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Catalytic Transformation of HODAs Using an Efficient *meta*-Cleavage Product Hydrolase-Spore Surface Display System

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Abstract

The accumulation of 2-hydroxy-6-oxohexa-2,4-dienoic acids (HODAs) in the process of aromatics transformation will hinder the mineralization rate. In this study, a novel type of biocatalyst, *meta*-cleavage product (MCP) hydrolase (MfphA and BphD) displayed on the surface of *Bacillus subtilis* 168 spores, was developed for the transformation of HODAs. The successful display of CotG-MfphA and CotG-BphD fusion protein on the surface of spore were confirmed by western blot analysis and activity measurement. The optimal transformation conditions by spore surface-displayed MfphA and BphD were found to be 70 ^{o}C and pH 7. The thermal and pH stability analysis exhibited that spore surface-displayed MfphA and BphD were stable and retained more than 80% of relative activities even at 80 ^{o}C and pH 10. Meanwhile, recycling experiments showed that the conversion percentage of HODA by surface-displayed MfphA and BphD were not significantly decreased throughout the reutilization process, which still retained 45% and 70% at the tenth cycle, respectively. To the best of our knowledge, this is the first report concerning the *B. subtilis* 168 spore surface-displayed MCP hydrolases. The high activities and good recycle performance suggested that this novel biocatalyst system could serve as a suitable alternative for HODAs transformation.

Keywords: Surface display system; *meta*-Cleavage product hydrolase; 2-Hydroxy-6-oxohexa-2,4-dienoic acid (HODA); Catalytic transformation

1. Introduction

2-Hydroxy-6-oxohexa-2,4-dienoic acid (HODA) and substituted HODAs are the *meta*-cleavage products in the microbial metabolic pathway of aromatic compounds such as catechols and polychlorinated biphenyls [1,2]. Many studies reported that the HODAs accumulation occurred in the transformation process of polychlorinated biphenyls, which could limit the aromatics mineralization process [3,4]. Thus, it is desirable to remove the HODAs from the polluted environments. Several *meta*-cleavage product (MCP) hydrolases (EC 3.7.1.8) that catalyzed hydrolytic reaction of C5-C6 bond cleavage of HODAs have been reported, such as BphD from *Burkholderia cepacia* LB400, BphD from *Rhodococcus* sp. RHA1, MhpC from *Escherichia coli* W3110, MfphA and BphD from *Dyella ginsengisoli* LA-4 [2,5-9]. The research on the transformation of HODAs was generally performed with purified MCP hydrolases or whole cells as biocatalysts [2,7,10]. However, the purification of enzymes is time-consuming and costly. Furthermore, purified enzymes are unstable for harsh environment and easily lose their activity [11]. In addition to the HODAs biodegradation process, MCP hydrolases also show potential application in fine chemical industry field due to their versatile functions such as hydroxamic acid formation, carbon-carbon bond formation, and hydrolysis of esters and thioesters [8,12]. Therefore, developing a novel biocatalytic system for MCP hydrolases is highly recommended for both pollutants degradation and fine chemicals production purposes.

Recently, surface display has emerged as an important technology, which has a wide range of biotechnological applications, including the development of vaccines, peptide and antibody libraries, bioremediation, biocatalysis, and biosensing [13-17]. Among numerous systems that have been employed in surface display, bacterial spore surface display systems have been widely used due to

the advantages of high stability and their robust resistance to heat, radiation and chemicals in a harsh environment [18,19]. Spores of *Bacillus* species, including *B. subtilis*, *B. clausii*, *B. coagulans*, *B. cereus*, and *B. natto*, are generally recognized as safe bioresources and used as additives in food, oral bacteriotherapy and bacterioprophylaxis [18]. Among all the *Bacillus* species, *B. subtilis* is widely studied for surface display due to the detailed knowledge of its spore structure and availability, as well as the mature genetic manipulation [20,21]. *B. subtilis* spores are surrounded by a coat, which is a proteinaceous structure with two layers and composed of at least 20 polypeptides [22]. Of these, CotB, CotC and CotG have successfully been used as anchoring motifs for display of either antigens or bioactive molecules such as Neu5Ac aldolase, β-galactosidase, and NADPH-cytochrome P450 oxidoreductase [11,12,23,24]. However, the MCP hydrolases displayed on the surface of spores have not been investigated as far as we know.

In this work, a novel surface display system based on *B. subtilis* 168 for MCP hydrolases using CotG as an anchoring motif was constructed, and the transformation characteristics of HODAs have been investigated. Furthermore, the thermal stability, pH stability and reusability of the surface display system were also studied, which displayed higher stability/reusability than purified and SBA-15 immobilized enzymes. Our work highlights the potential application of *B. subtilis* surface display system on the transformation of HODAs.

2. Materials and methods

2.1 Chemicals

HODA, 5-methyl-HODA, 5-fluoro-HODA, 5-chloro-HODA, and 5-bromo-HODA were enzymatically synthesized using BphC as previously reported [25]. Catechol, 4-methylcatechol, 4-fluorocatechol, 4-chlorocatechol, 4-bromocatechol, kanamycin and

isopropyl-β-D-thiogalactopyranoside (IPTG) were purchased from J&K Scientific Ltd. (China). *Hin*d III, *Spe* I and *Xho* I were purchased from TaKaRa (Dalian, China), which were used according to the instructions of the manufacturer. The other chemicals were of analytical grade.

