Whole-cell catalysis by surface display of fluorinase on *Escherichia coli* using N-terminal domain of ice nucleation protein

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Research

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Abstract

Background

Fluorinases play a unique role in producing fluorinated organic molecules through a biological method. Whole-cell catalysis is a better choice in the large-scale fermentation processes, and over 60% of industrial biocatalysis uses this method. However, the in vivo catalytic efficiency of fluorinases is stuck with the mass transfer of the substrates.

Results

A gene sequence encoding a protein with fluorinase function was fused to the N-terminal of ice nucleation protein, and the fused protein was expressed in *Escherichia coli* BL21(DE3) cells. SDS-PAGE and Immunofluorescence microscopy were used to demonstrate the surface localization of the fusion protein. The fluorinase-containing surface display system with improved whole-cell catalytic efficiency and stability showed low growth pressure on the protein expressing host. The conversion rate of 5′-fluorodeoxyadenosine (5′-FDA) from S-adenosyl-L-methionine (SAM) achieved 55%.

Conclusions

Here, we created the fluorinase-containing surface display system on *E. coli* cells for the first time. The fluorinase was successfully displayed on the surface of *Escherichia coli* and maintained its catalytic activity. The surface display offers a new solution for the industrial application of biological fluorination.

1. Introduction

Fluorine is a very special element because of its unique properties, such as the strong electronegativity and small atomic radius. The van der Waals radius of fluorine is close to the hydrogen, which makes fluorine substitutions of hydrogen reasonable in space structure [1]. The C-F bond is one of the strongest chemical bonds, which makes organic fluorides tend to be biologically inert [2]. Moreover, fluorinated chemicals are more lipophilic than the nonfluorinated counterparties [3]. Due to these capabilities, fluorine was used to modify the physical and chemical properties of compounds, and has attracted increasingly attention in pharmaceuticals, chemicals and materials fields [4].

Fluorine is the most common halogen element on Earth’s crust. However, only a few natural organofluorides were identified because of the low bioavailability of fluorine in nature. Chemical methods have been developed to introduce fluorine into organic compounds, which often use toxic fluorination reagents [5]. In contrast, fluorinases, which catalyze the formation of C-F bond, offer a more environment-friendly approach to incorporate inorganic fluoride ions into organic compounds. After the first fluorinase was reported in 2002 [6], [18F]-radiolabelled products, which was preferred for Positron Emission Tomography imaging, was synthesized with the participation of fluorinase [7, 8]. Despite the tremendous potential of industrial application, there is still a lot of work to be done before fluorinases can be applied.
on an industrial scale. The current research on the catalytic conversion of fluorination have been done to improve enzymatic activity and stability [9-11]. Immobilization of the fluorinase was carried out to improve the stability of enzyme, meanwhile, to make it easier to remove the catalyst [9, 12, 13]. However, the process of immobilization was also accompanied by the tedious purification steps of enzyme. By contrast, cell fermentation is more effective in large-scale applications. Previous studies have found that two shortcomings have severely limited the in vivo catalyzed fluorination. On the one hand, the transport of S-adenosylmethionine to the cell interior was blocked by cell membrane [14]. On the other hand, the intracellular fluoride concentration in wild-type E. coli was very low because of a CrcB channel protein, which was responsible for the excretion of fluoride ions [15]. To solve the difficulty that the substrate cannot be enriched in vivo, Markakis et al. constructed the engineered E. coli strain to achieve fermentation production by adding a SAM transporter and eliminating fluoride efflux capacity [16]. Although in vivo fluorination of E. coli has been achieved, the engineered cells were more sensitive to toxic fluoride ions [17]. In addition, the 5′-FDA product was accumulated inside the cell, which may cause trouble for later product extraction [16]. By the way, enzymes inside the cells can not be reused as extraction of the product requires cell disruption. The industrial application of fluorinase needs more exploration.

Surface display technology allows peptides and small proteins to be located on the cell surface and has been widely applied in live vaccines, whole-cell biocatalysts, biodetoxification, peptide library screening and biosensors [18-21]. Apart from eliminating the step of enzyme purification, the strategy can cut down restriction of substrates transport across the membrane. It is suggested that the biofluorination can be combined with surface display technology. However, the research on the display of fluorinases on the surface of microorganisms has not been reported. It is also challenging to obtain an active fusion enzyme. For example, Gustavsson et al. reported an inactive surface expression of ω-transaminase because of the dimer structure was difficult to form on the host surface [22]. Whether the hexamer structure of fluorinase can be successfully displayed on the surface of the host requires further research [23].

