

Article

Functional Autodisplay of Phenolic Acid Decarboxylase using a GDSL Autotransporter on *Escherichia coli* for Efficient Catalysis of 4-Hydroxycinnamic Acids to Vinylphenol Derivatives

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Abstract: Bioproduction of vinylphenol derivatives, such as 4-vinylguaiacol (4-VG) and 4-vinylphenol (4-VP), from 4-hydroxycinnamic acids, such as ferulic acid (FA) and *p*-coumaric acid (*p*CA), employing whole cells expressing phenolic acid decarboxylases (PAD) as a biocatalyst has attracted much attention in recent years. However, the accumulation of 4-VG or 4-VP in the cell may cause high cytotoxicity to *Escherichia coli* (*E. coli*) and consequently cell death during the process. In this study, we firstly report the functional display of a phenolic acid decarboxylase (BLPAD) from *Bacillus licheniformis* using a GDSL autotransporter from *Pseudomonas putida* on the cell surface of *E. coli*. Expression and localization of BLPAD on *E. coli* were verified by SDS-PAGE and protease accessibility. The PelB signal peptide is more effective in guiding the translocation of BLPAD on the cell surface than the native signal peptide of GDSL, and the cell surface displaying BLPAD activity reached 19.72 U/OD₆₀₀. The cell surface displaying BLPAD showed good reusability and retained 63% of residual activity after 7 cycles of repeated use. In contrast, the residual activity of the intracellular expressing cells was approximately 11% after 3 cycles of reuse. The molar bioconversion yields of 72.6% and 80.4% were achieved at the concentration of 300 mM of FA and *p*CA in a biphasic toluene/Na₂HPO₄-citric acid buffer system, respectively. Its good reusability and efficient catalysis suggested that the cell surface displaying BLPAD can be used as a whole-cell biocatalyst for efficient production of 4-VG and 4-VP.

Keywords: phenolic acid decarboxylase; cell surface display; 4-vinylguaiacol; 4-vinylphenol; GDSL autotransporter; biotransformation

1. Introduction

4-Vinylguaiacol (4-VG), also known as 2-methoxy-4-vinylphenol, is a high value-added product widely used in food, cosmetic, pharmaceutical, and chemical industries [1,2]. It was also used as a precursor for the synthesis of bio-based polymers such as oxygenated polystyrenes [3,4]. Recently, it was reported that 4-VG could be efficiently biotransformed into vanillin by the carotenoid cleavage oxygenase family enzymes [5–7]. 4-Vinylphenol (4-VP, 4-hydroxystyrene) is a monomer for production of polymers and a petroleum-based feedstock for resins, elastomers, and adhesives [8,9]. The bioproduction of 4-VG or 4-VP from ferulic acid (FA) or *p*-coumaric acid (*p*CA) employing

whole cells harboring intracellular phenolic acid decarboxylases (PAD) as biocatalysts has attracted much attention recently due to some advantages over the free phenolic acid decarboxylases, such as unnecessary cell lysis and enzyme purification, thereby significantly reducing production cost [8,10]. However, the accumulation of 4-VG or 4-VP in the cell may cause a high cytotoxicity to *Escherichia coli* (*E. coli*) and, consequently, cell death during the process [11,12]. In addition, reactivity of whole-cell catalysts containing intracellular enzymes also hampered the barriers between enzymes and their substrates [13,14].

Cell surface display systems overcome some of the limitations associated with free enzymes or intracellular enzyme catalysis systems by exposing the heterologous enzymes on the outer membrane of the cell by fusing with anchoring proteins [15]. The enzymatic reaction takes place outside the cell membrane. So, neither substrate nor product needs to be internalized or excreted through the cell membrane; therefore, cytotoxicity of the end product to the cell is alleviated, and biocatalysis is favored [16]. These systems have a wide range of biotechnological applications in many areas such as whole-cell biocatalysis for bioconversion, biosensing, biosorption, and immobilization [17–23]. The GDSL autotransporter is a distinctive autotransporter that consists of a lipolytic enzyme of the GDSL family of lipases or esterases as a passenger domain at the N-terminus and a β -barrel domain at C-terminus, which is necessary for secretion of the passenger protein to the outer membrane [24]. Unlike typical autotransporters, the passenger domain in the GDSL autotransporter is not cleaved off and covalently attach to the respective C-terminal β -domain after translocation on the exterior membrane, which makes it an excellent vehicle to display various enzymes on the surface of *E. coli* used for whole-cell biocatalysis [25].

Herein, we firstly report the functional display of a phenolic acid decarboxylase (BLPAD) from *Bacillus licheniformis* (*B. licheniformis*) on the *E. coli* cell surface for efficient production of 4-VG and 4-VP. BLPAD displayed high organic solvent tolerance and outstanding catalytic activity in the bioconversion of FA and *pCA* into 4-VG and 4-VP, respectively [26]. The translocator domain in a GDSL autotransporter is employed as an anchoring motif to display BLPAD on the cell surface. Two signal peptides, including the native GDSL signal and *pelB* signal sequences, were evaluated for their effectiveness in guiding outer membrane translocation of the target protein on the *E. coli* cell surface. We demonstrated that BLPAD was targeted on the surface of *E. coli* by the autotransporter pathway in its active form, it was more efficiently guided by the *pelB* signal peptide than native signal peptide, and the cell surface displaying BLPAD was effective as a whole-cell catalyst in the bioproduction of 4-VG and 4-VP from FA and *pCA*, respectively.

