

[Supplementary materials]

**Isolation of a potential anchoring motif based on proteome
analysis of *Escherichia coli* and its use for cell surface display**

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Construction of gene expression system with OmpX own promoter and cultivation

Endogenous promoter of *ompX* in *E. coli* MG1655 was amplified by PCR with forward primer (5-AGAATTCATACCCAATGCTGGTTGAGC) and reverse primer (5-TGAATTCAACCACCTCAAATGTGATTCAAATAAGTCC). PCR products were digested with EcoRI restriction enzyme and then cloned into the EcoRI site of pTrcOmpXK₁₂₂EX to yield pPOmpXK₁₂₂EX.

The constructed plasmid were transformed into *E. coli* XL10-Gold and cells were cultivated in Luria-Bertani medium (10 g/L of tryptone, 5 g/L of yeast extract, and 5 g/L of NaCl) supplemented with 50 µg/L of ampicillin at 37°C and 200 rpm. The OmpX promoter is a constitutive promoter, so cells were cultivated at 25°C for 6 hr and 14 hr without IPTG induction. The cells harboring pTrcOmpXK₁₂₂EX were cultivated as described in manuscript.

Measurement of lipase activity

The cells displaying lipase were harvested by centrifugation for 5 min at 3,500 × g and 4°C, washed with distilled water, and lyophilized with a freeze dryer for 48 h. Lipase activity was assayed by a spectrophotometric method using *p*-nitrophenyl decanoate as a substrate, as described previously (1) One unit (U) of lipase activity was defined as the amount of enzyme releasing 1 µmol of *p*-nitrophenol per min (2). The specific activity was defined as the lipase activity per milligram of lyophilized cells. All measurements were performed independently in triplicate and the standard deviations were determined.

[refs]

1. Lee, S. H., Choi, J. I., Han, M. J., Choi, J. H., & Lee, S. Y. (2005). Display of lipase on the cell surface of *Escherichia coli* using OprF as an anchor and its application to enantioselective resolution in organic solvent. *Biotechnology and Bioengineering*, 90(2), 223-230.
2. Ahn, J. H., Pan, J. G., & Rhee, J. S. (1999). Identification of the *tliDEF* ABC transporter specific for lipase in *Pseudomonas fluorescens* SIK W1. *Journal of Bacteriology*, 181(6), 1847-1852.

[Supplementary figure]

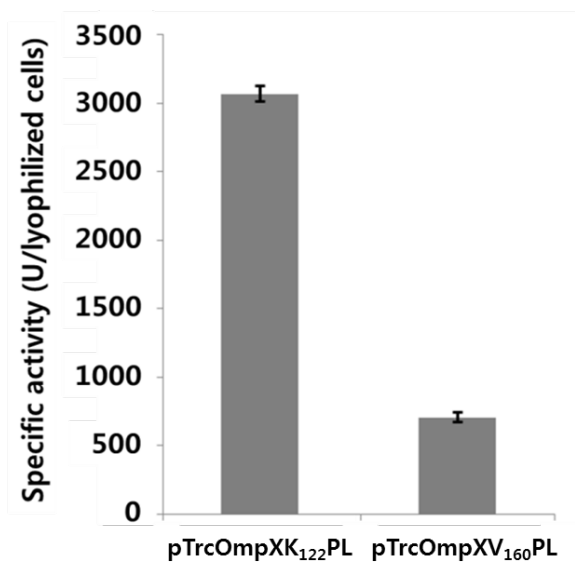


Fig. S1. Comparison of lipase activity with *E. coli* displaying OmpXK₁₂₂-lipase and OmpXV₁₆₀-lipase.

