic, and acidophilic

xylanases (Gupta *et al.* 2000; Liu *et al.* 2010a; Valenzuela *et al.* 2010).

The *cex* gene from *Cellulomonas fimi*, which contains endo-1,4-$\beta$-xylanase and exo-1,4-$\beta$-D-glucanase activities, has been characterized and expressed in *Escherichia coli*. The results show that Cex displays low exo-1,4-$\beta$-D-glucanase activity on microcrystalline celluloses (Tomme *et al.* 1995). Three-dimensional structure of Cex has also been reported and indicates that Cex consists of a catalytic domain (CexCD) and a cellulose-binding domain (CexCBD) connected by the proline-rich and serine/threonine-rich linker (PT-Linker). Although a recombinant version of Cex (p33) was constructed without CexCBD, it still exhibits high specific activity on xylan (White *et al.* 1994, 1996; Notenboom *et al.* 1998a,b, 2000). To allow economical extracellular production of the enzyme, Cex with the *ompA* leader sequence has been conducted in *E. coli* (Lam *et al.* 1997). High-level expression of intracellular enzyme leads to lower yield because of rapid cell death, which correlates with the rapid increase in the OmpA/Cex fusion precursor in the inner membrane (Fu *et al.* 2005).

The cell-surface display of heterologous proteins has several advantages in biotechnological applications, such as whole-cell biocatalysis for bioconversion, peptide library screening, biosensors and immobilization (Lee *et al.* 2003). Much attention has been devoted to the development of various anchoring motifs for representative cell-surface display (Lee *et al.* 2003). Several genes encoding membrane-anchored proteins, such as *pgsA* encoding poly-$\gamma$-glutamate synthetase (PGA) from *Bacillus subtilis* (Ashiuchi *et al.* 2001; Narita *et al.* 2006; Tateno *et al.* 2007), ice nucleation protein (INP) from *Xanthomonas* (Wu *et al.* 2006) and *estA* encoding an autotransporter from *Pseudomonas aeruginosa* (Wilhelm *et al.* 1999), have been developed for these applications in *E. coli*. In this study, the successful cell-surface display of Cex, encoding xylanase as a fusion with PgsA, is described. The properties of cell-surface displayed and the purified Cex are further compared.

## Materials and methods

### Bacterial strains, plasmids and growth conditions

*Cellulomonas fimi* ATCC 484 was used for *cex* gene cloning. This strain was incubated at 30°C with constant agitation in liquid medium (w/v, 0·3% beef extract and 0·5% peptone, pH adjusted to 7·0). Overexpression of Cex was conducted using pET23, and p4pgsA overexpression vectors with *E. coli* C41 (DE3) and BL21 (DE3). Expressions of cloned genes in both pET23 and p4pgsA plasmids are from the inducible T7 promoter by the addition of isopropyl $\beta$-D-thiogalactoside (IPTG).

### Gene cloning and plasmid construction

Bacterial genomic DNA was isolated using a Genomic DNA mini kit (Geneaid Biotech, Taipei County, Taiwan). The plasmid p4pgsA was provided by Dr W.-T. Wu of National Cheng Kung University. The *pgsA* gene (GenBank accession no. AL009126) was cloned into the *Nde*I–*Bam*HI site of pET43.1a (Novagen, Madison, WI, USA) to obtain p4pgsA plasmid. The primers used to amplify the *cex* gene from *C. fimi* ATCC 484 (GenBank accession no. L11080) by PCR were as follows: Cex-F: CGGAATTCATGGCGACCACGCTCAAGGAGG containing an *Eco*RI restriction site and Cex-R: CCCAAGCTTGTCGACGACCTTCTCGTACGACTC containing a *Hin*dIII restriction site. The PCR products of the 1·7-kb *cex* gene were digested by *Eco*RI and *Hin*dIII. The products were then introduced into the pET23 (Novagen) and p4pgsA plasmids, respectively, with digestion of the *Eco*RI-*Hin*dIII site to obtain the pETCex and pP4Cex plasmids (Fig. 1). An N-terminus primer including six histidines fusion was further designed as follows: Cex-hF: GAATTCATGCATCATCATCATCATCATGCGACCACGCTCAAGGAGG containing an *Eco*RI restriction site. The Cex-R sequence with *Hin*dIII restriction site was used as the C-terminus primer. Likewise, the PCR products from the digestion of *Eco*RI–*Hin*dIII were brought into the pET23 and p4pgsA plasmids to obtain the pETCex-h and pP4Cex-h plasmids.