2.2 Bacterial strain and growth condition

B. subtilis 168 was cultivated in glucose-yeast extract-salt (GYS) medium as previous described [11]. After cultivation in GYS medium at 37 °C for 24 h, the spores and sporangial cells of *B. subtilis* 168 with recombinant plasmids were harvested by centrifugation and resuspended with 0.1 M phosphate sodium buffer (pH 7.0). The purification of spore was carried out as previously described without phenylmethylsulfonyl fluoride (PMSF) [11].

2.3 Plasmids and strain construction

To display *mfphA* and *bphD* on the surface of *B. subtilis* 168 spores, we constructed two genetic fusions: *cotG-mfphA* and *cotG-bphD*. The DNA fragment with the *cotG* promoter and structure gene from the genome of *B. subtilis* 168 was amplified by primers P1 and P2, digested with *Hin*d III and *Spe* I, and ligated into pEasy-Blunt cloning vector to generate the plasmid pEasy-Blunt-*cotG*. The *mfphA* gene was amplified using primers P3 and P4 using plasmid pEBLX-*mfphA* as the template, digested with *Spe* I and *Xho* I, and cloned into the same restriction endonuclease sites of plasmid pEasy-Blunt-*cotG* to yield pEasy-Blunt-*cotG-mfphA* (a flexible linker [Gly-Gly-Gly-Gly-Ser] was inserted at the C terminus of the *cotG* structural gene product). The *bphD* gene was amplified using primers P5 and P6 with plasmid pET28a-*bphD* as the template, and digested as described with pEasy-Blunt-*cotG-mfphA* to yield pEasy-Blunt-*cotG-bphD*.

The fusion genes *cotG-mfphA* and *cotG-bphD* were digested with *Hin*d III and *Xho* I, and inserted into the shuttle vector pEB03 using the same enzymes to yield recombinant plasmids

pEB03-*cotG-mfphA* and pEB03-*cotG-bphD*. The recombinant plasmids pEB03-*cotG-mfphA* and pEB03-*cotG-bphD* were then separately transformed into *B. subtilis* 168 by electroporation transformation [26]. Spectinomycin (50 μg/mL) was added for selection of recombinant *B. subtilis* 168 harboring shuttle plasmid pEB03-*cotG-mfphA* or pEB03-*cotG-bphD*.

2.4 Western blot analysis

Spore coat protein was extracted from 100 μL purified spore suspension (1×10^{10} spores/mL) by sodium dodecyl sulfate (SDS)-dithiothreitol (DTT) treatment at 65°C [27]. The western blot analysis was performed using standard protocols [24]. The penta-His antibody (TaKaRa, Dalian, China) and HRP-rabbit anti-mouse IgG (TaKaRa, Dalian, China) were used for immunodetection of the fusion protein. The color reaction of horseradish peroxidase was done according to the manufacturer's instructions.

2.5 Activity assays of spore surface-displayed MfphA and BphD

The standard activity assays were performed at 30 ^{o}C in a total volume of 2.0 mL, consisting of phosphate sodium buffer (0.1 M, pH 7.0), HODAs (40 μM), and the purified spore suspension containing MfphA/BphD. The reaction was initiated by adding the purified spore suspension containing MfphA/BphD. After 10 min incubation, the reaction mixture was centrifuged (8,739×*g*, 15 min, 20 ^{o}C). The residual concentration of HODAs was measured by UV-vis spectrophotometer (JASCO V-560, Japan) at specific wavelengths (HODA at 375 nm, 5-methyl-HODA at 381 nm, 5-fluoro-HODA at 385 nm, 5-chloro-HODA at 380 nm, and 5-bromo-HODA at 380 nm). Extinction coefficients of the yellow-colored HODAs were determined as described by Zhou et al. [9]. One unit of enzyme activity was defined as the amount of enzyme required to consume 1 μmol of the HODA

per minute. The number of spores was calculated by direct counting with a Burker chamber under an optical microscope.

2.6 Biotransformation of HODA by spore surface-displayed MfphA and BphD

To optimize the biotransformation conditions, 10 mL of reaction mixture in a 50 mL Erlenmeyer flask was used with variations as follows. Both the concentrations of spore surface-displayed MfphA and BphD were 2 U/mL. Temperatures ranged from 30 to 80 ^{o}C. The buffer solutions with different pH used in this study were 0.1 mM PBS (pH 7) and 0.2 mM Tris-HCl (pH 8-10). Conversion rate (%) was calculated the following equation:

$$\text{Conversion rate (\%)} = \frac{A_0 - A_1}{A_0} \times 100\%$$

where A_0 and A_1 represent the initial and residual concentration of HODAs, respectively. All of the experiments were performed in duplicates and the average values were used in calculations.

2.7 Stability of spore surface-displayed MfphA and BphD

The thermal stabilities of spore surface-displayed MfphA and BphD were determined by incubating the reaction mixtures at 30, 40, 50, 60, 70 and 80 ^{o}C for 10 min at pH 7, and then residual activities against HODA were determined. The variable pH stability studies were performed by incubating the surface-displayed MfphA and BphD at different buffers (pH from 7 to 10) for 60 min at 70 ^{o}C. Reusability experiments were conducted by reusing the spore surface-displayed MfphA and BphD for ten cycles. After each biotransformation batch, the reaction mixtures were centrifuged and the supernatants were removed.