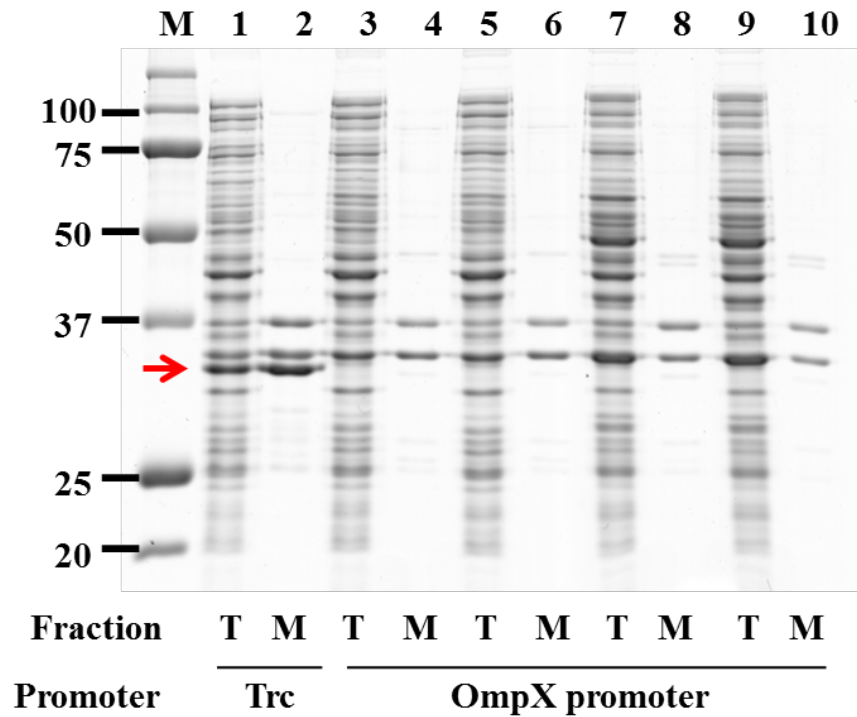


Fig. S2. SDS-PAGE analysis of production of OmpX (K122) fused endoxylanase under the OmpX own promoter. Fraction T and M represent the total fraction and Membrane fraction, respectively. Red arrow indicates the OmpXK₁₂₂-fused endoxylanase. For OmpX own promoter expression system, two clones (#1 and #2) were picked from the same plate and used for production analysis.

Lane M: Molecular weight size markers (kDa)

Lane 1: pTrcOmpXK₁₂₂EX, 4 hr culture after induction and total protein fraction

Lane 2: pTrcOmpXK₁₂₂EX, 4 hr culture after induction and Outer membrane fraction

Lane 3: pPOmpXK₁₂₂EX, 6 hr culture without induction and total protein fraction (#1)

Lane 4: pPOmpXK₁₂₂EX, 6 hr culture without induction and Outer membrane fraction (#1)

Lane 5: pPOmpXK₁₂₂EX, 6 hr culture without induction and total protein fraction (#2)

Lane 6: pPOmpXK₁₂₂EX, 6 hr culture without induction and Outer membrane fraction (#2)

Lane 7: pPOmpXK₁₂₂EX, 14 hr culture without induction and total protein fraction (#1)

Lane 8: pPOmpXK₁₂₂EX, 14 hr culture without induction and Outer membrane fraction (#1)

Lane 9: pPOmpXK₁₂₂EX, 14 hr culture without induction and total protein fraction (#2)

Lane 10: pPOmpXK₁₂₂EX, 14 hr culture without induction and Outer membrane fraction (#2)

[Supplementary Table 1]

List of the membrane proteins identified by LC-MS/MS.