Herein, a fluorinase mutant (Faa) from Amycolatopsis sp. CA-128772 was first dispalyed on Escherichia coli using the N-terminal domain of ice nucleation protein, and then whole-cell transformation was carried out to obtain 5′-fluorodeoxyadenosine. To our knowledge, this is the first application of surface display technology for biological fluorination.

2. Results And Discussion

2.1 Location of fluorinase in pET28a-INP-Faa-Expressing Cells

The ice nucleation proteins are membrane proteins commonly found in genera Pseudomonas, Panteola (Erwinia), and Xanthomonas, which mediates the formation of ice nucleus for ice growth [24]. The protein sequence can be divided into N-terminal, C-terminal and relatively conserved central domain. The N-
terminal containing 170 amino acid residues has been used as a linkage to the outer membrane of host cells to display target proteins and showed excellent biocatalysis [25, 26].

The N-terminal domain of ice nucleation protein was linked with Faa to create INP-Faa by genetic engineering. To investigate the location of INP-Faa expression in the E.coli cells, the protein from cytoplasm, inner membrane and outer membrane fractions were verified by SDS-PAGE (Figure 1). The theoretical molecular weight of INP-Faa protein is about 50 KDa while that of Faa is about 32 KDa, and the corresponding bands could be identified in the total protein section, which means that the proteins were expressed normally. In contrast to the control group (Faa), the 50 KDa band related to the INP-Faa was clearly observed in the column of outer membrane fractions. This result indicated that the fluorinase can be displayed with the N-terminal of ice nucleation protein from Pseudomonas borealis on the surface of E.coli cells.

2.2 Immunofluorescence microscopy

In order to further confirm that INP-Faa was indeed displayed on the outside of the outer membrane, cell immunofluorescence assay was carried out with his-tag antibody and Alexa Fluor 555-labeled Donkey Anti-Mouse IgG (H+L). Under normal conditions, large molecules such as antibodies cannot penetrate the cell membrane, which means that only proteins on the cell surface can be stained. As expected, cells harboring the INP-Faa showed detectable fluorescence signal, indicating that the fusion protein was displayed successfully on the cell surface (Figure 2). The small amount of fluorescence in the control may be caused by cell death.

2.3 Whole-cell assay

Regardless of the normal expression and correct localization of the fusion protein, obtaining the fusion protein with activity is also affected by the secondary structure. The crystal structure of wild type fluorinase from S. cattleya has been resolved by Dong et.al, and the structure revealed a hexamer composed of two trimeric units [23]. Thus, it is a challenge to obtain active INP-Faa on the cell surface. To our great joy, fluorinase activity of INP-Faa can be measured by HPLC (Figure 3). For Faa cells, the catalytic activity of whole-cell was lower than that of disrupted cell suspension, which means that the permeability of cell membrane does limit the catalytic ability of whole-cell. Since the protein expression intensity of different cells is different, it may affect the ability of the apparent whole-cell catalysis. To verify the contribution of surface display enzymes to whole-cell catalysis, the disrupted cell suspension which contained all enzymes expressed in the cell was set as a relative activity of 100%. For INP-Faa cells, the majority of activity was detected in the precipitate. This result indicated that most of the target protein has been localized in the cell membrane, and after fragmentation, it formed a precipitate along with large cell debris. The lower cell activity may be due to the fact that the correct display of the enzyme requires a certain period of time [27].

Thus, cells with increased induction time were used to measure changes in the catalytic ability of whole cells (Figure 4). As expected, the display efficiency of whole cells gradually increased with the increase of
induction time. The proportion of whole-cell catalysis was stable after 20 hours of induction, the result meant that the display efficiency of fluorinase has reached its maximum, as reported in the literature, is about 60% [28].

This confirms that the N-terminal of ice nucleation protein is an effective protein for surface display. The fluidity of cell membrane makes fluorinase monomers can be close to each other to form an active unit. In addition, we speculate that hexamer is not a necessary active structure for fluorinase as the huge difficulty in forming hexamer on the surface of cell membrane.

2.4 Stability study of whole-cells

One of the issues that cannot be ignored in cell-free biosynthesis is the instability of the enzyme. For a better comparison, the activity between the cell-free biosynthesis of Faa and whole-cell transformation of INP-Faa cells were measured for 7 days (Figure 4). The whole-cell relative activity of INP-Faa cells kept 120% in 7 days. In contrast, cell-free system activity of Faa was reduced by 93%. These results indicated that compared with cell-free catalysis, the INP-Faa cells retained a good catalytic ability and greatly enhanced the stability of the enzyme.