2. Results and Discussion

2.1. Construction of the Vector System for Phenolic Acid Decarboxylase Autodisplay on *Escherichia coli*

Two plasmids, pET22b-*pelblpad* and pET22b-*gdsblpad*, containing BLPAD chimeras with a *PelB* signal peptide or a GDSL autotransporter signal peptide (SP_{GDSL}) were constructed to display phenolic acid decarboxylase on the cell surface of *E. coli*. The wild GDSL autotransporter consists of signal peptide (SP_{GDSL}) at the N-terminus followed by a passenger domain (esterase protein), the α -helical linker peptide (LK), and C-terminal β -barrel domain (Figure 1A). SP_{GDSL} and the passenger domain were replaced with a *PelB* signal peptide sequence and BLPAD protein in the BLPAD chimera with a *PelB* signal peptide (Figure 1B), or, alternatively, only the passenger domain was replaced with BLPAD protein in the BLPAD chimera with a GDSL autotransporter signal peptide (Figure 1C). In this GDSL autotransporter-based cell surface display system, the translated fusion protein will be transported into the periplasmic space via Sec machinery guiding the signal peptide [27]. After the signal peptide is cleaved, the fusion protein can be translocated across the outer membrane with the help of the β -barrel domain. Finally, the N-terminal exogenous protein remains covalently attached to their respective C-terminal β -barrel domains on the cell surface [24]. Therefore, the functional displayed phenolic acid

decarboxylase (BLPAD) from *B. licheniformis* on the *E. coli* cell surface was used as a whole-cell catalyst for the efficient production of 4-VG and 4-VP in this study (Figure 2).

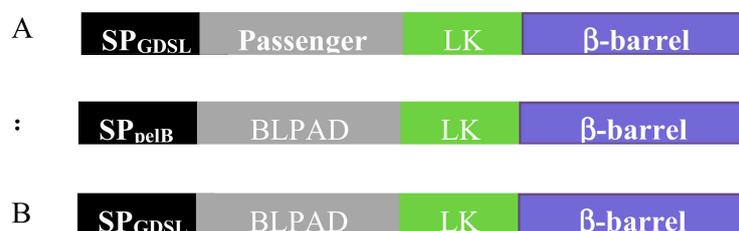


Figure 1. Schematic representation of the hybrid constructs for cell surface display of phenolic acid decarboxylase (BLPAD). (A) The wild GDSL autotransporter. (B) The BLPAD chimera with a PelB signal peptide sequence. (C) The BLPAD chimera with a GDSL autotransporter signal peptide sequence. SP_{GDSL} : signal peptide of GDSL autotransporter; SP_{peLB} : PelB signal peptide; LK: the α -helical linker peptide; BLPAD: *B. licheniformis* phenolic acid decarboxylase; and β -Barrel: the translocator domain of GDSL autotransporter.

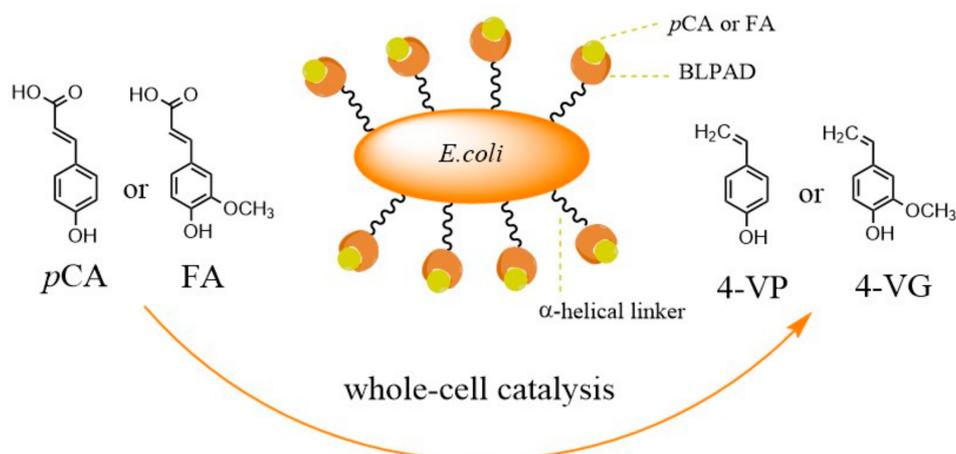


Figure 2. Schematic diagram of the protein surface displayed on the cell and the catalysis of 4-hydroxycinnamic acid to vinyl phenol derivatives.