### Expression of Cex and purification of recombinant Cex

*Escherichia coli* BL21 (DE3) and C41 (DE3) were transformed with plasmids harbouring the *cex* gene and inoculated from a single colony into broth for overnight culture. These cultures were used to inoculate LB medium and grown at 37°C until $OD_{550}$ reached 0·5–0·6. Cultures were induced by the addition of IPTG to 0·5 mmol $l^{-1}$ and further incubated at 37°C. The *E. coli*-surface displayed xylanase was expressed as a fusion with PgsA, with and without a His-tag present at the beginning of the *cex* ORF, by introducing plasmids pP4Cex and pP4Cex-h, respectively, for whole-cell activity analysis. The cultures were centrifuged at 10 000 ***g*** and 4°C for 10 min and cells resuspended in 50 mmol $l^{-1}$ sodium phosphate buffer (pH 6·0), after which it was disrupted by sonication (Sonics & Materials, Newtown, CT, USA). The membrane fractions were prepared and isolated according to the method developed by Narita *et al.* (2006). Protein expression of both soluble and membrane-associated protein was monitored by SDS-PAGE (10% gels) and Coomassie Blue staining. Western blot analysis of Cex was performed as described by Sambrook *et al.* (1989) using monoclonal antibody (LTK BioLaboratories, Taipei, Taiwan) raised against His-tag and a secondary goat anti-mouse

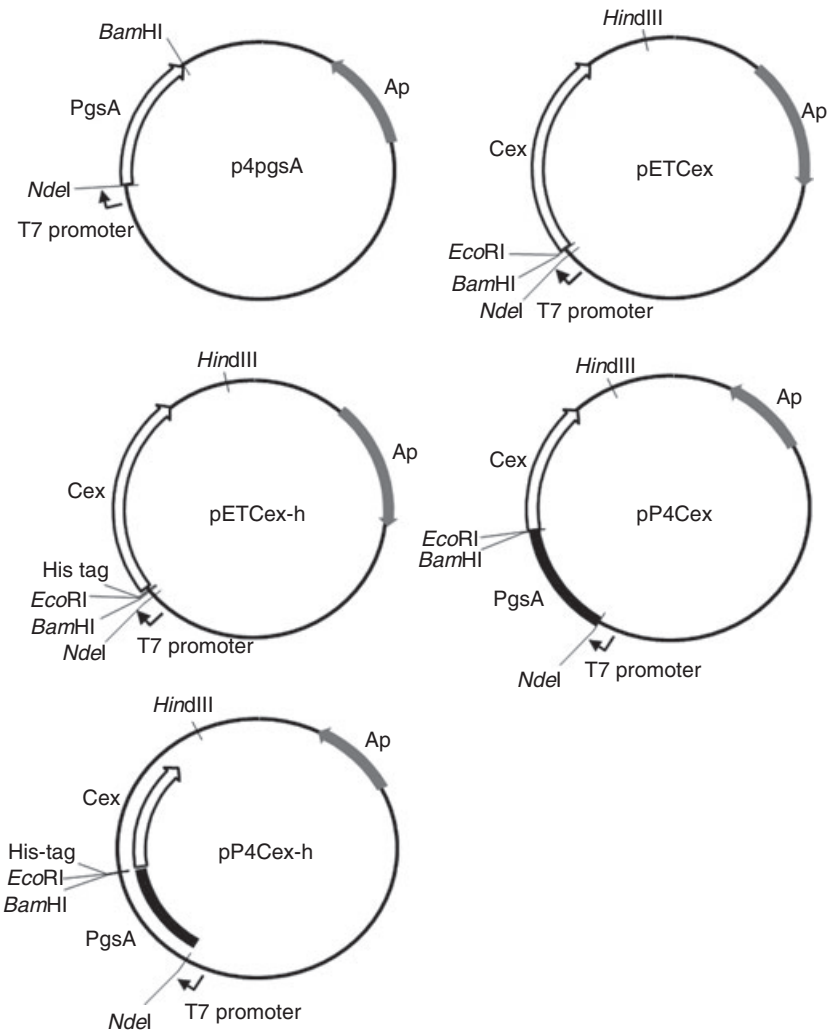


**Figure 1** Plasmid maps of p4pgsA, pETCex, pETCex-h, pP4Cex, and pP4Cex-h for the overexpression of the *cex* gene in *Escherichia coli*. The pETCex-h plasmid includes the six histidines at the N-terminus of Cex. The pP4Cex and pP4Cex-h plasmids harbour the Cex which fused the PgsA as the anchor protein. The PgsA-Cex fusion protein of pP4Cex-h plasmid was connected by hexa-His.