2.5 Stability of cultures expressing INP-Faa

The over-expression of foreign protein will cause a certain burden on the growth of the host [29]. To evaluate the influence of the surface display system on cell stability, the growth kinetics of cells carrying pET28a-Faa and pET28a-INP-Faa were compared (Figure 5). The INP-Faa cells achieved greater cell density (OD_{600} ≈ 6.9) while Faa cells can only attain 5.2 OD_{600} after 33 h of culture. The results indicated that the surface display of foreign protein can reduce its toxicity to cells.

2.6 Synthesis of 5′-FDA by INP-Faa-Expressing Cells

For applications, whole-cell catalysis has great advantages, including recyclability and reusable, but the efficiency of substrate utilization must also be considered. 5′-FDA was synthesized by INP-Faa displayed on E.coli cells. During 28 hours of whole-cell catalytic transformation, 1 mM SAM was fluorinated to get maximum amount of 5′-FDA (0.55 mM) at 37 hours. Compared with the immobilization method reported by previously literature, the surface display system showed a comparable conversion rate and reached 55% [12]. The reason for the low yield may come from unstable of SAM as the hydrolysis reaction to produce adenine at alkaline conditions [30]. This study was first to confirm the potential of surface display technology in whole-cell fluorination catalysis.

3. Conclusion

The cell surface display system provides a new height for whole-cell catalysis. In this study, the fluorinase mutant from Amycolatopsis sp. CA-128772 was displayed on the surface of E.coli by the N-terminal of domain of ice nucleation protein. The fusion protein showed fluorinase activity and high stability. In
addition, the 5'-FDA was synthesized by the bacteria-displaying fluorinase without enzyme-extracting and purifying. Furthermore, the surface display system reduced the pressure of cell growth, this means that more cells can be obtained, which is beneficial to large-scale applications.

4. Materials And Methods

4.1 Materials.

Isopropyl β-D-thiogalactopyranoside (IPTG), 5'-fluorodeoxyadenosine (5'-FDA), S-adenosyl-L-methionine (SAM), KF were obtained from commercial corporations (Aladdin, Sigma-Aldrich, Macklin, etc.). The mutant sequence of fluorinase named Faa comes from our previous studies, which showed improved catalytic efficiency. The E. coli strain BL21(DE3) was used as the host cell, and the ice nucleation protein (NCBI accession number: ACB59244.1) from Pseudomonas borealis was used as carrier protein. Plasmid pET28a was used as the expression vector.

4.2 Construction of a fluorinase expression plasmid on the E. coli cell surface

The optimized gene sequence of ice nucleation protein was synthesized by the Tsingke Biotechnology Co. with pET28a between the Ncol and BamHI restriction sites to created pET28a-INP. The gene corresponding to the Faa was obtained by PCR using the following pair of primers: Faa-F 5'-ggatcttccagagatgagctcATGGCGAAACCTAGCCGC-3' (the underline denotes SacI recognition sites) and Faa-R 5'-ctgccgttcgacgatACGCTGCAACAACGCGAA-3' (the underline denotes HindIII recognition sites). Then the PCR product was digested by SacI and HindIII restriction enzyme and connected to pET28a with the corresponding sites to form pET28a-INP-Faa. The plasmid was transformed into E. coli BL21 (DE3), and the recombinant strain was screened by colony PCR using primers T7 (5'-TAATACGACTCACTATAGGG-3') and T7t (5'-GCTAGTTATTGCTCAGCGG-3'). The control stain harboring pET28-Faa was also created.

4.3 Location of fluorinase in pET28a-INP-Faa-Expressing Cells

The recombinant strain with pET28a-INP-Faa was cultivated in LB broth containing 50 µg ml⁻¹ kanamycin at 37 °C. IPTG was added at a final concentration of 0.2 mM for 4 h of induced expression at 30 °C when the OD₆₀₀ reached 0.6-0.8. The cells were harvested at 6000 rpm for 10 min at 4 °C and washed three times with Tris-HCL (pH7.8, 50 mM).