2.2. Expression and Localization of the Autodisplayed Phenolic Acid Decarboxylase on *E. coli*

The two constructed plasmids were transformed into *E. coli* strains BL21(DE3) and BL21(DE3) pLysS, respectively, and BLPAD was induced as described in the Materials and Methods section. To investigate localization of BLPAD in *E. coli*, whole-cell lysates and membrane fractions of the recombinant *E. coli* expressing BLPAD were analyzed by SDS-PAGE. After 4 h induction, the target protein of about 60 kDa was clearly presented in whole-cell lysates, the total membrane fraction, and the outer membrane fraction of induced cells. The molecular weight of the target protein was equal to the sum of three molecular weights of the BLPAD chimera combined with the C-terminal β -barrel domain of GDSL and linker peptide, indicating a successful expression of BLPAD as a fusion protein on the cell surface (Figure 3).

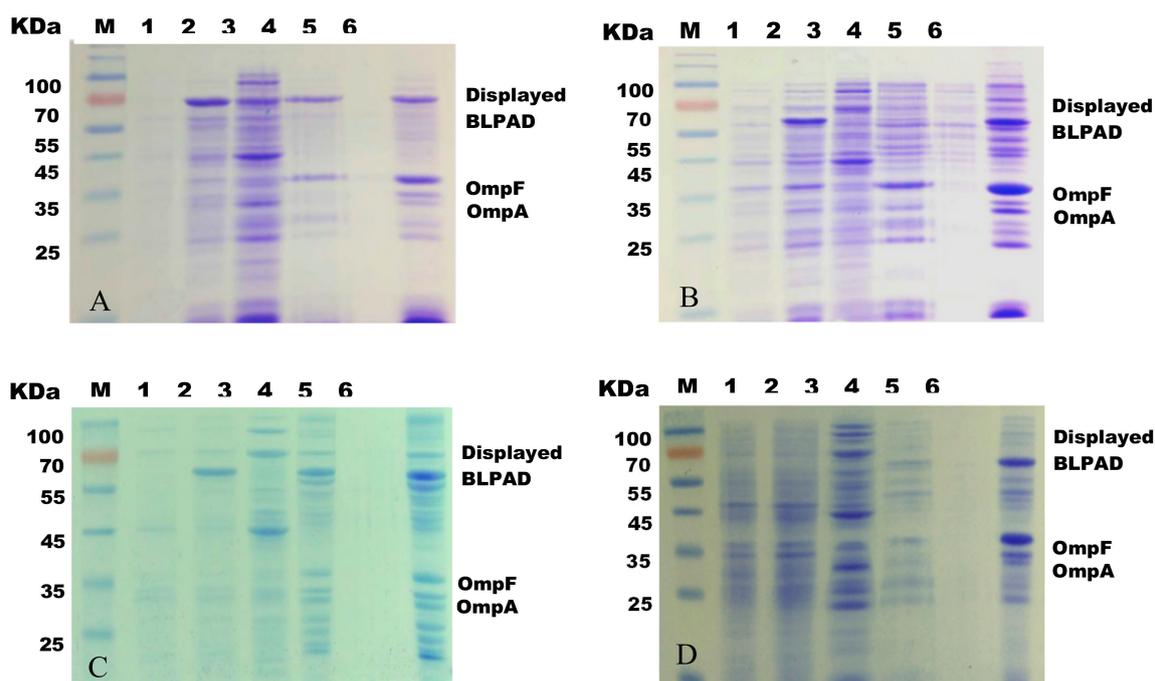


Figure 3. Expression and membrane localization of the surface-displayed cells. (A,B) BL21(DE3) cells and BL21 (DE3) pLysS cells carrying a pET22b-pelblpad vector. (C,D) BL21(DE3) cells and BL21 (DE3) pLysS cells carrying a pET22b-gdslpad vector, respectively. M: protein marker; Lane 1–6: whole-cell lysates of no induced cell, whole-cell lysates of induced cell, soluble fraction, total membrane fraction, inner membrane fraction, and outer membrane fraction.

To verify whether BLPAD was directed toward the external side or the periplasmic side of the outer membrane, whole cells of *E. coli* BL21(DE) or BL21(DE3) pLysS strains expressing BLPAD were pretreated by proteinase K. Then, the outer membrane fraction was isolated from the proteinase K treated cells. SDS-PAGE analysis showed that the corresponding protein band of BLPAD chimera on the outer membrane almost disappeared after 0.1 mg/mL proteinase K treatment (Figure 4).