IgG-conjugated with horseradish peroxidase (HRP) (Chemicon International, Temecula, CA, USA). ChemiLuminescence (ECL) was used for detection. Purification of xylanase was carried out using Ni-NTA columns as described by the manufacturer (Invitrogen, CA, USA).

### Fluorescence microscopy

*Escherichia coli* C41 (DE3) was transformed with pP4Cex-h and inoculated with a single colony for overnight culture. This was transferred into an LB medium and grown until $OD_{550}$ reached 0·5–0·6 at 37°C. The culture was induced by the addition of IPTG to 0·5 mmol $l^{-1}$, followed by incubation at 37°C for 3 h. The cell-surface display of xylanase was harvested and centrifuged at 8000 ***g*** (Kubota, Osaka, Japan). The cells were resuspended in 50 mmol $l^{-1}$ sodium phosphate buffer containing 1% bovine serum albumin (BSA) at 30°C for 2 h. The sample was allowed to react with both primary mouse anti-his monoclonal antibody and secondary goat anti-mouse IgG-antibody conjugated with fluorescein isothiocyanate (FITC) at 30°C for 2 h, respectively (Chemicon International, CA, USA). After washing three times with phosphate-buffered saline (PBS) buffer, the cells were resuspended and observed using fluorescence microscopy. Xylanase expression in *E. coli* harbouring pP4Cex-h was detected by fluorescence microscopy and compared with that of the p4pgsA. Fluorescence photomicrography was implemented on the specimens using a fluorescence microscope (Leica RXA, Mannheim, Germany). The red filter (Chroma, Brattleboro, VT, USA) with peak excitation at 450–490 nm and peak suppression at 525–575 nm was used to detect Cex.

### Enzyme assay and determination of temperature, pH optima and thermostability

*Escherichia coli* BL21 (DE3) and C41 (DE3) harbouring pP4Cex-h and p4pgsA were induced by the addition of

IPTG to 0·5 mmol $l^{-1}$, followed by incubation at 37°C for time-course activity analysis to determine the cell-surface display of xylanase. One millilitre samples was harvested and centrifuged at time intervals. The cells were maintained in 50 mmol $l^{-1}$ PBS buffer, pH 6·0 supplemented with 1% xylan (w/v) (Sigma-Aldrich, St Louis, MO, USA) at 37°C for 2 h. The enzymatic activity of xylanase was assayed by the detection of reducing sugars using 3,5-dinitrosalicyclic acid (DNS) method at 540 nm. The growth rate of cells was monitored at 550 nm with a spectrophotometer. *Escherichia coli* C41 (DE3) at an $OD_{550}$ of 1 per litre of LB medium corresponded to 316 mg of dry cells. One unit (U) was defined as 1 $\mu$mol of reducing sugar (D-xylose equivalent) released per minute.

The Cex protein from the expression of pETCex-h was purified by the Ni-NTA purification system. Protein concentration was determined by Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA) using BSA as a standard. The enzyme was maintained in 50 mmol $l^{-1}$ PBS buffer, pH 6·0. One per cent xylan (w/v) was used as substrate. The thermostability of the enzyme (either purified or cell-surface displayed) was carried out by incubation at 60°C up to 120 min. The enzymes samples were immediately chilled, and activity was assayed at 37°C using 1% xylan for 2 h. The xylanase activity was analysed by the detection of reducing sugars using as described earlier.

