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Development of a tannase biocatalyst based on bio-imprinting for the production of propyl gallate by transesterification in organic media

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ABSTRACT

A bio-imprinting technique was applied to activate tannase in order to enhance its biocatalytic activity. Specifically, the effects of three bio-imprinting methods (i.e. substrate imprinting, pH imprinting, and interfacial imprinting) on the activating factor of tannase were investigated. The results show that bio-imprinting methods can activate tannase remarkably, and they were combined to develop a tannase biocatalyst with a 40% conversion rate of substrate, 100-fold higher than that of the control. This approach can be used to construct an effective way to produce propyl gallate as well as to exploit readily available tannic acid. The immobilized bio-imprinted tannase can catalyze the synthesis of propyl gallate from tannic acid by transesterification in organic media. This work not only presents an effective means of making use of various tannic acid-rich agro-forestry residues, but also broadens the field of applications of the bio-imprinting technique.

1. Introduction

Enzymes are well recognized as practical biocatalysts of immense potential and, in particular, are being increasingly used on an industrial scale for bio-transformation [1], preparation of fine chemicals, and synthesis of enantiopure pharmaceuticals [2–4]. In the future, more and more biological resources will be transformed into high value-added products by biocatalysts. These biocatalytic syntheses will replace conventional chemical syntheses, which often employ corrosive reagents and require high energy input.

Propyl gallate (PG) is a very important gallic acid ester usually used as an antioxidant in foods, cosmetics, hair products, adhesives, and the lubricants industry [5,6]. It has also been the focus of attention because of its possible pharmaceutical applications [7–10]. Most available propyl gallate is synthesized mainly from gallic acid by the conventional chemical method, which uses concentrated sulfuric acid as a chemical catalyst. It would be preferable to replace the chemical synthesis with a green biocatalytic method.

Tannase, tannin acyl hydrolase (E.C. 3.1.1.20), can not only hydrolyze the ester and depside bonds in tannic acid (TA) to release gallic acid and glucose, but can also catalyze the transesterification or esterification syntheses of gallic acid esters [11] in anhydrous media. Due to the presence of many phenolic hydroxyl groups, TA is a specific substrate of tannase. The conversions of TA to gallic acid [12] or propyl gallate [13] by tannase have recently been reported. The bioconversion of TA to propyl gallate (shown in Scheme 1) [13,14] entails fewer procedures and lower cost than the conventional chemical synthesis. However, the biocatalytic activity of natural tannase in the transesterification is so low that the conversion ratio of substrate (CR) prevents it from being a viable industrial application. To improve the CR, most previous studies have focused on the immobilization of tannase [12,13,15]. Notwithstanding, the CR of immobilized tannase is still restricted by the limitations of the natural enzyme structure.

A bio-imprinting technique is, in general, based on modifying the conformation of an enzyme by changing the pH or by adding substrate or corresponding analogs, surfactants, and other entities to the aqueous phase. The aim is to make the enzyme adopt a conformation with specific nano-sized cavities that enable precise coupling between enzyme and substrate. These cavities can be retained when the enzyme is transferred from aqueous media to anhydrous media due to their dynamical rigidity in the organic phase [16]. An imprinted enzyme may have high catalytic specificity, affinity, and stability [17]. Therefore, the technique can break through limitations in the natural enzyme structure. Thus far, bio-imprinting techniques have been used to improve several biocatalysts. For example, the biocatalytic activities of chymotrypsin [2] and lipase [18–20] were improved based on bio-imprinting with the substrate (i.e. substrate imprinting). Lipase was also modified based on bio-imprinting with the surfactant (i.e. interfacial