To demonstrate location of INP-Faa, harvested cells were fractionated to obtain cytoplasam and membrane fractions according to the method proposed by Jochen [31]. The cells were resuspended in PBS buffer (pH8.0, 50 mM) to set OD₆₀₀ as 5.0 and then crushed by constant cell disruption systems (Constant Systems) at 30 Kpsi. The suspension was centrifuged at 6,000 rpm for 10 min to remove undisrupted cells and large cell debris. The clarified extract was then centrifuged at 34500 rpm for 1 h (Himac CP100WX, Hitachi, Japan) to obtain proteins from the periplasm and cytoplasam. The insoluble part was suspended in PBS containing MgCL₂ (0.01mM) and Triton X-100 (2%) and incubated at 25 °C
for 30 min to dissolving inner membrane, and then the resuspended components was centrifuged at 34500 rpm for 1 h to get the outer membranes which was insoluble. The different fractionated samples were mixed with protein loading buffer and boiled for 2 min, then the mixture was determined by 10% SDS-PAGE.

**4.4 Immunofluorescence microscopy**

The INP-Faa with a his-tag was constructed for Immunofluorescence microscopy (Additional file 1). The *E. coli* cells displaying fluorinase were harvested by centrifugation at 3800 rpm at 4°C for 10 min, washed three times with Tris-HCL (pH7.8, 50 mM) and fixed in 2% formaldehyde for 10 min at room temperature. The fixed cells were washed and blocked in PBS buffer containing 2% BSA at 4°C for 12 h, then his-tag antibody (Beyotime Biotechnology) was added (1:100) for another 4 h of incubation at room temperature. Alexa Fluor 555-labeled Donkey Anti-Mouse IgG (H+L) was incubated for 4 h with the cells after 3 washes. Finally, the cells were washed and examined by fluorescence microscope.

**4.5 Whole-cell assay**

For activity assay, the induced cells were resuspended in Tris-HCL (pH7.8, 50 mM) and divide into two equal parts, one of which was crushed. Then the disrupted cell suspension was divided into supernatant and precipitate by 6000 rpm centrifugation at 4°C. Subsequently, the precipitate was resuspended with an equal amount of buffer. Equal amount of cell suspension, supernatant and precipitation suspension was mixed with 20 mM KF and 1 mM SAM and incubated at 37°C for 1 h. The samples were detected by HPLC after termination of the reaction by boiling for 2 min.

**4.6 Stability study of whole-cells**

The induced cells (OD$_{600}$=10) containing pET28a-INP-Faa were stored in Tris-HCL (pH7.8, 50 mM) at 4°C for 7 days, and 800 µL of samples were removed each day to mix with 200 µL of substrate mixture containing 1M KF and 5 mM SAM and incubated at 37°C for 2 hours incubation at 37°C. The stability assays of Faa were also carried out using the cell-free extracts.

**4.7 Stability of cultures expressing INP-Faa**

The cells harboring pET28a-INP-Faa or pET28a-Faa were grown in 50 mL LB broth with 50 µg ml-1 kanamycin at 200 rpm at 37°C until the OD$_{600}$ reached 0.6, and then 0.2 mM IPTG was added for protein expression at 30°C. The optical density of the cultures was monitored until both cultures reached the stagnate phase.

**4.8 Synthesis of 5’-FDA by INP-Faa-Expressing Cells**

The reaction to investigate the ability to synthesize 5’-FDA by INP-Faa whole-cell system was carried out in Tris-HCL buffer (pH7.8, 50 mM) containing KF (200 mM), SAM (1 mM) and cells (OD$_{600}$=5) at 37°C. The generation of 5’-FDA was detected. The cells with pET28a-INP-Faa was induced for 24 hours.
Abbreviations

SAM: S-adenosyl-L-methionine

5′-FDA: 5′-fluorodeoxyadenosine

IPTG: Isopropyl-β-D thiogalactopyranoside

SDS-PAGE: Sodium dodecylsulphate polyacrylamide gel electrophoresis

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Contributions

XM and LW conceived and designed the experiments, FXM carried out the experimental work and wrote the manuscript, JMM and HW supervised the project. All authors read and approved the final manuscript.

References

1. O'Hagan D. Understanding organofluorine chemistry. An introduction to the C-F bond. Chem Soc Rev 2008, 37(2):308-319.

2. Wu YJ, Davis CD, Dworetzky S, Fitzpatrick WC, Harden D, He H, Knox RJ, Newton AE, Philip T, Polson C et al. Fluorine substitution can block CYP3A4 metabolism-dependent inhibition: Identification of (S)-N-[1-(4-fluoro-3-morpholin-4-ylphenyl)ethyl]-3-(4-fluorophenyl)acrylamide as an orally bioavailable KCNQ2 opener devoid of CYP3A4 metabolism-dependent inhibition. J Med Chem 2003, 46(18):3778-3781.

