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# Enhanced Display of Lipase on the *Escherichia coli* Cell Surface, Based on Transcriptome Analysis▽†

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**A cell surface display system was developed using *Escherichia coli* OmpC as an anchoring motif. The fused *Pseudomonas fluorescens* SIK W1 lipase was successfully displayed on the surface of *E. coli* cells, and the lipase activity could be enhanced by the coexpression of the *gadBC* genes identified by transcriptome analysis.**

Cell surface display is a technique for expressing target molecules fused to an anchoring motif on the surface of various host cells (2, 9, 15, 21, 22). This technique has numerous biotechnological applications, including vaccine development, antibody production, peptide library display and screening, bioremediation, bioadsorption, biocatalysis, and biosensor development.

We previously reported that lipase could be successfully displayed using FadL and OprF as anchoring motifs (12, 13, 14). When we displayed the *Pseudomonas fluorescens* SIK W1 lipase (TliA) on the surface of *Escherichia coli* W3110 (derived from prototropic strain K-12 $\lambda^-$ $F^-$) by using these anchoring motifs, the lipase activity was very low. Thus, a new surface anchoring system was developed in this study.

For the efficient display of lipase, a C-terminal deletion fusion strategy using *E. coli* OmpC as an anchoring motif was first considered (23). Based on the predicted secondary structure and information found in literature (3, 11, 23), the truncated *ompC* gene encoding 288 amino acids from the N terminus was amplified and fused with the *tliA* gene (13; see also the supplemental methods). The fusion fragment was cloned into pTac99A (17) to make pTacOmpC288PL.

As expected, lipase activity was detected on the surface of *E. coli* cells harboring pTacOmpC288PL by using a spectrophotometric method (13; see also the supplemental methods) (Fig. 1). Compared with other lipase display systems, the lipase activity obtained with the OmpC display system was >10-fold higher than those obtained with OprF and FadL display systems; the specific activities were 1,940 ± 118, 172 ± 19, and 62 ± 13 U/mg lyophilized cells by the use of OmpC, OprF, and FadL as anchoring motifs, respectively. These results suggest that the C-terminal deletion fusion strategy using the *E. coli* OmpC as an anchoring motif provides an efficient way for displaying functional lipase on the surface of *E. coli*.

For the enhanced display of lipase, transcriptome profiling was performed. All procedures, including RNA preparation, cDNA synthesis, DNA hybridization, and data analysis, were carried out as described previously (1; see also the supplemental methods). The transcriptome profiles of lipase-displaying cells and control cells at 5 h after IPTG (isopropyl-β-D-thiogalactopyranoside) induction were compared. It was found that 1,165 out of 4,021 genes were differentially expressed by >2-fold (see Table S2 in the supplemental material). There is no general rule for selecting the gene amplification targets based on transcriptome profiling. However, one can select those genes that are significantly up- or downregulated as primary amplification targets, among which those relevant to possibly accomplishing the objective can be first tried (4, 18, 19).

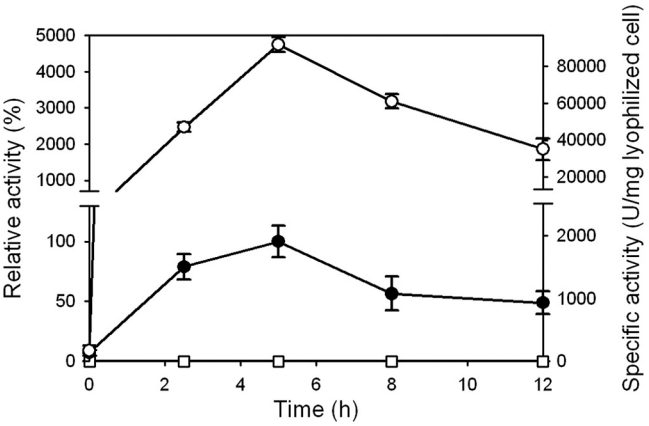


FIG. 1. Activities of lipase displayed on the surface of *E. coli* W3110 cells harboring pTac99A (open squares), pTacOmpC288PL (closed circles), and pTacOmpC288PL plus pHNCgadBC (open circles). Relative activity was calculated by assuming the lipase activity of the strain harboring pTacOmpC288PL at 5 h as 100%.