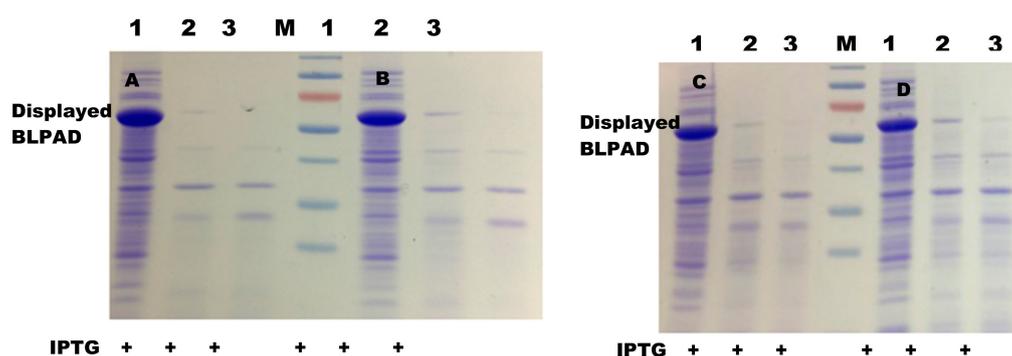


Figure 4. Protease accessibility analyses of the surface-displayed cells. (A,B) BL21(DE3) cells and BL21 (DE3) pLysS cells carrying a pET22b-pelblpad vector. (C,D) BL21(DE3) cells and BL21 (DE3) pLysS cells carrying a pET22b-gdslpad vector, respectively. M: protein marker, Lane 1–3: the outer membrane fractions from no proteinase K treated cells, with 0.05 mg/mL proteinase K treated cells, and with 0.1 mg/mL proteinase K treated cells, respectively. PrK: proteinase K; IPTG: isopropyl β -D-thiogalactoside.

As demonstrated previously, proteinase K is not able to pass the outer membrane and enter the periplasm [28]. These results clearly confirmed that the expressing BLPAD was exposed on the surface; therefore, BLPAD on the outer membrane degraded after treatment with proteinase K. After

confirming successful display of BLPAD on the *E. coli* cell surface, whole-cell catalytic activities of four recombinant strains with or without proteinase K treatment were also assayed in this study. As shown in Figure 5, after induction, the activities of BL21(DE3) and BL21(DE3) pLysS strains carrying the pET22b-pelblpad vector reached 19.72 and 19.38 U/OD₆₀₀, respectively, and the corresponding strains carrying the pET22b-gdslblpad vector reached 10.90 and 14.74 U/OD₆₀₀, respectively. In contrast, the strains transformed with blank vector almost had no activity, and the activities of proteinase K treated strains were also tremendously reduced (Figure 5).

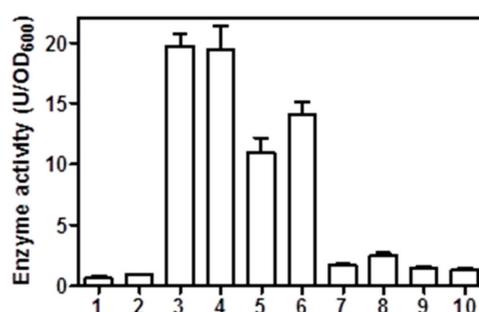


Figure 5. Phenolic acid decarboxylase activity of the surface-displayed cells. 1 and 2: BL21(DE3) and BL21(DE3)pLysS3 cells carrying a blank pET-22b vector, respectively; 3 and 4: BL21(DE3) and BL21(DE3) pLysS cells carrying a pET22b-pelblpad vector, respectively; 5 and 6: BL21(DE3) and BL21(DE3) pLysS cells carrying a pET22b-gdslblpad vector; 7 and 8: proteinase K treated BL21(DE3) and BL21(DE3) pLysS cells carrying a pET22b-pelblpad vector, respectively; and 9 and 10: proteinase K treated BL21(DE3) and BL21(DE3) pLysS cells carrying a pET22b-gdslblpad vector, respectively. Catalytic activity was measured under standard assay conditions.

GDSL autotransporters have proved to be excellent vehicles for the *E. coli* surface display of various enzymes, particularly for members of the lipase and esterase families [27,29–31]. However, not all proteins can successfully be autodisplayed on the cell surface in active forms due to limitations of the passage domains, such as molecular weight and number of disulfide bonds [32,33]. The high catalytic activities of BLPAD autodisplayed *E. coli* cells demonstrated that the GDSL autotransporter system is applicable to display BLPAD with the β -barrel structural motif in an active form for application of whole-cell biocatalysts. The N-terminal signal peptide is needed by Sec machinery for translocation across the inner membrane [28]. Comparably, expressing cells carrying the pET22b-pelblpad vector with the pelB signal sequence displayed higher catalytic activity than the expressing cells carrying pET22b-gdslblpad vector with the native signal sequence, suggesting that the pelB signal sequence is more effective in guiding the transfer of BLPAD into the periplasmic space (Figure 5).