## Results

### Construction and characterization of recombinant Cex proteins

A number of studies have described the application of *pgsA* for whole-cell-surface display (Narita *et al.* 2006; Tateno *et al.* 2007). In this study, the p4pgsA plasmid was derived from pET43 by inserting *pgsA* into the *Nde*I-*Bam*HI site downstream of the T7 promoter, so that PgsA expression was inducible by IPTG. Two sets of primers were designed for cloning the *cex* gene from *C. fimi* ATCC 484 to generate the pP4Cex and pP4Cex-h plasmids which contained the PgsA/Cex and the PgsA/His/-Cex fusions, respectively (Fig. 1). Analysis of Cex expression by SDS-PAGE showed that pP4Cex-h *E. coli* C41 (DE3) clearly expressed more proteins than the pP4Cex transformant (Fig. 2a). The expected band of 86 kDa had a 2·4-fold greater intensity in extracts of cells containing pP4Cex-h than those containing pP4Cex. The primer set containing just the His-tag was further used to amplify the *cex* gene and this was introduced into pET23 for the production of His-Cex (pETCex-h). Ni-NTA purified His-Cex was detected as a single band on the SDS-PAGE with an approximate molecular mass of 47 kDa (Fig. 2b).

Whole-cell and membrane fractions of *E. coli* C41 (DE3) (pP4Cex-h) were isolated and the fusion proteins were detected by Western blot to verify the expression of Cex on the cell surface (Fig. 2c). Although the nonspecific protein was detected in whole-cell protein extracts, only one band was identified after the purification of the membrane proteins. The proteins detected from the transformed *E. coli* C41 (DE3) (pP4Cex-h) had a molecular weight corresponding to 86kDa in both whole-cell and membrane fractions, while no obvious band was detected in the *E. coli* C41 (DE3) (p4pgsA) extracts (data not shown).

Immunofluorescence was used to demonstrate expression of the PgsA/His/Cex fusion construct (Fig. 3). Fluorescence was clearly observed in these transformants

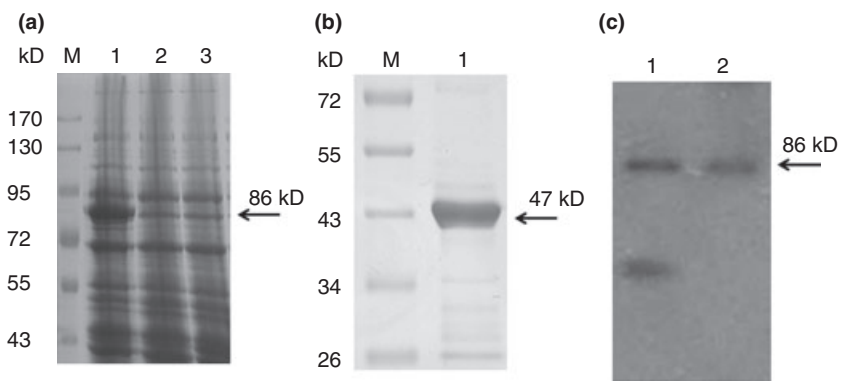


**Figure 2** Overexpression and confirmation of recombinant Cex. (a) The SDS-PAGE analysis of cell-surface display of Cex was stained with Coomassie blue. Lane 1, total proteins of *Escherichia coli* C41 (DE3) (pP4Cex-h); Lane 2, total proteins of *E. coli* C41 (DE3) (pP4Cex); Lane 3, total proteins of *E. coli* C41 (DE3) (p4pgsA). (b) The recombinant Cex of *E. coli* C41 (DE3) (pETCex-h) was purified through nickel column. The SDS-PAGE was stained with Coomassie blue. (c) Western blot analysis of the cell-surface displayed Cex. Lane 1, total proteins of *E. coli* C41(DE3) (pP4Cex-h); Lane 2, membrane fraction of *E. coli* C41(DE3) (pP4Cex-h).