3. Results

3.1 Construction of surface display system

To construct the surface display system, the outer coat protein CotG (23.9 kDa) expressed at the coat of *B. subtilis* 168 was used as an anchoring motif for the display of MfphA and BphD. A flexible linker composed of 5 amino acids (Gly-Gly-Gly-Gly-Ser) was inserted between the C terminus of *cotG* gene product and the N terminus of *mfphA* and *bphD* gene product to obtain the *cotG-mfphA* and *cotG-bphD* fusion gene, respectively. For the detection of surface displayed protein, the genes *mfphA* and *bphD* fused to a DNA fragment encoding six histidines (His_6) at N terminus were amplified, respectively. The His_6 was served as an epitope for the rabbit anti-His probe antibody. A *cotG*-specific promoter was employed in the surface display system as previously reported [10]. Western blot analysis of spore coat proteins purified from recombinant strains revealed the presence of a 58 kDa band (lane 3) which reacted with rabbit anti-His probe antibody (Figure 1). The molecular weight of CotG-MfphA and CotG-BphD was about 58 kDa. Thus, the 58 kDa bands in lane 3 could be the fusion protein of CotG-MfphA and CotG-BphD with a His_6 at N terminus. Meanwhile, no signal was detected in the spores of *B. subtilis* 168 (lane 2), indicating that there was no His_6 in the proteins of *B. subtilis* 168. Thus, it was confirmed that MfphA and BphD were successfully displayed on the spore surface of *B. subtilis* 168.

In order to further investigate if surface display system was successfully constructed, the activities of spore surface-displayed MfphA and BphD against HODAs were also examined. The reaction scheme of HODAs by spore surface-displayed MfphA and BphD was shown in Figure 2. Since not all proteins were extractable from the *B. subtilis* spore coat, the units of MfphA and BphD were normalized with respect to the numbers of spores. The spores not harboring the recombinant plasmid had no activities (data not shown). As seen in Table 2, both spore surface-displayed MfphA and BphD showed high activities against HODAs. BphD exhibited the highest activity ($1.32\ 10^{-8}$ U

spore^{-1}) against HODA in 10 min which was 1.02, 1.11, 1.08, and 1.05-fold higher than that of 5-methyl-HODA, 5-fluoro-HODA, 5-chloro-HODA, and 5-bromo-HODA, respectively. A similar trend was also observed for MfphA, which was consistent with the free MfphA expressing in *E. coli* BL21 (DE3) [9]. Moreover, the activity of BphD against HODA was 1.29-fold higher than that of MfphA. These results indicated that both MfphA and BphD were successfully displayed in an active form.

3.2 The effects of temperature and pH on HODAs transformation

To investigate the HODA transformation characterizations by surface-displayed MfphA and BphD, the effects of temperature and pH on HODAs transformation were tested. As shown in Figure 3 and 4, using HODA (40 μM) as substrate, the highest conversion efficiency of HODA (40 μM) was detected at 70 ^{o}C and pH 7 by both MfphA and BphD, corresponding to 70% and 92%, respectively. In the temperature ranging from 30 to 80 ^{o}C, the conversion efficiencies by MfphA and BphD were more than 60% and 50%, respectively. Within the pH range of 7 to 10, the conversion efficiencies of HODA by MfphA and BphD were 60% and 80%, respectively. These results indicated that the spore surface-displayed MfphA and BphD exhibited high biotransformation capability towards HODA over wide ranges of temperature and pH.

3.3 Stability of spore surface-displayed MfphA and BphD

The stability of surface display system is one of the most important parameters for application [28]. The stability of spore surface-displayed MfphA and BphD was examined. Figure 5 showed the residual relative activities of the MfphA and BphD after 60 min incubation at different temperatures. The surface-displayed MfphA and BphD were stable at 30 ^{o}C, 40 ^{o}C, 50 ^{o}C and 60 ^{o}C. Both of the surface-displayed enzymes maintained more than 90% of corresponding initial activities at 30 ^{o}C in

60 min. Surprisingly, even the temperature was increased to 70 °C and 80 °C, the spore surface-displayed MfphA and BphD did not show much loss of activity (less than 20%). As shown in Figure 6, within the pH range of 7 to 10, both the surface-displayed MfphA and BphD were stable and retained a relative activity of more than 85% in 60 min.

3.4 Reusability of spore surface-displayed MfphA and BphD

The reusability of the surface-displayed protein was also an important index during the process of application. To investigate the reusability of spore surface-displayed MfphA and BphD, the reactions were conducted under the optimal transformation conditions (Figure 7). After each reaction cycle, the spore surface-displayed MfphA and BphD were isolated by centrifugation and washed with 0.1 M phosphate sodium buffer (pH 7.0). The spore surface-displayed MfphA and BphD maintained high relative activities towards HODA within 3 reaction cycles. From the fourth to the tenth cycle, the catalytic efficiencies of surface-displayed MfphA and BphD were gradually decreased to 45% and 70% by MfphA and BphD, respectively, at the end of tenth cycle.