2.3. Bioconversion of Ferulic Acid by Whole-Cell Catalysis of Expressing *E. coli*

Four different BLPAD autodisplayed *E. coli* cells were compared to catalyze ferulic acid into 4-VG, in the monoaqueous phase or in the biphasic toluene/Na₂HPO₄-citric acid buffer system, in order to evaluate their bioconversion capability as a whole-cell biocatalyst. As shown in Figure 6, the molar conversion yields of the expressing BL21 (DE3) and BL21 (DE3) pLysS strains carrying a pET22b-pelblpad vector reached 62% or 40% in the monoaqueous phase, respectively, or almost 100% in the biphasic system after a 50 min reaction. Meanwhile, the corresponding values for expressing BL21 (DE3) and BL21 (DE3) pLysS strains carrying a pET22b-gdslblpad vector were 27% and 37% in the monoaqueous phase or 86% and 81% in the biphasic system, respectively. The higher conversion yields of the cells carrying a pET22b-pelblpad vector than the cells carrying pET22b-gdslblpad vector were attributed to higher catalytic activities of the whole cells carrying a pET22b-pelblpad vector as mentioned above (Figure 5). The lower yields presented in the monoaqueous system when compared to the biphasic system mainly were due to toxicity of product accumulated in the aqueous phase in the

monoaqueous system [9,11]. In the biphasic system, the product could be extracted from the aqueous phase into the organic phase. Then, the residual concentration of product is lower in the aqueous phase of biphasic system compared to the aqueous phase of monophasic system. Biotransformation of hydroxycinnamic acid to 4-vinyl phenol derivatives was also feasibly affected by the toxicity of substrate, particularly as the substrate concentration increased [34]. The bioconversion efficiency of expressing BL21 (DE3) cells carrying a pET22b-pelblpad vector was further evaluated under a higher concentration of initial substrate to test the behavior of this system with different substrates. As shown in Table 1, the molar bioconversion yields of whole-cell catalysis for FA and *p*CA reached up to 72.6% and 80.4%, respectively, at the concentration of 300 mM in a biphasic toluene/Na₂HPO₄-citric acid buffer system. In contrast, the molar bioconversion yields of whole-cell catalysis for caffeic acid (CA) and sinapic acid (SA) were only 7.24% and 4.48%, respectively (Table 1). Whole-cell biocatalysis by using *E. coli* cells overexpressing the intracellular BLPAD protein or containing blank plasmid, as described previously [26], was also performed in the same reaction conditions. Molar bioconversion yields of *E. coli* cells overexpressing intracellular BLPAD reached 94.9% ± 4.8% and 99.8% ± 5.3% for FA and *p*CA at the concentration of 300 mM under same conditions, respectively. Additionally, the corresponding values for caffeic acid (CA) and sinapic acid (SA) were 25.8% ± 3.7% and 8.9% ± 2.5%, respectively. No product was detected when using *E. coli* cells containing a blank plasmid in the same reaction conditions. Comparatively, the bioconversion efficiencies of hydroxycinnamic acid in high concentration conditions were relatively lower than intracellular expressing BLPAD *E. coli*, but they were much higher than in previous literature using recombinant *E. coli* harboring intracellular PADs [8,9]. These results indicated that the displayed BLPAD had a high potential as a whole-cell biocatalyst for 4-VG and 4-VP bioproduction.