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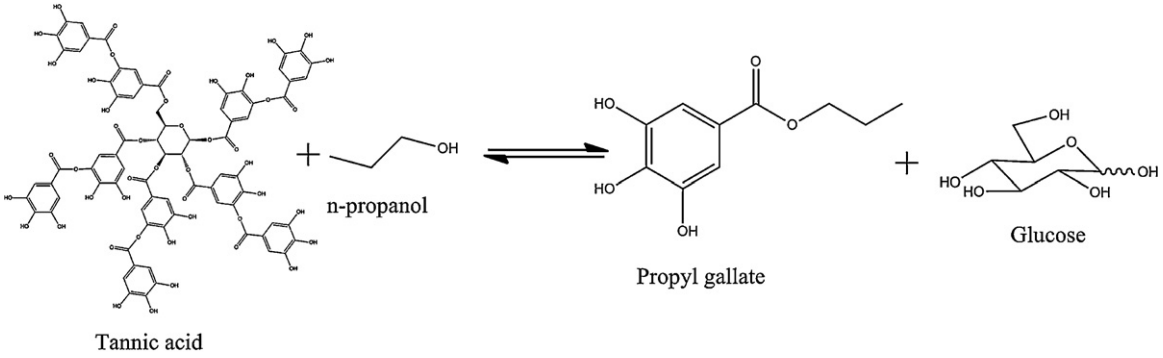

Scheme 1. Transesterification reaction to form propyl gallate from tannic acid.

imprinting) [4,21–23]. An enzyme with a 'pH memory' can be activated by pH tuning (i.e. pH imprinting). Furthermore, the strategy of combinational imprinting has also been applied to enhance the catalytic performance of lipase [24,25]. These improved biocatalysts exhibit surprisingly high activities compared to the corresponding controls. However, improvements in tannase activity based on a bio-imprinting technique have not been reported previously.

In this study, the bio-imprinting technique has been used to enhance the transesterification activity of tannase. Three bio-imprinting methods (i.e. substrate imprinting, pH imprinting, and interfacial imprinting) were investigated to develop a promising biocatalyst that could be used to synthesize propyl gallate from TA in organic media. This report explores an effective way to biotransform TA coupled with a practical method for synthesis of propyl gallate on an industrial scale.

2. Materials and methods

2.1. Reagents

Tannase was purchased from Jinan Huazuan trading Co., Ltd., China. Commercial TA ($C_{76}H_{52}O_{46}$); GA monohydrate ($C_7H_6O_5{\cdot}H_2O$), Triton X-100, citric acid monohydrate, and celite were purchased from Sinopharm Chemical Reagent Co., Ltd. (SCRC), China. TA, GA, and citric acid were of analytical grade. All other solvents and reagents were obtained commercially and were of analytical grade.

2.2. Bio-imprinting

50 mg TA and 16 IU tannase were uniformly dissolved in 5 mL of 90 mM citrate acid buffer at pH 6.0, followed by the addition of 0.15 mL of 0.3% Triton X-100 surfactant and 0.5 g celite, and the mixture was frozen at −20 °C overnight. Finally, the sample was lyophilized in a freeze dryer (VirTis, SP Scientific USA) to a powder, and then stored at −20 °C until use. The bio-imprinting process and parameters were the same throughout unless stated otherwise. The activating factor (AF) refers to the enhancement in CR of the bio-imprinted tannase relative to the corresponding control, and it is calculated as the ratio of the CR of imprinted tannase to that of the control. The controls were prepared according to different requirements.

2.3. Transesterification synthesis of propyl gallate

An aliquot of imprinted biocatalyst containing 0.05 g TA was added to a 25-mL flask with approximately 10 mL of solvent mixture composed of 1 mL of n-propanol, 9 mL of hexane, and 100 μL of distilled water. The transesterification reaction was conducted at 40 °C and 200 rpm for 24 h. CR is the mole ratio of 10% propyl gallate formed to TA in the initial reaction mixture.