4. Discussion

The *meta*-cleavage product (MCP) hydrolases (EC 3.7.1.9), which are identified in the degradation pathway of aromatic compounds, can catalyze the rare carbon-carbon bond cleavage of the 2-hydroxy-6-oxohexa-2,4-dienoate (HODA) [2,5-9]. Moreover, they could also catalysis C-C bond formation in organic media [12]. In previous studies, free MCP hydrolases, immobilized MCP hydrolases, and whole cells containing MCP hydrolases were used for HODAs degradation [2,7,10]. In recent years, biological surface display has been used in various areas. Many enzymes such as levansucrase, organophosphorous hydrolase, and lipase have been displayed on the cell surface in Gram-negative bacteria [13,29,30]. In contrast to Gram-negative bacteria, the cell surface display in

Gram-positive bacteria enjoyed some advantages, which made the bacteria suitable for variety of biotechnological applications [31]. However, only a few existing studies of the cell display of contaminate-specific proteins onto Gram-negative bacteria were available [11,32]. Besides, the cell display in Gram-negative bacteria mainly focused on biosynthesis and heavy metal ions removal [11,32,33]. Little information on the biodegradation by displaying contaminate-specific proteins in Gram-negative bacteria has been reported. In this study, we introduced a novel surface display system using *B. subtilis* 168 for displaying MCP hydrolases and investigated the catalytic capability of this system using HODA and substituted HODAs as substrates. To our best knowledge, this was the first report about displaying MCP hydrolases on the surface of bacterial spores to degrade HODAs.

CotG, a 23.9-kDa outer coat protein, was a convincing example of a coat protein that resulted in the display of functional enzymes [11,12]. In this study, we used CotG as the anchoring motif for the display of MCP hydrolases on the surface of *B. subtilis* 168 spores. In the previous study, the strategies of genetic construction to display the heterogenous protein on spores were utilizing low-copy-number expression vector, thus low activity of the displayed enzymes were generally obtained [11]. For example, Xu et al., compared the expression vectors pHP13 (low-copy-number) and pEB03 (high-copy-number) for the displaying Neu5Ac aldolase, indicating that the activity of Neu5Ac aldolase obtained using pEB03 as the expression vector was 6-fold higher than that obtained using pHP13 as the expression vector [11]. Thus, the vector pEB03 with high-copy-number has been used as the expression vector for displaying the fusion genes *cotG-mfphA* and *cotG-bphD* to ensure effective expression of the enzymes during the sporulation process. This surface display system was similar with that reported for displaying Neu5Ac aldolase on the surface of *B. subtilis* WB 600

spores [11]. A 58 kDa fusion protein obtained by western blot analysis confirmed that MfphA and BphD were successfully displayed on the spore surface of *B. subtilis* 168, due to the His_6 of this fusion protein reacted with rabbit anti-His probe antibody.

In previous studies, MfphA and BphD have been purified and immobilized on SBA-15 and carbon nanotubes [9,34]. Compared with these previous studies, spore surface-display based immobilization represented a promising alternative for HODAs degradation, because it excluded the purification and immobilization process. Meanwhile, the natural robustness of spores in the presence of heat, solvent, pH, oxidizing agents and salts has been also reported [35]. In our previous studies, the pH stability of the free and SBA-15 immobilized MfphA for HODA hydrolysis indicated that both of them retained less than 20% of relative activity at pH 8 [9]. However, both the spore surface-displayed MfphA and BphD were stable and retained more than 85% within the pH ranges (from 7 to 10). In the case of thermostabilities, the immobilized MfphA on SBA-15 retained 33% of its original activity at 50 ^{o}C [9]. Meanwhile, carbon nanotube immobilized BphD retained 40% of its original activity after incubation at 60 ^{o}C for 25 min [34]. In this work, the spore surface-displayed MfphA and BphD exhibited higher thermal resistance, which were very stable at mesophilic temperatures (from 30 to 50 ^{o}C) and retained above 90% of their initial activity. These results indicated that MfphA and BphD displayed on the surface of spores could enhance both thermal and pH stabilities. Generally speaking, thermostability of an enzyme is often accompanied with good performance under harsh conditions, as well as long-term survival under mild conditions [36]. As shown in Figure 3, although the optimal reaction temperature was around 70 ^{o}C, the surface-displayed MfphA and BphD also exhibited relatively high conversion percentage to HODA

at 30 °C. In addition, they also displayed the best thermostabilities at 30 °C. Therefore, it suggested that this novel biocatalyst system could serve as a suitable alternative for HODAs transformation.

The reusability of a biocatalyst is one of the most important factors to be considered in its industrial application. Spores have the characteristic of easy purification by centrifugation or filtration. Therefore, the spore surface-displayed MfphA and BphD could be easily separated from the catalytic system to be used for circular catalysis process [11]. The transformation rates of HODA by MfphA and BphD were not significantly affected throughout the reutilization process, which still retained 45% and 70% transformation activities of HODA by MfphA and BphD at the tenth cycle, respectively. Previous studies indicated that the immobilized MfphA on SBA-15 retained 30% activity at tenth cycle because the MfphA was only adsorbed by relatively weak electrostatic interactions with the silica walls [9]. Surface-displayed Neu5Ac aldolase in the spores of *B. subtilis* WB 600 could be used for up to 3 reaction cycles without a significant decrease in the conversion rate [11]. Therefore, the spore-displayed enzymes could help to meet the ever-increasing industrial demand for preparation and stabilization of biocatalysts and may be generally applicable to numerous biocatalytic reactions.