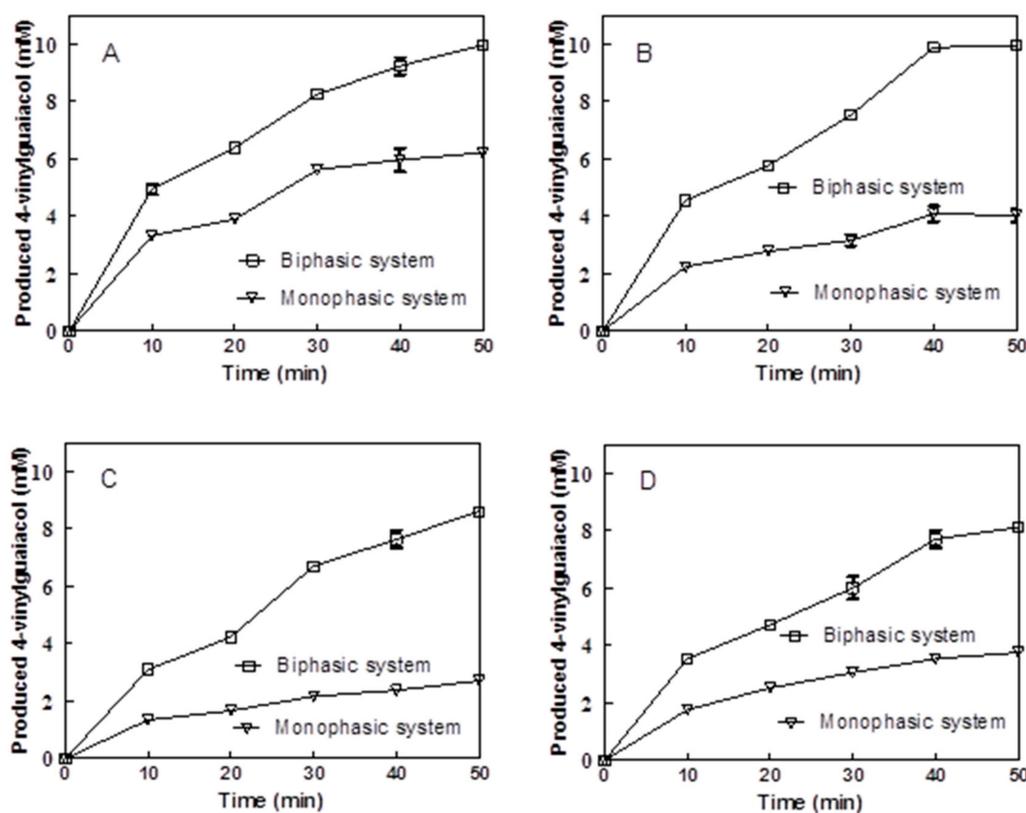


Figure 6. Bioconversion of ferulic acid (FA) in monophasic and biphasic systems with four different surface-displayed cells. (A,B) BL21(DE3) cells and BL21 (DE3) pLysS cells carrying a pET22b-pelblpad vector, respectively. (C,D) BL21(DE3) cells and BL21 (DE3) pLysS cells carrying a pET22b-gdslpad vector, respectively.

Table 1. Bioconversion of 4-hydroxycinnamic acid by *Escherichia coli* BL21 (DE3) cells carrying a pET22b-pelblpad vector after one hour of incubation. pCA: *p*-coumaric acid; CA: caffeic acid; and SA: sinapic acid.

Substrate Concentrations (mM)		50	100	150	200	250	300
Overall conversion yields (%)	pCA	100 ± 4.6	100 ± 3.8	100 ± 4.1	98.1 ± 5.2	88.3 ± 2.7	80.4 ± 4.1
	FA	100 ± 3.9	100 ± 3.1	94.2 ± 3.8	81.4 ± 2.9	79.2 ± 3.2	72.6 ± 3.6
	CA	29.3 ± 2.5	26.1 ± 2.2	16.2 ± 1.4	12.9 ± 2.0	7.8 ± 1.6	7.2 ± 1.1
	SA	9.1 ± 1.2	8.8 ± 0.9	8.5 ± 1.2	7.3 ± 1.6	6.3 ± 1.3	4.5 ± 0.9

Recyclability of biocatalysts is often a desired feature for the economics of industrial biocatalysis, which may reduce the high costs of biocatalysts in general [34]. However, the toxicity effect of vinylphenol derivatives could cause serious damage to the intracellular decarboxylase-containing cells. Ben-Bassat et al. reported that after a single 4-VP production cycle, the intracellular decarboxylase-containing cells were not viable when streaked on agar plates, which limited cell recycling [9]. The autodisplayed BLPAD showed good recyclability in this study. As shown in Figure 7, the BLPAD displayed cells were successfully recycled for 7 cycles with a residual activity of 63% (The activity at the first run was taken as 100%). In contrast, the recyclability of the intracellular expressing cells was rather low, and their residual activity reduced to approximately 11% after three reuse cycles. This result indicated that BLPAD anchored on the cell surface appeared to be more stable compared to free intracellular enzyme.

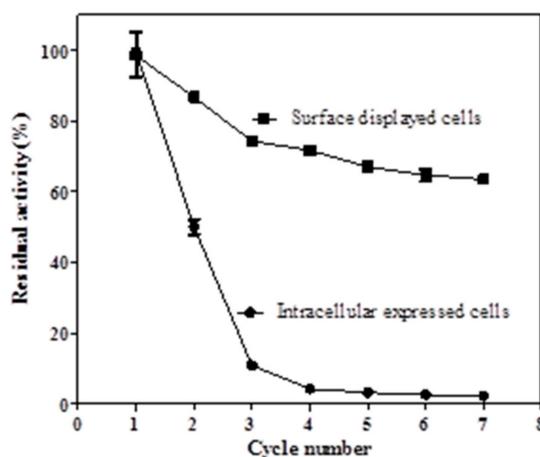


Figure 7. Reusability of the surface-displayed and intracellular expressed cells.

3. Materials and Methods

3.1. Bacterial Strains, Vectors, and Chemicals

E. coli strains BL21 (DE3) and BL21 (DE3) pLysS (TransGene, Beijing, China) were used for expression and whole cell activity assays of the surface-displayed BLPAD. pET-22b (Novagen, Copenhagen, Denmark) was used as an expression vector. pET28b-blpad and pET-29a-GDSL, harboring the genes of BLPAD and GDSL autotransporter, respectively, were used as templates for amplifying the gene fragments of BLPAD and the translocator domain, respectively [25,26]. Lysozyme and proteinase K were obtained from Sangon Biotech (Shanghai, China). FA, pCA, CA, and SA were purchased from Sinopharm Group (Beijing, China). 4-VG and 4-VP were obtained from Sigma-Aldrich (St. Louis, MO, USA). FastPfu DNA polymerase and restriction enzymes were all obtained from Takara (Dalian, China).