2.4. Assay of propyl gallate

Samples were analyzed by HPLC (Waters 600, Waters, USA) equipped with a Waters 996 photodiode array detector (PDAD) and Phenomenex C18 column (250 mm × 4.60 mm, 4 μm). The mobile phase consisted of 50 mL of methanol, 50 mL of water, and 10 μL of acetic acid. The operating temperature was maintained at 35 °C. 20 μL of sample were injected and peaks were detected at 274 nm with propyl gallate as the control at a flow rate of 1 mL/min. Sample preparation was subjected to the following protocol: (1) 200 μL reaction solution were diluted 10-fold with methyl alcohol (HPLC grade), (2) the diluents were filtered using an organic membrane with a pore size of 0.45 μm. All experiments were performed in duplicate unless stated otherwise.

3. Results and discussion

3.1. Effect of tannic acid on the activity of imprinted tannase

Fig. 1 shows that the TA-imprinted tannase (TE) has a lower CR than the control (non-imprinted tannase with distilled water as solvent, Ew). However, when TE and Ew were individually fixed on the celite, the immobilized TE (TEi) achieved a 4.86-fold enhancement in CR compared to the immobilized Ew (Ei). When citric acid buffer replaced the distilled water in the TE solution, the buffer-TA-imprinted tannase (BTE) attained a CR of 7.56%, approximately 150-fold higher than that of TE. Further experiments showed that TA addition results in a steep reduction in the pH value of distilled water, whereas it hardly changes the pH value of the citric acid buffer (data not shown). The effect of the quantity of TA added on the AF of tannase is shown in Fig. 2a. When the mass of TA was below 0.04 g, the AF increased with TA mass. When the mass of TA was 0.04 g, the imprinted tannase achieved a CR of 5.7% and an AF of 1.4.

Comparison of the CR values of TE and Ew indicates that TA appears to inactivate tannase. As shown by further experiments, TA inhibits the biocatalytic activity of tannase by changing the pH value in the hydrated shell around the enzyme. In contrast, comparison of the CR values of immobilized TE and immobilized Ew implies that TA can activate immobilized tannase, significantly enhancing the biocatalytic activity of TE by reducing molecular aggregation. The aggregation results from the interaction of TA with free tannase. The difference in CR between BTE and TE suggests that more efficient substrate imprinting may require a favorable conformation modulated by pH imprinting. Fig. 2a indicates that TA substantially improves the biocatalytic activity of tannase, and the AF of tannase is affected by the TA concentration. This may

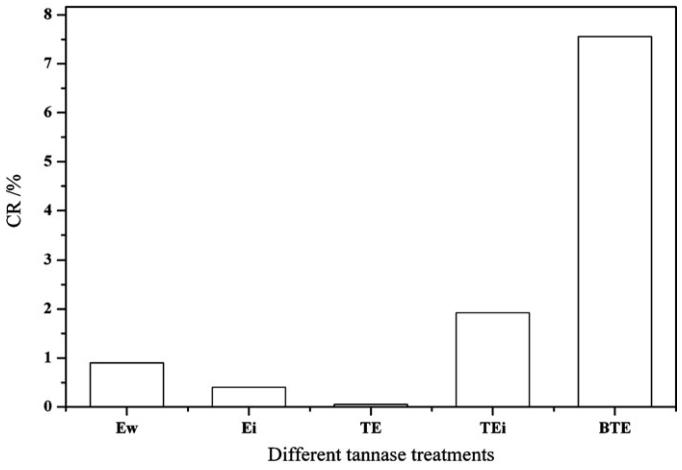

Fig. 1. Comparison of the effects of different treatments on the CR of tannase. Ew: The non-imprinted tannase, 16 IU tannase were uniformly dissolved in 5 mL distilled water, and then the solution was frozen overnight and lyophilized for 24 h. TE: The TA-imprinted tannase, 16 IU tannase and 0.05 g TA were uniformly dissolved in 5 mL distilled water, and then the mixture was frozen overnight and lyophilized for 24 h. BTE: The buffer- and TA-imprinted tannase, 16 IU tannase and 0.05 g TA were uniformly dissolved in 5 mL 50 mM pH 5.5 citric acid buffer, and then the mixture was frozen overnight and lyophilized for 24 h. Ei and TEi are Ew and TE immobilized on celite, respectively. These tannases were applied to catalyze the synthesis of propyl gallate by transesterification. The effects of different treatments on the biocatalytic activity of tannase have been estimated by comparing the CRs. The catalytic reaction conditions were the same as those given in Section 2.