5. Conclusion

Two novel systems using spore surface-displayed MfphA and BphD were constructed for the transformation of HODA. Both MfphA and BphD were successfully displayed in an active form on the spore surface of *B. subtilis* 168 according to the western blot analysis and activity measurement. The conditions of HODA biotransformation by spore surface-displayed MfphA and BphD were characterized. The thermal and pH stabilities of spore surface-displayed MfphA and BphD were both improved. Meanwhile, the spore surface-displayed MfphA and BphD could be reused up to 10 times

with 45% and 70% residual activities, respectively. This work will provide the potential application of spore surface display system in the fields of pharmaceuticals, fine chemicals, agrochemicals, and other demanding industries.

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1 Table 1 Strains, plasmids and primers used in this work

Strain, plasmid, primer	Description	Source
Strain		
Bacillus subtilis 168	Type strain	Bacillus Genetic Stock Center (BGSC)
Plasmid		
pEBLX-*mfphA*	pEBLX with gene *mfphA*	[7]
pET28a-*bphD*	pET28a with gene *bphD*	[7]
pEasy-Blunt	Cloning vector	Transgene
pEasy-Blunt-*cotG-mfphA*	pEasy-Blunt with fusion gene *cotG-mfphA*	This study
pEasy-Blunt-*cotG-bphD*	pEasy-Blunt with fusion gene *cotG-bphD*	This study
pEB03	High-copy-number *E. coli-B. subtilis* shuttle vector	[10]
pEB03-*cotG-mfphA*	pEB03 with fusion gene *cotG-mfphA*	This study
pEB03-*cotG-bphD*	pEB03 with fusion gene *cotG-bphD*	This study
Primers		
P1 [a]	5′-GCCTTT*AAGCTT*AGTGTCCCTAGCTCCGAG-3′	This study
P2 [a,b]	5′-CTATTG*ACTAGT*TGAACCCCCACCTCCTTTGTATTTCTTTTTGACTA-3′	This study
P3 [a,c]	5′-CTCC*ACTAGT***CATCATCATCATCATCACAT**GACGGCACTTACCGA-3′	This study
P4 [a]	5′-CGAT*CTCGAG*TCATTCATGCTTGAGAAAAT-3′	This study
P5 [a,c]	5′-GCCCG*ACTAGT***CATCATCATCATCATCACAT**GTCCAAGCCAGATA-3′	This study
P6 [a]	5′-AATT*CTCGAG*TCAGGGCAGCGGCAGCGGTT-3′	This study

2 [a] The italicized letters indicate the introduction of restriction sites.

3 [b] The underlined letters indicate the introduction of a flexible linker at the C terminus of the *cotG* structural gene product.

4 [c] The bold letters indicate the introduction of His-tag at the C terminus of the *mfphA* or *bphD* structural gene product.

5

1 Table 2 The activity of MfphA and BphD displayed on the spore surface

Substrate	MfphA (10^{-8} U spore^{-1})	BphD (10^{-8} U spore^{-1})
HODA	1.02 ± 0.08	1.32 ± 0.24
5-methyl-HODA	1.00 ± 0.07	1.29 ± 0.15
5-fluro-HODA	0.83 ± 0.04	1.19 ± 0.11
5-chloro-HODA	0.89 ± 0.05	1.22 ± 0.12
5-bromo-HODA	0.96 ± 0.06	1.26 ± 0.14

2

3

Figure Captions

Figure 1 The western blot analysis of recombinant spores expressing *cotG-mfphA* and *cotG-bphD* fusion proteins. (A) line 1, molecular mass standards; line 2, spores of *B. subtilis* 168; line 3, spores expressing *cotG-mfphA* fusion protein; line 4, Perfect Protein™ Marker. (B) line 1, molecular mass standards; line 2, spores of *B. subtilis* 168; line 3, spores expressing *cotG-bphD* fusion protein; line 4, Perfect Protein™ Marker.

Figure 2 The reaction scheme of HODAs by spore surface-displayed MfphA and BphD.

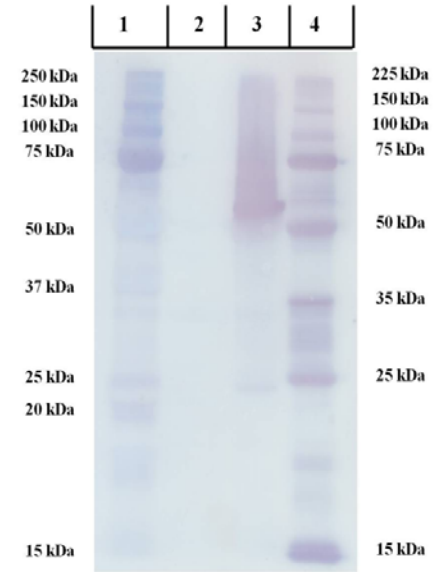
Figure 3 Effect of temperature on the biotransformation of HODA by spore surface-displayed MfphA (A) and BphD (B).

Figure 4 Effect of pH on the biotransformation of HODA by spore surface-displayed MfphA (A) and BphD (B).