3. Smart BE. Fluorine substituent effects (on bioactivity). J Fluorine Chem 2001, 109(1):3-11.

4. Charvillat T, Bernardelli P, Daumas M, Pannecoucke X, Ferey V, Besset T. Hydrogenation of fluorinated molecules: an overview. Chem Soc Rev 2021; doi:10.1039/D0CS00736F
5. Shimizu M, Hiyama T. Modern synthetic methods for fluorine-substituted target molecules. Angew Chem Int Ed Engl 2004, 44(2):214-231.

6. O'Hagan D, Schaffrath C, Cobb SL, Hamilton JT, Murphy CD. Biochemistry: biosynthesis of an organofluorine molecule. Nature 2002, 416(6878):279.

7. Lowe PT, Dall'Angelo S, Mulder-Krieger T, AP IJ, Zanda M, O'Hagan D. A New Class of Fluorinated A2A Adenosine Receptor Agonist with Application to Last-Step Enzymatic [(18) F]Fluorination for PET Imaging. ChemBiochem 2017, 18(21):2156-2164.

8. Martarello L, Schaffrath C, Deng H, Gee AD, Lockhart A, O'Hagan D. The first enzymatic method for C-F-18 bond formation: the synthesis of 5'-[F-18]-fluoro-5' deoxyadenosine for imaging with PET. J Labelled Compd Rad 2003, 46(13):1181-1189.

9. Tu CH, Zhou J, Peng L, Man SL, Ma L. Self-assembled nano-aggregates of fluorinases demonstrate enhanced enzymatic activity, thermostability and reusability. Biomater Sci-Uk 2020, 8(2):648-656.

10. Sun H, Zhao H, Ang EL. A coupled chlorinase-fluorinase system with a high efficiency of trans-halogenation and a shared substrate tolerance. Chem Commun 2018, 54(68):9458-9461.

11. Sun HH, Yeo WL, Lim YH, Chew XY, Smith DJ, Xue B, Chan KP, Robinson RC, Robins EG, Zhao HM et al. Directed Evolution of a Fluorinase for Improved Fluorination Efficiency with a Non-native Substrate. Angew Chem Int Edit 2016, 55(46):14275-14278.

12. Sergeev ME, Morgia F, Javed MR, Doi M, Keng PY. Polymer-immobilized fluorinase: Recyclable catalyst for fluorination reactions. J Mol Catal B-Enzym 2013, 92:51-56.

13. Li NN, Hu BJ, Wang AM, Li HMM, Yin YC, Mao TY, Xie T. Facile Bioinspired Preparation of Fluorinase@Fluoridated Hydroxyapatite Nanoflowers for the Biosynthesis of 5'-Fluorodeoxy Adenosine. Sustainability-Basel 2020, 12(1).

14. Driskell LO, Tucker AM, Winkler HH, Wood DO. Rickettsial metK-encoded methionine adenosyltransferase expression in an Escherichia coli metK deletion strain. J Bacteriol 2005, 187(16):5719-5722.

15. Stockbridge RB, Lim HH, Otten R, Williams C, Shane T, Weinberg Z, Miller C. Fluoride resistance and transport by riboswitch-controlled CLC antiporters. Proc Natl Acad Sci U S A 2012, 109(38):15289-15294.

16. Markakis K, Lowe PT, Davison-Gates L, O'Hagan D, Rosser SJ, Elfick A. An Engineered E. coli Strain for Direct in Vivo Fluorination. ChemBiochem 2020, 21(13):1856-1860.

17. Baker JL, Sudarsan N, Weinberg Z, Roth A, Stockbridge RB, Breaker RR. Widespread genetic switches and toxicity resistance proteins for fluoride. Science 2012, 335(6065):233-235.
18. Urbar-Ulloa J, Montano-Silva P, Ramirez-Pelayo AS, Fernandez-Castillo E, Amaya-Delgado L, Rodriguez-Garay B, Verdin J. Cell surface display of proteins on filamentous fungi. Appl Microbiol Biotechnol 2019, 103(17):6949-6972.

19. Jing KJ, Guo YL, Ng IS. Antigen-43-mediated surface display revealed in Escherichia coli by different fusion sites and proteins. Bioresour Bioprocess 2019, 6.

20. Lin P, Yuan HB, Du JK, Liu KQ, Liu HL, Wang TF. Progress in research and application development of surface display technology using Bacillus subtilis spores. Appl Microbiol Biot 2020, 104(6):2319-2331.