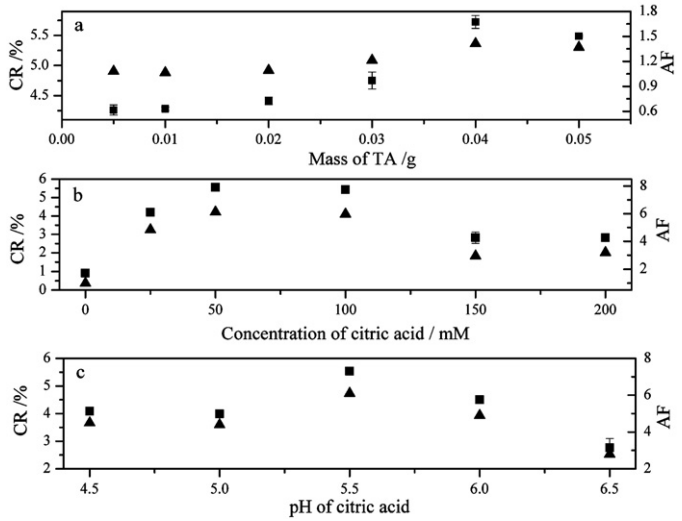

Fig. 2. Effects of TA and pH on the biocatalytic activity of tannase. (a) With 50 mM pH 5.5 citric acid buffer as solvent, 16 IU tannase were imprinted with 0.005–0.05 g TA, and the control CR was 4%. (b) With 0.04 g TA as template, 16 IU tannase were imprinted with 5 mL 0–200 mM citric acid at pH 5.5, and the control CR was 0.9%. (c) With 0.04 g TA as template, 16 IU tannase were imprinted with 5 mL pH 4.5–6.5 citric acid at a concentration of 50 mM, and the control CR was also 0.9%. Substrate amounts in each test were 0.05 g in the catalytic reaction system. All of the tannases were applied to catalyze the synthesis of propyl gallate by transesterification. The effects of TA and pH on the biocatalytic activity of tannase were estimated by comparing their CRs and AFs. The catalytic reaction conditions were the same as listed in Section 2. Squares and triangles refer to CR and AF, respectively (the same as below).

be because the coupling of TA with tannase induces the enzyme to form an efficient and specialized active center with the assistance of pH imprinting, and to construct a transition complex of substrate-enzyme at the appropriate concentration of TA (shown in step 3 in Fig. 3) [26]. However, as the concentration of TA increases, TE becomes so 'sticky' that it prevents diffusion of the substrate and product into and out of the complex, respectively,