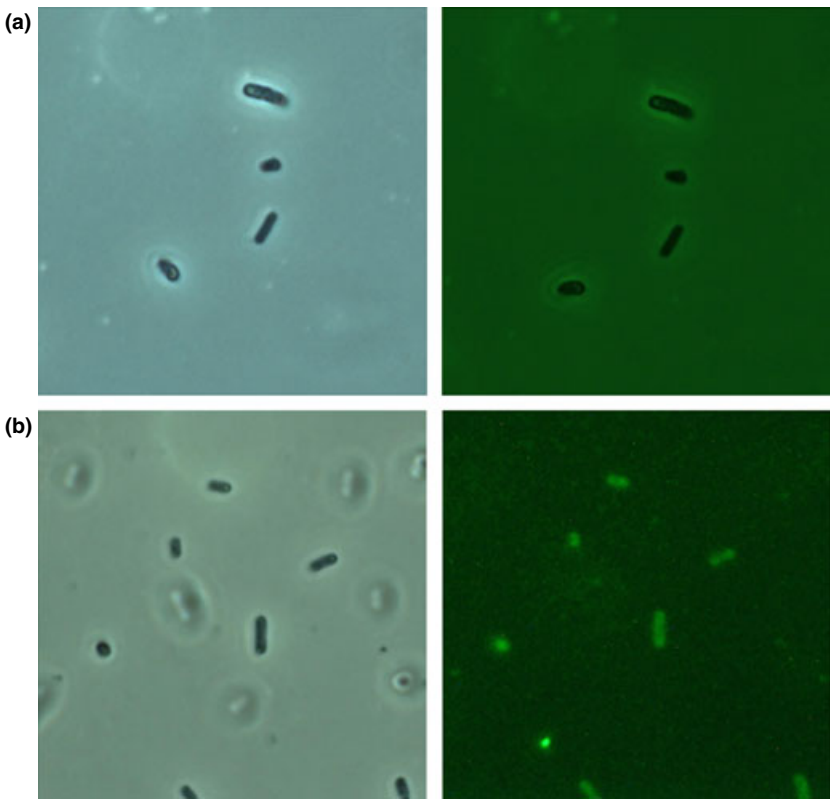


**Figure 3** Immunofluorescence of the cell-surface display was analysed by fluorescence microscopy. (a) *Escherichia coli* C41 (DE3) (p4pgsA). (b) *Escherichia coli* C41 (DE3) (pP4Cex-h). *Escherichia coli* C41 (DE3) was determined by fluorescence (right panel) and bright field microscopy (left panel). The photograph was taken at 1600 × magnification.

indicating that the cell-surface display of PgsA/His/Cex fusion was extensively expressed in the *E. coli* C41 (DE3). Together the cell fractionation and immunofluorescence results demonstrated that Cex with PgsA fusion was successfully expressed on the surface of *E. coli*.

### Analysis of xylanase activity and properties in *Escherichia coli*

The activity of the whole-cell of *E. coli* C41 (DE3) harbouring pP4Cex-h with cell-surface display of Cex was examined to investigate the xylan-degrading ability of the cell-surface displayed enzyme. Cell growth and xylanase activity was monitored for 72 h and the results are shown in Fig. 4. *Escherichia coli* was grown at 37°C until the culture reached an $OD_{550}$ of 0·6, then induced by addition of 0·5 mmol $l^{-1}$ IPTG. *Escherichia coli* was harvested as a whole-cell biocatalyst and xylanase activity was detected. The xylanase activity of the *E. coli* (pP4Cex-h) depended on the growth phase; when the culture approached the stationary phase, the xylanase activity slowly increased. The xylanase-surface display achieved the highest activity of 54 U $g^{-1}$ dry cell weight at 60 h and 37°C.

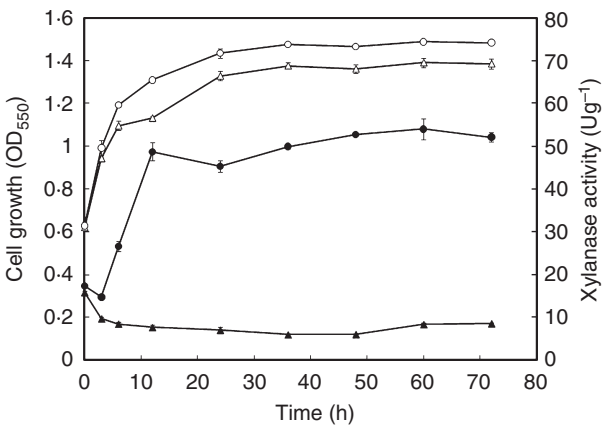


**Figure 4** Submerged cultures of *Escherichia coli* C41 (DE3) (p4pgsA) and *E. coli* C41 (DE3) (pP4Cex-h) were incubated at 37°C on a 200 rev $min^{-1}$ rotary shaker for 3 days. Cell growth of (○) *E. coli* C41 (DE3) (pP4Cex-h) and (△) *E. coli* C41 (DE3) (p4pgsA); xylanase activity of (●) *E. coli* C41 (DE3) (pP4Cex-h) and (▲) *E. coli* C41 (DE3) (p4pgsA). The cell growth of *E. coli* C41 (DE3) was detected by measuring the absorbance at 550 nm using a spectrophotometer. The xylanase activity was determined using 3,5-dinitrosalicyclic acid method.