Figure 5 Thermal stability of spore surface-displayed MfphA (A) and BphD (B). 30 °C (■); 40 °C (●); 50 °C (▲); 60 °C (▼); 70 °C (◆); 80 °C (◄).

Figure 6 The pH stability of spore surface-displayed MfphA (A) and BphD (B). pH 7 (■); pH 8 (▲); pH 9 (●); pH 10 (▼).

Figure 7 Reuse of spore surface-displayed MfphA (A) and BphD (B) for the biotransformation of HODA.



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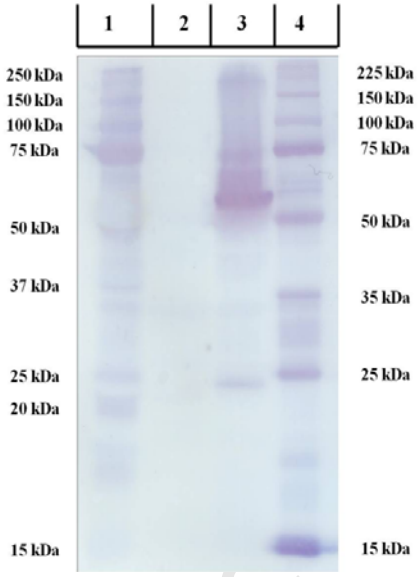

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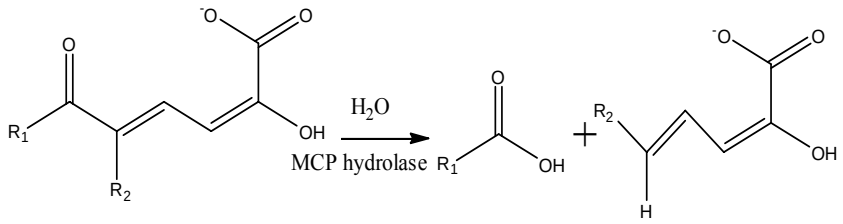
Figure 1

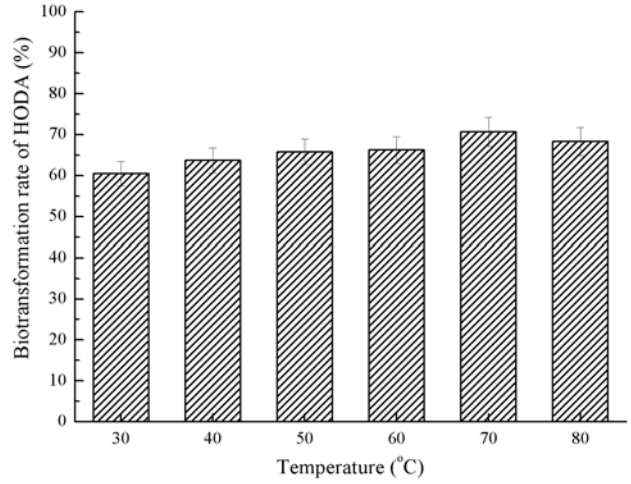
1



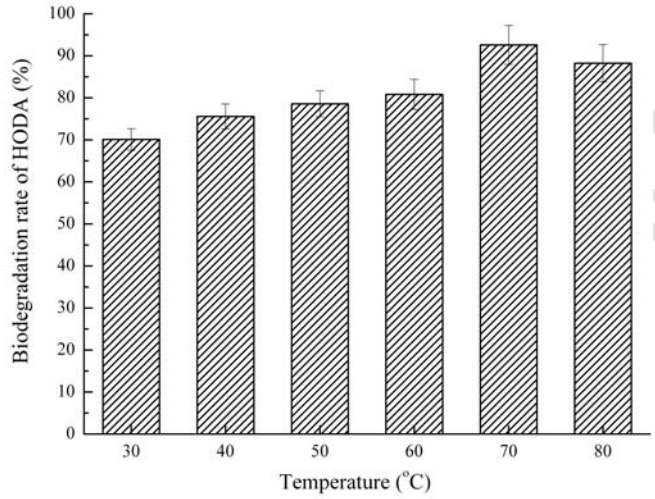
2

3 Figure 2

4

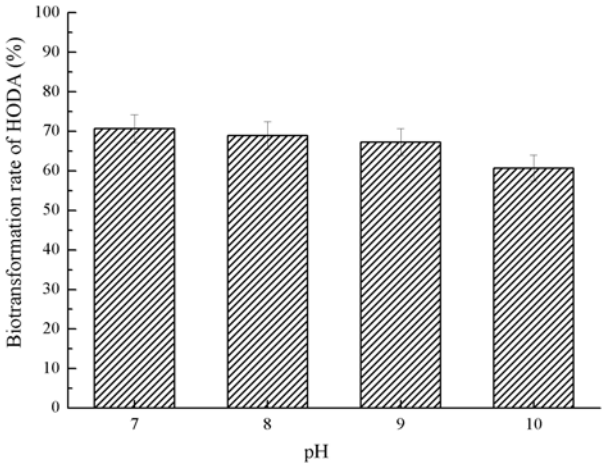


(A)



(B)