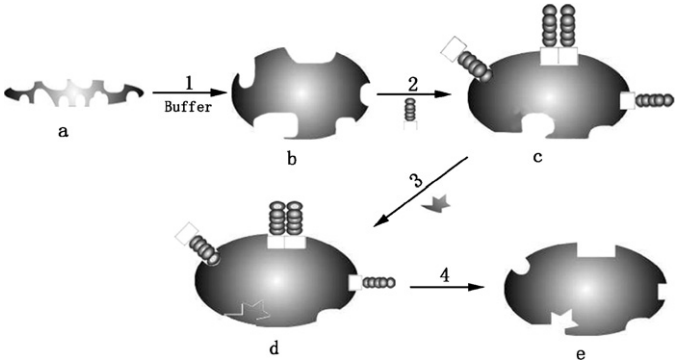

Fig. 3. Schematic diagram of the three bio-imprinting processes. (a) Natural tannase, (b) pH-imprinted tannase, (c) pH- and surfactant-imprinted tannase, (d) combinational bio-imprinted tannase with pH-, surfactant- and TA-imprinting and (e) unloaded bio-imprinted tannase with high biocatalytic activity. Step 1: pH-imprinting step, the molecular conformation of natural tannase changes with variations in the ionic state of tannase caused by bio-imprinting with 90 mM pH 5.5 citric acid buffer. Step 2: Interfacial-imprinting step, binding of surfactant to tannase further stabilizes the molecular conformation of buffer-imprinted tannase, and improves the interfacial qualities of the enzyme. Step 3: Substrate-imprinting step, the specific catalytic site of tannase and the transition complex of enzyme–substrate are introduced with coupling of TA and tannase. Step 4: The removal step, unloaded complex bio-imprinted tannase is formed through removal of all ligands in organic solution.

and thus the apparent activity is reduced. Previous studies have reported that some enzymes are hyper activated by a substrate analog on the basis of bio-imprinting [20,27–29]. In addition, some researchers [18–20] have directly used the substrate as the imprinting template. However, to our knowledge, substrate imprinting has not been reported before as a means of improving the biocatalytic activity of tannase. From the above discussion, it appears that TA can activate tannase significantly. At the same time, it meets the requirements (i.e. resemblance to substrate or competitive inhibitor and solubility in the aqueous phase [29]) of a favorable imprinting template. If TA can be used as the template in bio-imprinting, the step of removing the bio-imprinting template (shown in step 4 in Fig. 3) is avoided. This not only simplifies the imprinting process, but also decreases the loss of enzyme resulting from the removal step. Therefore, substrate imprinting is very suitable for industrial applications.

3.2. *Effect of pH on the activity of imprinted tannase*

Fig. 2b shows that the AF increases as the concentration of citric acid buffer increases from 0 to 50 mM, reaches a maximum at 50 mM, and then decreases slowly after 50 mM. The AF increases when the pH value of the citric acid buffer is below 5.5, at which it reaches a maximum (shown in Fig. 2c). This suggests that the AF significantly depends on the pH value and the buffer concentration. Previous studies have reported that some enzymes were hyper-activated by changing the pH value in the aqueous microenvironment around the enzyme [14,16]. In this work, the optimal pH value of tannase ranges from 4 to 6, and when pH value of the citric acid buffer was 5.5, the protein molecules may be assembled into a favorable conformation with high activity. This favorable structure may also be ascribed to modulation of the ionic state of the enzyme caused by variation of the buffer concentration. When the enzyme is transferred into an anhydrous medium from an aqueous solution, this structure is memorized due to its rigidity in the organic phase (shown in step 1 in Fig. 3).

To obtain the optimal AF for TA imprinting together with pH imprinting, Box–Behnken factorial design was applied on the basis of the single-factor experiments aforementioned. Table 1 shows the independent variables and their coding levels. Table 2 presents

Table 1
Defining variables and coding.

Factor	−1	0	+1
TA mass, X_1 (g)	0.01	0.03	0.05
Buffer concentration, X_2 (mM)	20	70	120
pH value, X_3	5	5.5	6

Table 2
Box–Behnken factorial design and the values of responses.

RUN	X_1	X_2	X_3	Y
1	−1	−1	0	1.98
2	−1	1	0	2.35
3	1	−1	0	0.31
4	1	1	0	1.79
5	0	−1	−1	0.36
6	0	−1	1	1.19
7	0	1	−1	1.67
8	0	1	1	5.21
9	−1	0	−1	3.45
10	1	0	−1	0.75
11	−1	0	1	3.63
12	1	0	1	7.54
13	0	0	0	4.02
14	0	0	0	4.61
15	0	0	0	4.16

Table 3
ANOVA for the responses.