3.2. Construction of Plasmids for Cell Surface Display of Phenolic Acid Decarboxylase (BLPAD)

A schematic representation of the constructs for cell surface display of BLPAD is shown in Figure 1. The translocator domain in the GDSL autotransporter (Genbank No. JQ855507) from *Pseudomonas putida* was used as an anchoring motif to display BLPAD on the cell surface. The gene of GDSL autotransporter encodes a preprotein of 627 amino acids, which comprises a 24 amino acid N-terminal signal peptide, a 283 amino acid GDSL esterase passenger domain (P25-Q307), a 51 amino acid linker region, and a 272 amino acids C-terminal located autotransporter β -barrel domain. Two chimeras with different signal sequences were constructed as follows. For translocation via the signal sequence of pelB, the mature sequence of BLPAD was PCR amplified using pET28b-blpad as the template and blpad-EF/blpad-ER as primers (Table 2). PCR was performed under the following conditions: one cycle at 94 °C for 5 min, 55 °C for 30 s, and 72 °C for 6 min; 30 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 6 min; and a final extension at 72 °C for 10 min. The restrictive sites of *Bam*H1 and *Nhe*I were introduced into the PCR fragment at 5' and 3' ends, respectively. The fragment encoding for the translocator domain of the GDSL autotransporter was also PCR amplified using pET-29a-GDSL as the template and EstA-EF/EstA-ER as primers (Table 2). The restrictive sites *Nhe*I and *Xho*I were introduced into the PCR fragment at 5' and 3' ends, respectively. These two resulting PCR fragments were digested with *Bam*H1/*Nhe*I and *Nhe*I/*Xho*I, respectively, and ligated to pET-22b to give pET22b-pelblpad. For translocation via the signal sequence of the GDSL autotransporter, the mature sequence of BLPAD and the signal sequence of the GDSL autotransporter at the N-terminus were amplified by primer extension PCR using pET28b-blpad as the template and blpad-F1 to F4/blpad-ER as primers (Table 2). The restrictive sites of *Nde*I and *Nhe*I were introduced into the PCR fragment at 5' and 3' ends, respectively. This PCR fragment was digested with *Nde*I/*Nhe*I and ligated, similar to digested pET22b-pelblpad, to give pET22b-gdslblpad.

Table 2. Oligonucleotide primers used in this study.

Primers	Sequences (5'–3')	Restrictive Sites
blpad-EF	CGGGATCCTATGAATCAAGATGTA AA AAGAGTTT	<i>Bam</i> H I
blpad-ER	CAGGCTAGCTACCCGCTTTCCTGCCCT	-
EstA-EF	GTAGCTAGCCTGATCGCCGACTATGGCTA	-
EstA-ER	CCGCTCGAGTCAGAAATCCAGGCTGACC	<i>Xho</i> I
F1	GCAGCCAGGCATTCGCTGCCATGAATCAAGATGTA AA AAGAG	-
F2	GGCCACATTGGCGCTGGCGTGCAGCCAGGCATTCGCTGCC	-
F3	CCGTTATTGCGCTTTACCCTGGCCACATTGGCGCTGGCGT	-
F4	GGGAATTCATATGCGAAAGCACC GTTATTGCGCTTTACCCT	<i>Nde</i> I

3.3. Expression and Membrane Fraction Isolation

Constructed plasmids pET22b-pelblpad and pET22b-gdslblpad were transformed into *E. coli* BL21(DE3) and *E. coli* BL21(DE3) pLysS strains, respectively. The recombinant strains were cultured in 50 mL of Luria–Bertani (LB) medium supplemented with ampicillin (100 μ g/mL) at 37 °C with shaking at 180 rpm. After OD₆₀₀ reached 0.6, the temperature was reduced to 25 °C, and the expression of recombinant BLPAD was induced with 0.2 mM isopropyl- β -D-thiogalactopyranoside (IPTG) for 5 h.

Membrane fraction isolation was performed as previously described [27]. After induction, the expressed cells were harvested by centrifugation (6000 \times *g*, 30 min, 4 °C). The pellets were resuspended in 5 mL lysis buffer (50 mM Tris–HCl, 5mM EDTA, 20% sucrose, and 0.2 mg/mL lysozyme, pH 8.0) and were disrupted by sonication in an ice bath. The cell lysate was centrifuged at 6000 \times *g* for 30 min at 4 °C. The resulting supernatant was ultracentrifuged at 100,000 \times *g* at 4 °C for 1 h. The supernatant after ultracentrifugation was kept as a soluble fraction for future analysis. The pellet (total membrane fraction) was resuspended and incubated in 5 mL solution containing 50 mM Tris–HCl (pH 8.0), 10 mM MgCl₂, and 1% TritonX-100 at room temperature for 20 min. The suspension was ultracentrifuged again as above. The resulting pellet was resuspended in 50 mM Tris–HCl (pH 8.0)

as the outer membrane fraction. The supernatant was kept as an inner membrane fraction for future analysis. Protein concentrations were determined using a BCA (Bicinchoninic acid) protein assay kit (Sangon, Shanghai, China) and BSA (Bovine Serum Albumin) as a standard. Equivalent amounts of the protein fractions were analyzed by protein electrophoresis using 12% separating gel.