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TABLE 1. Effect of manipulating the target genes identified by transcriptome profiling on the specific activities of lipase displayed on the *E. coli* cell surface

| Target gene | Change in gene expression levels ($log_2$)[a] | Lipase sp act (U/mg of lyophilized cells)[b] | | Relative lipase activity (%)[c] | |
|---|---|---|---|---|---|
| | | Knockout strain | Amplification strain | Knockout strain | Amplification strain |
| *pspABCDE* | 2.4 to 5.8 | 1,110 ± 78 | 1,180 ± 78 | 57.2 ± 4.02 | 60.8 ± 4.02 |
| *gadBC* | −2.6 to −2.1 | 815 ± 83 | 92,300 ± 407 | 42.0 ± 4.28 | 4,760 ± 21.0 |

[a] The values indicate the changes in the expression levels of the target genes in a recombinant *E. coli* strain displaying lipase compared with those of a control strain containing the backbone plasmid, determined by DNA microarray analysis.

[b] One unit of lipase activity was defined as the amount of enzyme releasing 1 micromole of *p*-nitrophenol per minute. All measurements were independently performed in triplicates. The values after the ± sign represent the standard deviation.

[c] Relative lipase activity was calculated by assuming the lipase activity measured in control strain without target gene manipulation (1,940 ± 118) as 100%.

Based on the transcriptome data, two operons, *pspABCDE* (1,924 bp) and *gadBC* (3,091 bp), containing the most up- and downregulated genes, were selected as target genes. It has been reported that the highly upregulated *pspABCDE* genes for phage shock proteins are related to protein export (5, 8). Thus, it seems that the expression levels of the *pspABCDE* genes are correlated with efficient secretion of lipase for its display on cell surface. On the other hand, the significantly downregulated *gadBC* genes are known to play an important role in glutamate decarboxylase-dependent acid tolerance (20). These target genes were coexpressed using pHNCpspABCDE and pHNCgadBC, respectively (see the supplemental methods). Recombinant *E. coli* W3110