The temperature profiles of the cell-surface displayed and the purified Cex were analysed and compared over the range of temperature from 30 to 80°C to characterize the whole-cell biocatalysis. As shown in Fig. 5, the optimal temperature was 40°C in the purified Cex, whereas it was 60°C for the cell-surface displayed Cex. The optimal

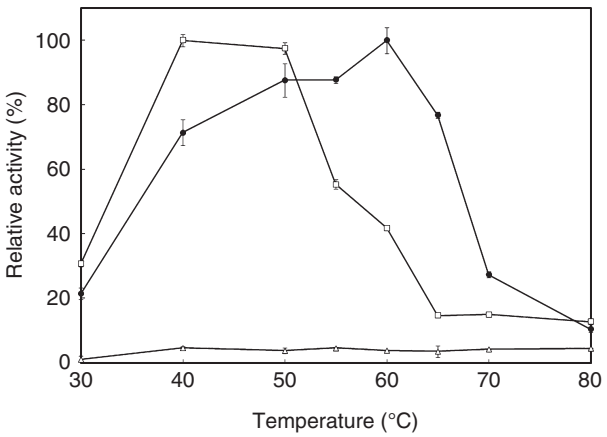


**Figure 5** Comparison of the temperature activity profiles of the cell-surface displayed and the purified Cex. The temperature activity was assayed at pH 6.0 in 50 mmol $l^{-1}$ sodium phosphate buffer at 30–80°C. The remaining xylanase activity was determined for the (●) cell-surface display of *Escherichia coli* C41(DE3) (pP4Cex-h), the (□) purified Cex from *E. coli* C41(DE3) (pETCex-h), and the (△) cell-surface display of *E. coli* C41(DE3) (p4pgsA).

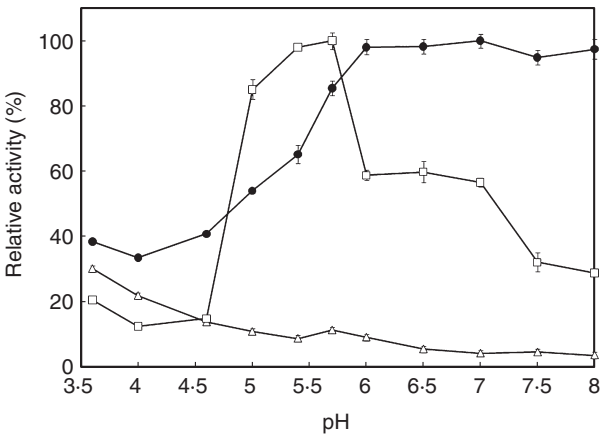


**Figure 6** Comparison of the pH-activity profiles of cell-surface displayed and the purified Cex. The pH activity was assayed at 37°C in sodium acetate buffer with pH ranging from 3.6 to 5.4 and sodium phosphate buffer ranging from pH 5.7 to 8.0. The remaining xylanase activity was determined for the (●) cell-surface display of *Escherichia coli* C41(DE3) (pP4Cex-h), the (□) purified Cex from *E. coli* C41(DE3) (pETCex-h), and the (△) cell-surface display of *E. coli* C41(DE3) (p4pgsA).

temperature of the cell-surface displayed enzyme was 20°C higher than that of purified Cex. Moreover, the cell-surface displayed Cex was active over a broader range of temperatures than the purified Cex. The residual activity of the cell-surface displayed Cex was maintained at above 70% between 40 and 65°C; however, the residual activity of the purified Cex rapidly decreased to 43% at 55°C. The relative activity of the cell-surface displayed Cex remained 77% at 65°C, whereas the relative activity of the purified protein was only 15%. The residual activities of the cell-surface displayed and the purified enzymes were both low at 30°C.

The effect of pH on the activity of the cell-surface displayed and the purified enzymes is shown in Fig. 6. The purified Cex reached maximum activity at pH 5·7, whereas the optimal pH of the cell-surface displayed Cex was 7·0. The cell-surface displayed Cex was less active at low pH, but maintained high relative activity under neutral and alkaline conditions. The residual activity of the cell-surface displayed Cex was almost 100% between pH 6 and 8, whereas the residual activity of the purified Cex was 59% at pH 6 and 29% at pH 8. At pH 4·6, the relative activity of the purified Cex showed a dramatic drop to 15%, whereas the cell-surface displayed Cex still retained 41% activity.