Figure 3



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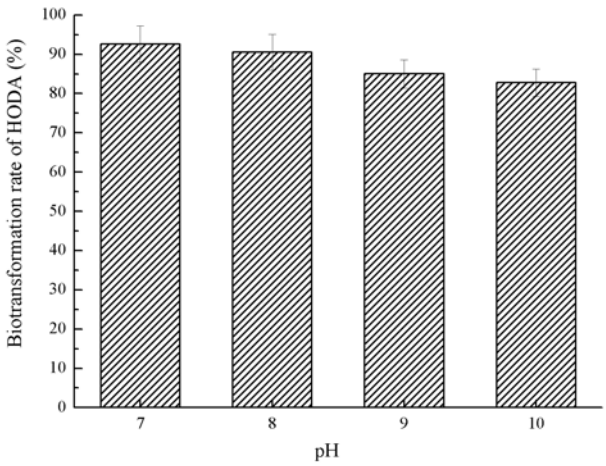
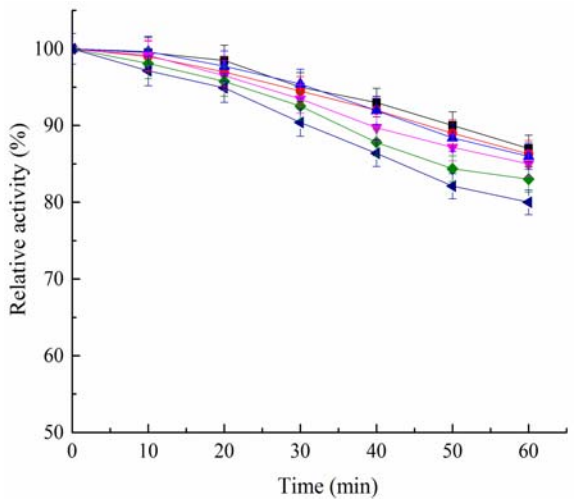


(B)

Figure 4



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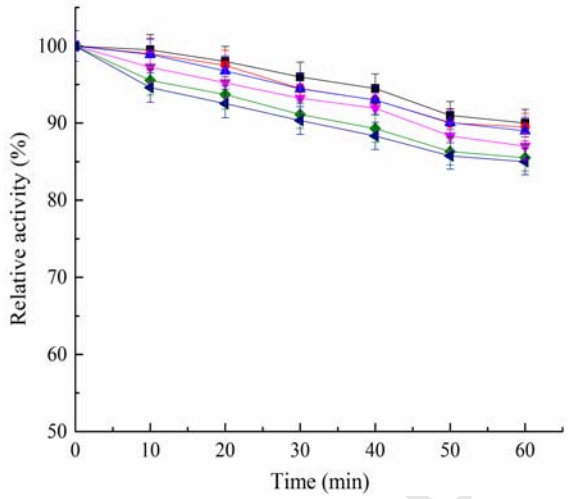
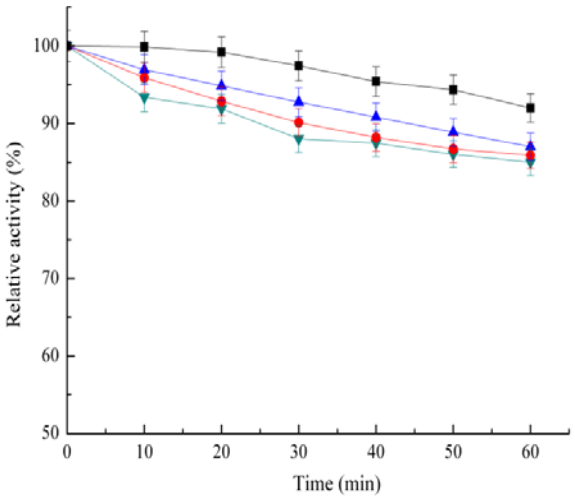


(B)

Figure 5



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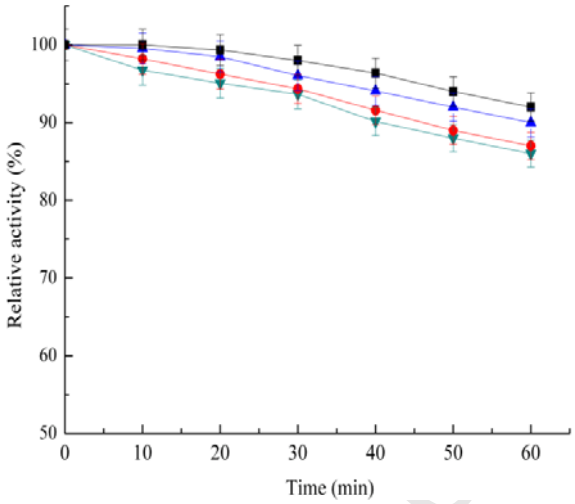

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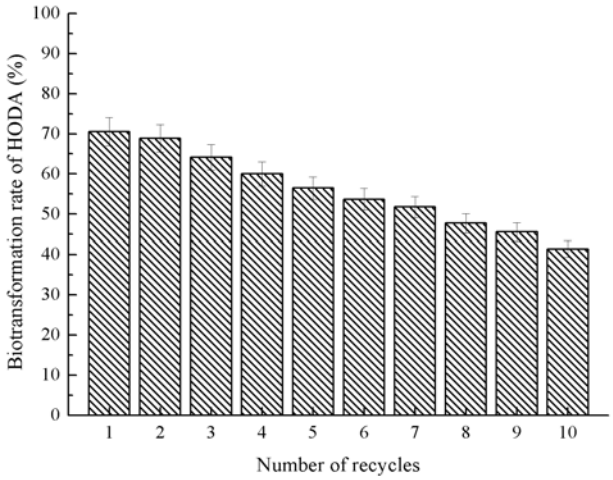
Figure 6