21. Yuan YC, Bai XL, Liu YM, Tang XY, Yuan H, Liao X. Ligand fishing based on cell surface display of enzymes for inhibitor screening. Analytica Chimica Acta 2021, 1156.

22. Gustavsson M, Muraleedharan MN, Larsson G. Surface expression of omega-transaminase in Escherichia coli. Appl Environ Microbiol 2014, 80(7):2293-2298.

23. Dong CJ, Huang FL, Deng H, Schaffrath C, Spencer JB, O’Hagan D, Naismith JH. Crystal structure and mechanism of a bacterial fluorinating enzyme. Nature 2004, 427(6974):561-565.

24. Vanderveer TL, Choi J, Miao D, Walker VK. Expression and localization of an ice nucleating protein from a soil bacterium, Pseudomonas borealis. Cryobiology 2014, 69(1):110-118.

25. Yuan YC, Bai XL, Liu YM, Tang XY, Yuan H, Liao X. Ligand fishing based on cell surface display of enzymes for inhibitor screening. Anal Chim Acta 2021, 1156:338359.

26. Liang B, Li L, Tang X, Lang Q, Wang H, Li F, Shi J, Shen W, Palchetti I, Mascini M et al. Microbial surface display of glucose dehydrogenase for amperometric glucose biosensor. Biosens Bioelectron 2013, 45:19-24.

27. Shimazu M, Mulchandani A, Chen W. Simultaneous degradation of organophosphorus pesticides and p-nitrophenol by a genetically engineered Moraxella sp with surface-expressed organophosphorus hydrolase. Biotechnol Bioeng 2001, 76(4):318-324.

28. Li L, Kang DG, Cha HJ: Functional display of foreign protein on surface of Escherichia coli using N-terminal domain of ice nucleation protein. Biotechnol Bioeng 2004, 85(2):214-221.

29. Palomares LA, Estrada-Mondaca S, Ramirez OT. Production of recombinant proteins: challenges and solutions. Methods Mol Biol 2004, 267:15-52.

30. Hoffman JL. Chromatographic Analysis of the Chiral and Covalent Instability of S-Adenosyl-L-Methionine. Biochemistry-Us 1986, 25(15):4444-4449.

31. Maurer J, Jose J, Meyer TF. Autodisplay: one-component system for efficient surface display and release of soluble recombinant proteins from Escherichia coli. J Bacteriol 1997, 179(3):794-804.
**Figures**

**Figure 1**

Expression of INP-Faa in different fractions of the induced cell. Lane 1: Total protein components of INP-Faa; lane 2: cytoplasmic proteins of INP-Faa; lane 3: inner membrane of INP-Faa; lane 4: outer membrane of INP-Faa; lane 5: Total protein components of Faa; lane 6: cytoplasmic proteins of Faa; lane 7 inner membrane of Faa; lane 8: outer membrane of Faa. The corresponding target protein was marked in red.

**Figure 2**

Immunofluorescence micrographs of BL21(DE3) A: Cells harboring INP-Faa with a his-tag probed with histag and antibody fluorescently stained with Alexa Fluor 555-labeled Donkey Anti-Mouse IgG (H+L). B: Cells harboring Faa with a his-tag probed with anti-his-tag and fluorescently stained with Alexa Fluor 555-labeled Donkey Anti-Mouse IgG (H+L).
Figure 3

The relative activity of different fractions to the cell fragmentation. The assays were carried out in Tris-HCL buffer (pH 7.8, 50 mM) containing 20 mM KF, 1 mM SAM and induced cells or cell fragmentation at equal quantity for 1h at 37 °C.
Figure 4

The relative activity of different fractions to the cell fragmentation. The assays were carried out in Tris-HCL buffer (pH 7.8, 50 mM) containing 20 mM KF, 1 mM SAM and induced cells or supernatant or cell debris of cell fragmentation at equal quantity for 1 h at 37 °C.
Figure 5

Stability of whole-cell catalyst The assays were carried out in Tris-HCl buffer (pH 7.8, 50 mM) containing 200 mM KF, 1 mM SAM and 8 OD600 induced cells of INP-Faa or cell-free extracts of Faa at equal quantity for 2 h at 37 °C.
Figure 6

Effects of surface display systems on host growth The cells were cultured at 37 °C in LB broth for about 2h, then 0.2 mM IPTG was added for protein expression at 30 °C. 2.6 Synthesis of 5'-FDA by INP-Faa-Expressing Cells

Figure 7

Accumulation of 5'-FDA in whole-cell catalysis

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