Gene	SWISS accession number	Product	pI	Mw	Score	Sequence coverage (%)
<i>aceA</i>	P0A9G6	Isocitrate lyase	5.16	47491.6	409	66
<i>acrA</i>	P0AE06	Acriflavine resistance protein A	7.69	42171.1	511	60
<i>atpA</i>	P0ABB0	ATP synthase subunit alpha	5.80	55187.8	191	17
<i>atpB</i>	P0AB98	ATP synthase subunit a	6.36	30283.4	203	25
<i>atpF</i>	P0ABA0	ATP synthase subunit b	5.99	17253.3	365	54
<i>bamA</i>	P0A940	Outer membrane protein assembly factor YaeT	4.93	90496.5	632	15
<i>bamB</i>	P77774	Lipoprotein YfgL	4.72	41861.3	586	54
<i>bamC</i>	P0A903	Lipoprotein 34	5.34	36819.6	471	50
<i>bamD</i>	P0AC02	UPF0169 lipoprotein YfiO	6.16	27811.8	292	60
<i>btuB</i>	P06129	Vitamin B12 transporter BtuB	5.23	68365.4	608	48
<i>dcrB</i>	P0AEE1	Protein DcrB	5.08	19775.1	599	66
<i>fadL</i>	P10384	Long-chain fatty acid transport protein	5.09	48511.5	206	13
<i>ftsZ</i>	P0A9A6	Cell division protein FtsZ	4.63	40298.6	664	65
<i>gatY</i>	P0C8J6	D-tagatose-1,6-bisphosphate aldolase subunit GatY	5.87	30792.6	109	35
<i>gatZ</i>	P0C8J8	D-tagatose-1,6-bisphosphate aldolase subunit GatZ	5.49	47078.8	399	52
<i>hemL</i>	P23893	Glutamate-1-semialdehyde 2,1-aminomutase	4.73	45336.4	437	32
<i>hemX</i>	P09127	Putative uroporphyrinogen-III C-methyltransferase	4.66	42936.9	830	61
<i>hslJ</i>	P52644	Heat shock protein HslJ	6.72	15155.6	187	52
<i>mipA</i>	P0A908	MltA-interacting protein	5.50	27813.5	436	69
<i>nagE</i>	P09323	PTS system N-acetylglucosamine-specific EIICBA component	5.78	68302.9	126	8
<i>ompA</i>	P0A910	Outer membrane protein A	5.99	37177.7	521	54
<i>ompF</i>	P02931	Outer membrane protein F	4.76	39309.1	528	61
<i>ompT</i>	P09169	Protease 7	5.76	35540.3	591	57
<i>ompW</i>	P0A915	Outer membrane protein W	6.03	22913.3	328	45
<i>ompX</i>	P0A917	Outer membrane protein X	6.56	18590.9	428	43
<i>pal</i>	P0A912	Peptidoglycan-associated lipoprotein	6.29	18812.2	243	45
<i>recA</i>	P0A7G6	Protein RecA	5.09	37949.6	450	48
<i>rlpA</i>	P10100	Rare lipoprotein A	5.51	37504.9	118	19
<i>rpsA</i>	P0AG67	30S ribosomal protein S1	4.88	61120.7	723	43
<i>rpsB</i>	P0A7V0	30S ribosomal protein S2	6.61	26726.8	32	12
<i>sdhA</i>	P0AC41	Succinate dehydrogenase flavoprotein subunit	5.85	64381.1	295	14
<i>tolC</i>	P02930	Outer membrane protein TolC	5.46	53708.2	604	49
<i>tsx</i>	P0A927	Nucleoside-specific channel-	5.07	33567.9	126	8

		forming protein Tsx					
<i>uspF</i>	P37903	Universal stress protein F		5.60	16006.5	31	13
<i>yajG</i>	P0ADA5	Uncharacterised lipoprotein YajG		8.69	20936.8	203	25
<i>yceB</i>	P0AB26	Uncharacterised lipoprotein YceB		6.14	20486.7	107	21
<i>lpoB</i>	P0AB38	Penicillin-binding protein activator LpoB		6.41	22501.4	229	36
<i>engD</i>	P0ABU2	GTP-dependent nucleic acid-binding protein EngD		4.87	39642.3	328	59
<i>ydgA</i>	P77804	Protein YdgA		5.07	54654.9	166	40
<i>yfgM</i>	P76576	UPF0070 protein YfgM		5.07	22162.4	110	27
<i>yiaF</i>	P0ADK0	Uncharacterised protein YiaF		6.08	25646.9	272	42