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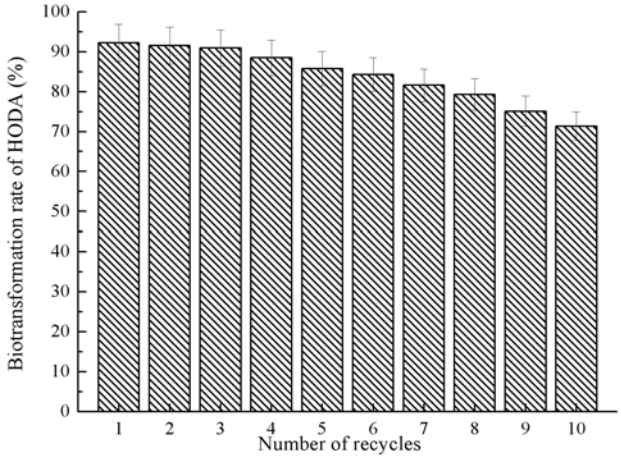


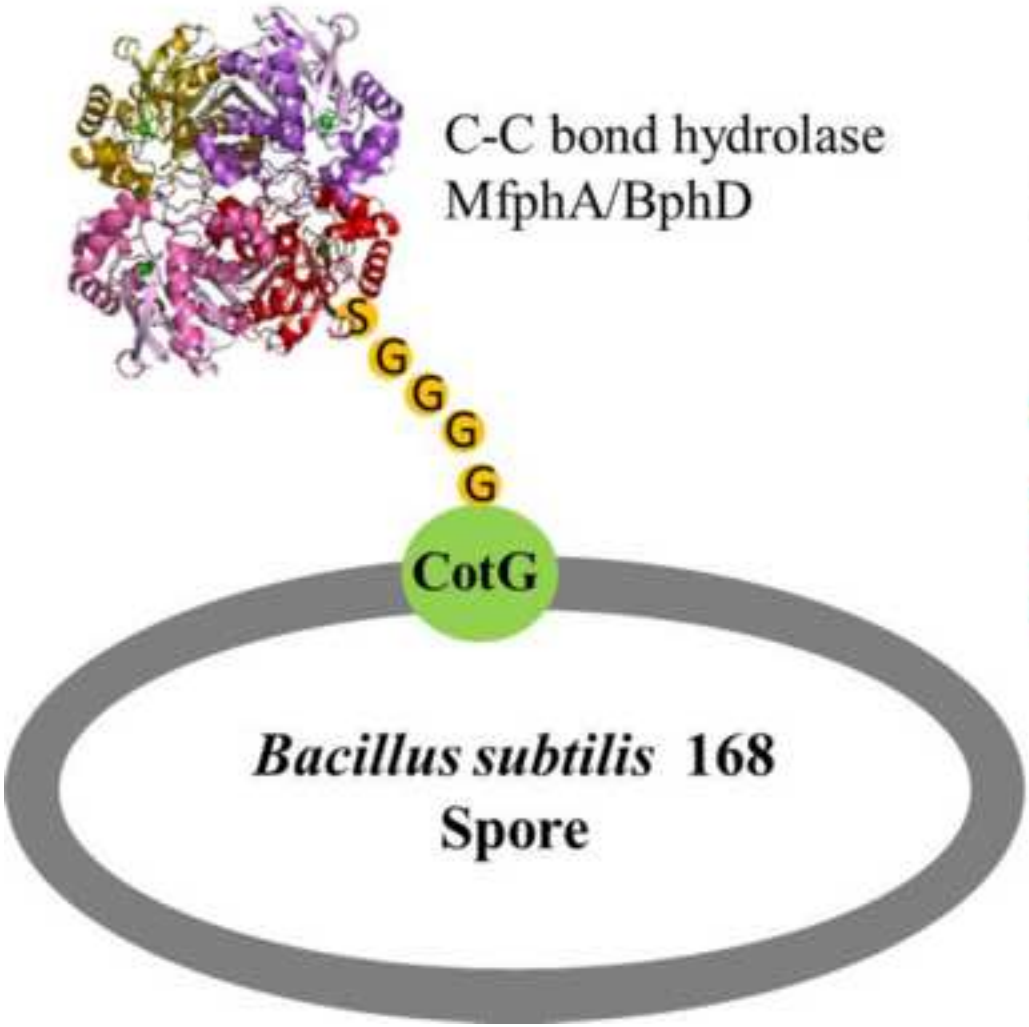
(B)

Figure 7

Highlight

- An effective *meta*-cleavage product hydrolase-spore surface display system was constructed.
- Thermostability, pH stability and reusability of BphD and MfphA were greatly improved by immobilization.
- Surface displayed BphD and MfphA possessed high activities on various substrates.

*Graphical Abstract (for review)



Surface display based biotransformation platform

- Highly active
- Highly thermal and pH resistance
- Good reusability (up to 10 cycles)