3.4. Protease Accessibility

The expressed cells were washed twice using phosphate buffered saline (PBS) and resuspended in 50 mM Tris-HCl buffer (pH 8.0) containing 5 mM CaCl₂ and 0.1 mg/mL proteinase K. The cell suspension was incubated at 37 °C and 150 rpm for 30 min. The reaction was stopped by addition of PMSF (Phenylmethanesulfonyl fluoride) solution (up to 5 mM). The cells were pelleted by centrifugation and washed with PBS again. The outer membrane fraction was isolated from the obtained pellet as described above and analyzed by SDS-PAGE.

3.5. Bioconversion of Ferulic Acid by Whole-Cell Catalysis of Expressing *E. coli*

Comparisons of bioconversion capabilities of four different BLPAD autodisplayed *E. coli* cells were performed in a 2 mL glass tube with Teflon seals at 37 °C and 400 rpm shaking for set times. The mixture for the monoaqueous phase consisted of 0.9 mL of 200 mM Na₂HPO₄-citric acid buffer (pH 6.0) containing 10 mM FA and 0.1 mL of BLPAD autodisplayed cells (OD₆₀₀ = 1). For a biphasic reaction system, an equal volume of toluene was added into above mixture. Triplicate samples were withdrawn at intervals (10 min), and the reaction was terminated by adding 50 µL of 50% trichloroacetic acid. Bioconversion of different substrates with higher initial concentrations by BLPAD autodisplayed *E. coli* cells carrying a pET22b-pelblpad vector was carried out in a biphasic reaction system, which consisted of 10 mL of toluene and 10 mL of Na₂HPO₄-citric acid buffer containing different initial concentrations of substrates and *E. coli* cells (2 OD₆₀₀ in final), at 200 rpm and 37 °C for 1 h [35]. Whole-cell catalysis by using *E. coli* cells overexpressing intracellular BLPAD protein or containing a blank plasmid as described previously [26] was also performed in the same reaction conditions. The substrate and product formed in aqueous and organic phases were quantified by high-performance liquid chromatography (HPLC) as previously described [26].

Reusability of the surface-displayed BLPAD was determined by measuring the activity of recombinant cells at a concentration of 50 mM FA in a monophasic system as described above. Recombinant *E. coli* cells with autodisplayed BLPAD were induced as above. Induction of recombinant *E. coli* cells expressing intracellular BLPAD was performed as previously described [26]. After induction, cells from the 50 mL culture were harvested by centrifugation at 6500 rpm and 4 °C for 10 min, washed twice with Na₂HPO₄-citric acid (200 mM, pH 6.0) buffer, and were resuspended in the same buffer. The reaction was carried out in 15 mL of reaction mixture at 200 rpm and 37 °C for 15 min. After each reaction, the cells were collected by centrifugation and washed with the same buffer twice. Then, the buffer containing 50 mL of FA was added to start a new batch of catalysis. The generated product was quantified by HPLC as previously described [26]. The residual activity was calculated by defining the activity of the first reaction as 100%.

3.6. Phenolic Acid Decarboxylase Activity Assay

The whole cell activity of BLPAD autodisplayed *E. coli* was assayed in the mixture containing 0.8 mL of 200 mM Na₂HPO₄-citric acid buffer (pH 6.0), 0.1 mL of 50 mM FA, and 0.1 mL of diluted cell suspension in a 10 mL glass tube with Teflon seals at 37 °C for 10 min. The BLPAD autodisplayed *E. coli* BL21 (DE3) cells were cultivated and induced as described above. One milliliter of methanol was added into the mixture to terminate the reaction. The generated product was quantified by HPLC as previously described [26]. One unit (U) of BLPAD activity was defined as the amount of enzyme that generated 1 µmol 4-VG per minute under the conditions described above. The value was presented as unit per milliliter of cells with one OD₆₀₀ density (U/OD₆₀₀) in order to compare the activity of different surface-displayed cells on the basis of the same number of cells used.

4. Conclusions

In this study, a phenolic acid decarboxylase from *B. licheniformis* was functionally autodisplayed on the *E. coli* cell surface using a GDSL autotransporter. Expression and localization of BLAPD on the cell surface of *E. coli* was verified by SDS-PAGE and protease accessibility. The pelB signal peptide is more effective in guiding the translocation of BLAPD on the cell surface than the native signal peptide of GDSL. Molar bioconversion yields of 72.6% and 80.4% were achieved at a concentration of 300 mM of FA and pCA in a biphasic system, respectively. Furthermore, the cell surface displayed BLPAD showed good reusability and retained 63% of residual activity after 7 cycles of repeated use, indicating that the cell surface displayed BLPAD can be used as a whole-cell biocatalyst for efficient production of 4-VG and 4-VP.

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Abbreviations

FA: ferulic acid; 4-VG: 4-vinyl guaiacol; pCA: p-coumaric acid; CA: caffeic acid; SA: sinapic acid; BLPAD: *Bacillus licheniformis* phenolic acid decarboxylases; OD600: optical density at 600 nm; PBS: phosphate buffered saline; PCR: polymerase chain reaction; IPTG: isopropyl- β -D-thiogalactopyranoside; SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis; LB medium: Luria-Bertani medium; HPLC: high-performance liquid chromatography.

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