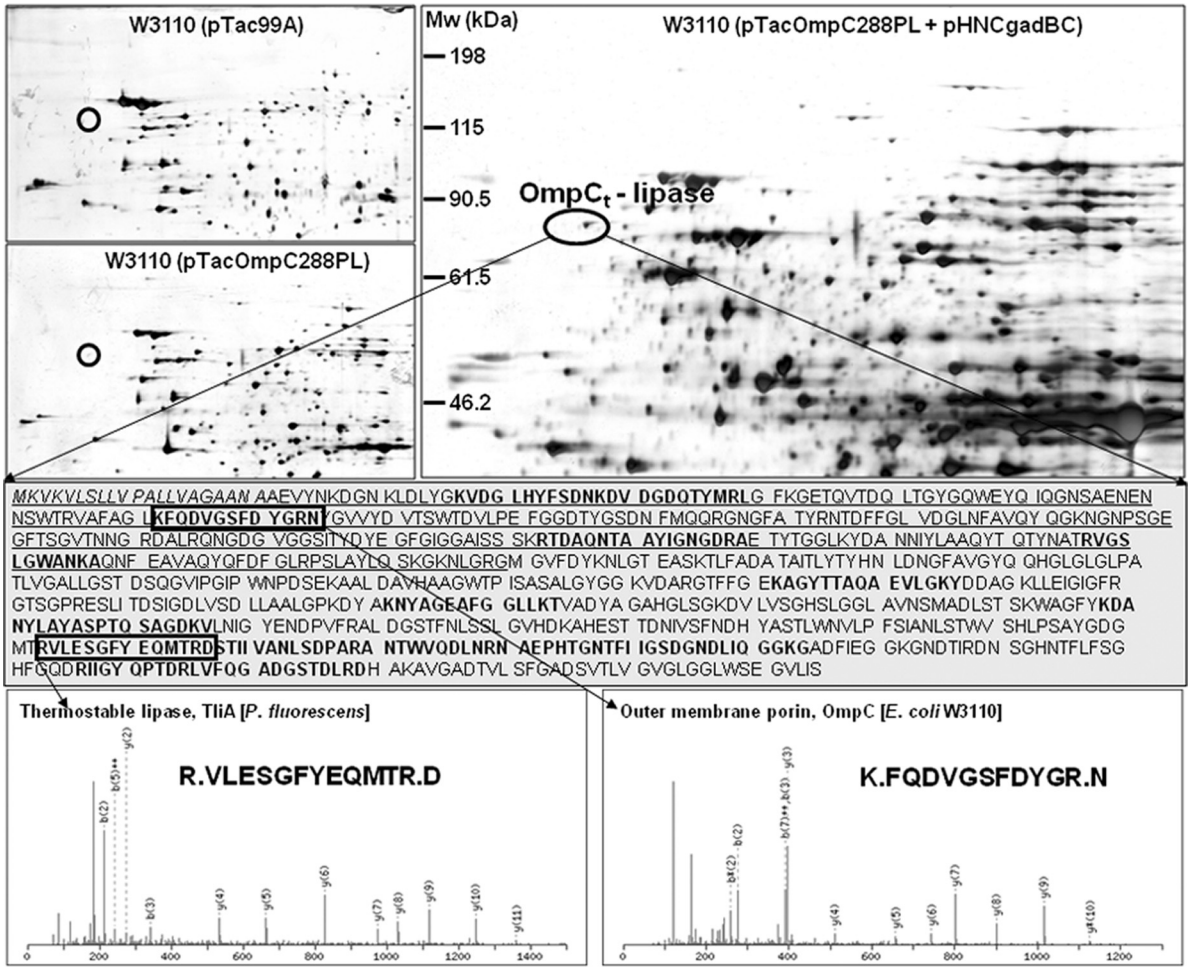


FIG. 2. Outer membrane proteome profiles obtained with *E. coli* W3110 harboring pTac99A, pTacOmpC288PL, and pTacOmpC288PL plus pHNCgadBC. Circles indicate the position of the truncated OmpC ($OmpC_t$)-lipase fusion protein. The middle panel shows the amino acid sequence of lipase fused to $OmpC_t$ (underlined). The signal sequence is indicated in italics. The nonredundant peptide sequences identified by nano-liquid chromatography-tandem mass spectrometry (LC/MS/MS) are indicated in boldface type. The representative peptide sequences of lipase and $OmpC_t$ are shown in the bottom panels.

harboring pTacOmpC288PL and pHNCpspABCDE exhibited 60.8% of the original lipase activity (Table 1). When the *pspABCDE* genes were deleted using the homologous recombination system (6), the lipase activity also decreased. Thus, the *pspABCDE* genes were not further considered target genes to be manipulated.

In contrast, when the *gadBC* genes were coexpressed using pHNCgadBC, the lipase activity dramatically increased by 48-fold (Fig. 1, Table 1). The *gadBC* knockout strain harboring pTacOmpC288PL showed decreased lipase activity. To see if this positive effect is transferrable, the effect of GadBC coexpression on the lipase display using different anchoring motifs was examined. It was found that the lipase activities were increased by 2.51- and 1.59-fold for the display systems employing OprF and FadL, respectively; the specific activities were 432 and 98.6 U/mg lyophilized cells when using OprF and FadL anchoring motifs, respectively, with GadBC coexpression. Thus, these results indicate that GadBC coexpression might generally applicable for the enhanced display of lipase on the *E. coli* cell surface.

To validate the effect of GadBC coexpression on lipase display, transcript and protein level analyses were carried out. Real-time PCR was performed as described previously (1; see also the supplemental methods). It was found that the expression levels of the *ompC* and *tliA* genes were highly increased by >20-fold by GadBC coexpression. For protein level analysis, outer membrane proteins were isolated, separated, and identified using two-dimensional gel electrophoresis coupled with mass spectrometry, as reported previously (10, 13; see also the supplemental methods). As shown in Fig. 2, the OmpC-lipase fusion protein of ca. 83 kDa (pI 4.8) was detected, and the amount of the protein increased 15-fold when the *gadBC* genes were coexpressed. Thus, these results clearly suggest that GadBC coexpression improves the expression and display of the OmpC-lipase fusion protein.

To examine whether biocatalytic ability is also enhanced by GadBC coexpression, enantioselective hydrolysis of 1-phenylethyl butyrate (1-phenylethyl butanoate) was performed (13; see also the supplemental methods). The conversion of reaction and the enantiomeric excess of the product (*R*)-phenylethanol obtained at 5 h were both improved by GadBC coexpression—over 30 and 12% of conversion, and 99 and 94% of enantiomeric excess with and without GadBC coexpression, respectively. This result suggests that the lipase-displaying cells coexpressing the *gadBC* genes have an advantage of enhanced functional expression of lipase on the cell surface. This enhanced lipase display is expected to be widely useful for the production of enantiomerically pure compounds as an effective biocatalytic system in the fields of fine chemicals, pharmaceuticals, agrochemicals, and other demanding industries.

There has so far been no report on the roles of GadBC in cell surface display systems. However, it has been reported that GadBC proteins are involved in stress responses such as acid shock, anaerobic phosphate starvation, salt stress, and stationary-phase stress (7, 16). Thus, cell surface display of lipase (and possibly other proteins as well) seems to act like a stress to the cell due to the greater than normal amount of protein export, translocation, and anchoring onto the cell envelope. Even though the exact mechanism needs to be elucidated, the results of this study suggest that coexpression of the *gadBC* genes, identified by transcriptome profiling, improves lipase display on *E. coli* cell surfaces. This is an additional example of employing transcriptome profiling for the identification of gene manipulation targets for improving the performance of microorganisms.

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