### Increasing thermostability of the *Escherichia coli*-surface display xylanase

In a previous study, the melting temperature $T_m$ of Cex was about 66°C in the presence of excess ligand and 2°C higher than that under ligand-free conditions (Nikolova *et al.* 1997). According to the analysis of the temperature profile of the enzyme performed here, the optimal temperature of the cell-surface displayed Cex was 60°C, some 20°C higher than that of the purified Cex. When the thermostability of the cell-surface displayed and the purified Cex were compared at 60°C, the result showed that the cell-surface displayed Cex was more thermostable than the purified Cex (Fig. 7). The half-life (time over which 50% activity is lost) of the cell-surface displayed Cex was 1·6-fold longer than that of the purified Cex at 60°C. These results reveal that anchoring Cex on the cell membrane can enhance enzymatic thermostability.

## Discussion

Several studies have been reported regarding the heterologous expression of xylanase in *E. coli* (Gupta *et al.* 2000; Srivastava and Mukherjee 2001). In the research described here, the *cex* encoding xylanase on the surface of *E. coli* C41 (DE3) was successfully expressed through fusion with PgsA. Based on the result of the Western blot and immunofluorescence analyses, the surface location of the PgsA anchor fused to the N-terminus of the Cex protein on the *E. coli* surface was demonstrated (Figs 2 and 3). The PgsA-Cex expression in *E. coli* without the His-tag showed less protein production (Fig. 2), which may be ascribed to the aberrant folding of the larger fusion protein. The requirement of an appropriate spacer between the targeting and anchor proteins for correct folding and

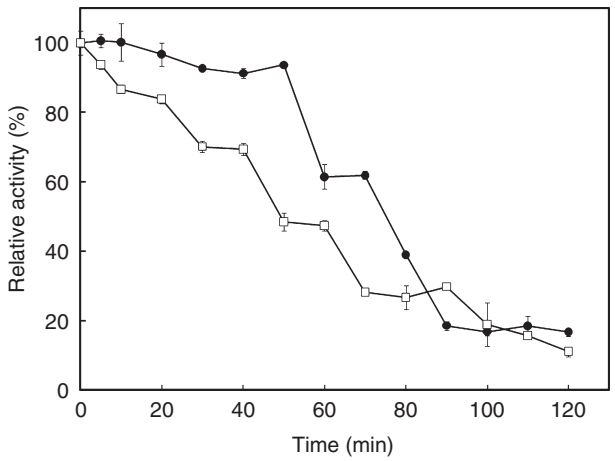


**Figure 7** Temperature stability of the cell-surface displayed and the purified Cex. The enzymes were incubated at 60°C from 0 to 120 min. The remaining xylanase activity was determined for the (●) cell-surface display of *Escherichia coli* C41 (DE3) (pP4Cex-h), and the (□) purified Cex from *E. coli* C41 (DE3) (pETCex-h) at 37°C using 1% xylan by 3,5-dinitrosalicyclic acid method.

overexpression has been previously suggested (Lee *et al.* 2003). Fusion tags, such as histidines, NusA, glutathione S-transferase and maltose-binding protein, are generally used in the gene fusion for purification. Shih *et al.* (2002) reported that 80% of genes had high levels of expression using at least one of the eight different carrier proteins for purification. In this study, PgsA-Cex with hexa-His as spacer was found to have a higher expression than that without hexa-His sequence. Therefore, the increase in Cex expression can be attributed to the His-tag gene fusion. This finding is consistent with the overexpression of Cex in *E. coli* with His-tag (Shih *et al.* 2002).

For convenient harvest and efficient production of proteins, the secretion of Cex has been established and described (Lam *et al.* 1997; Fu *et al.* 2005). However, overexpression and accumulation of an OmpA/Cex fusion precursor was found to result in the interference of SecTEG translocation machinery and lead to cell death (Lam *et al.* 1997). Therefore, Fu *et al.* (2006) designed a weaker promoter to achieve maximum overexpression of secreted Cex. In addition, *E. coli* C41 (DE3) and C43 (DE3) derived by mutation of BL21 (DE3) were constructed for overexpression of membrane and toxic proteins and can produce higher protein yields than BL21 (DE3) (Miroux and Walker 1996). Further study shows that C41 (DE3) and C43 (DE3) can minimize plasmid instability (Dumon-Seignovert *et al.* 2004). In this study, *E. coli* C41 (DE3) was adopted for the overexpression of PgsA-Cex surface display. During analysis of growth, the cells reached stationary phase after 12 h cultivation and cell mass remained constant (Fig. 4). This finding indicates that the lethal effect of the saturation of Sec translocation machinery did not occur. Plasmid pP4Cex-h was also introduced into *E. coli* BL21 (DE3). Although the growth rate of BL21 (DE3) (pP4Cex-h) was similar to that of C41 (DE3), the xylanase activity of the cell-surface display in C41 (DE3) was higher than that of BL21 (DE3) (data not shown). In strain C41 (DE3), the mutation of the *lac*UV5 promoter results in a lower transcription of T7 RNA polymerase and gives rise to a lower expression rate and successful overexpression of membrane protein. Hence, protein misfolding and aggregation is also reduced in *E. coli* C41 (DE3) compared to BL21 (DE3). These results indicate that C41 (DE3) was superior to BL21 (DE3) for the overexpression of membrane proteins (Wagner *et al.* 2008) and our results are consistent with this.

The stability of enzyme is very important in reducing the cost for industrial production because they can tolerate the harsh process conditions. On the other hand, the advantages of stable enzymes can shorten the catalytic time and reduce the enzyme dosage. Directed evolution and immobilization of enzymes have been regarded as the normal approach to enhance enzymatic stability. The properties of thermostable proteins obtained from directed evolution have been explained in term of structural features, such as of an increased protein hydrophobicity, additional salt bridges, a decreased number of internal cavities, increased helix stability and decreased chain flexibility, among others (Kumar and Nussinov 2001). In addition, the stability of enzymes through immobilization can be examined by covalent binding onto various supporting materials (Vallejo-Becerra *et al.* 2008; Kim *et al.* 2010). In this study, the different temperature and pH profiles were determined for the cell-surface displayed and the purified Cex. The cell-surface display of Cex caused a significant shift in the optimal temperature from 40 to 60°C (Fig. 5). Similarly, the optimal pH of the cell-surface displayed Cex was broader than that of the purified form (Fig. 6). In addition, the thermostability of the cell-surface displayed Cex was better than that of purified form with the cell-surface displayed Cex maintaining above 90% residual activity after 50 min incubation at 60°C, whereas the purified Cex only retained 48% activity (Fig. 7). A few other studies have reported that cell-surface display of enzymes can improve thermostability compared with purified enzymes. For example, Sed1 and Cwp2 are utilized as the anchor proteins to fuse lipase for surface display in *Pichia pastoris* and *Saccharomyces cerevisiae*, respectively (Liu *et al.* 2010b; Su *et al.* 2010). These surface proteins are covalently immobilized into the cell wall. The process resembles the immobilization of enzymes on supporting material by glutaraldehyde. In contrast, PgsA contains a large number of hydrophobic (34%) and charged amino acid residues (33%) to anchor

it to the cell membrane (Ashiuchi *et al.* 2001; Ashiuchi and Misono 2002). The location of PgsA-hexaHis-Cex in the membrane fraction of *E. coli* was verified by Western blot (Fig. 2). Furthermore, Cex also consists mainly of hydrophobic fractions (37%), as shown by VectorNTI software analysis. An increase in hydrophobic amino acid content can improve protein thermostability, which suggests that the fusion protein is embedded in the cell membrane by increasing the hydrophobic interaction (Haney *et al.* 1997; Luke *et al.* 2007). The phospholipid environment of the membrane provided the additional protection against the thermo-inactivation of the enzyme, which may achieve a rigid protein structure, thereby enhancing thermal tolerance, as well as broad pH activity (Kumar and Nussinov 2001).

In conclusion, an active xylanase was successfully established on the cell-surface of *E. coli* using PgsA as anchor protein. Based on the analysis of enzymatic properties, the cell-surface displayed Cex embedded in the membrane was superior to its purified form counterpart. The cell-surface displayed Cex has an increased optimal temperature range and pH profile, as well as thermostability. The results suggest that these properties are suitable for industrial applications, such as biobleaching and renewable energy production (Garg *et al.* 1996; Weiss *et al.* 2010), and whole-cell biocatalysis can reduce the operating cost and the usage of toxic chemicals.

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