Source	SS	MS	F	Pr > F
X_1	1.0629	1.0629	14.1342	0.0132
X_2	3.0522	3.0522	40.5883	0.0014
X_3	2.752	2.752	36.5967	0.0018
X_1*X_2	0.6302	0.6302	8.38	0.034
X_1*X_3	1.2736	1.2736	16.9361	0.0092
X_2*X_2	3.4953	3.4953	46.4817	0.001
Model	12.3779	1.3753	18.2893	0.0026
Error	0.376	0.0752		

the three factors and the three-level face-centered cube design, and the responses. Analysis of variance (ANOVA) shows that all factors (i.e. TA, buffer concentration, and buffer pH value) have a dramatic effect on the AF of tannase. Table 3 shows that TA imprinting significantly correlates with pH imprinting. This further proves that TA imprinting activates tannase more when it is coupled with pH imprinting. A second-order model was used to fit the response to the independent variables, and the model is shown as the following (R^2 97.05%):

$$Y = 1.448342 - 0.364497 * X_1 + 0.617674 * X_2 + 0.586515 * X_3 - 0.236851 * X_1 * X_1 + 0.396913 * X_1 * X_2 + 0.564261 * X_1 * X_3 - 0.972962 * X_2 * X_2 - 0.014462 * X_2 * X_3 - 0.146454 * X_3 * X_3 \quad (1)$$

where X_i refers to the independent variable coded value and Y is the response (CR). Based on Eq. (1), the optimal CR of imprinted tannase was predicted to be 8.2%, and the optimal imprinting medium composition included 0.05 g TA and 5 mL 90 mM pH 6.0 citric acid buffer. Verification experiments yielded a CR of 8.7% and an AF of 9.7, in agreement with the prediction.

3.3. Effect of temperature on the activity of imprinted tannase

The effect of temperature on the AF of tannase is shown in Fig. 4. In Fig. 4a, the highest CR, 11.81%, occurs at 25 °C, and it is 13-fold higher than that of Ew. The same result is seen in Fig. 5b. This demonstrates that a suitable imprinting temperature is very important in enhancing the AF of tannase. In fact, bio-imprinting is a process correlated with temperature because bio-imprinting is, in principle, based on molecular thermal motion. At high temperature the motion becomes too rapid to form the transition complex of tannase-TA efficiently, whereas at low temperature the motion is too slow to form an effective coupling of TA with tannase. It has also been reported that, if the imprinting temperature is too high, it has a negative impact on the biocatalytic activity of bio-imprinted lipase [19]. Therefore, it is believed that the proper imprinting temperature really does contribute to an improvement in CR of tannase.

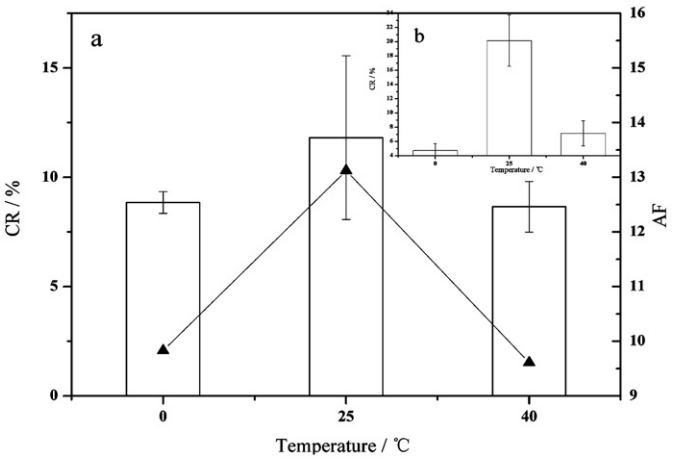

Fig. 4. Effect of the imprinting temperature on the biocatalytic activity of tannase. With 90 mM pH 6.0 citric acid buffer as solvent, 16 IU tannase were imprinted with 0.05 g TA at 0–40 °C. (a) The imprinted tannase catalyzed the synthesis of propyl gallate from TA by transesterification. (b) The imprinted tannase catalyzed the synthesis of propyl gallate from gallic acid by esterification. The effect of imprinting temperature on the biocatalytic activity of tannase was estimated by comparing the relevant CRs and AFs. The catalytic reaction conditions were the same as those in Section 2.

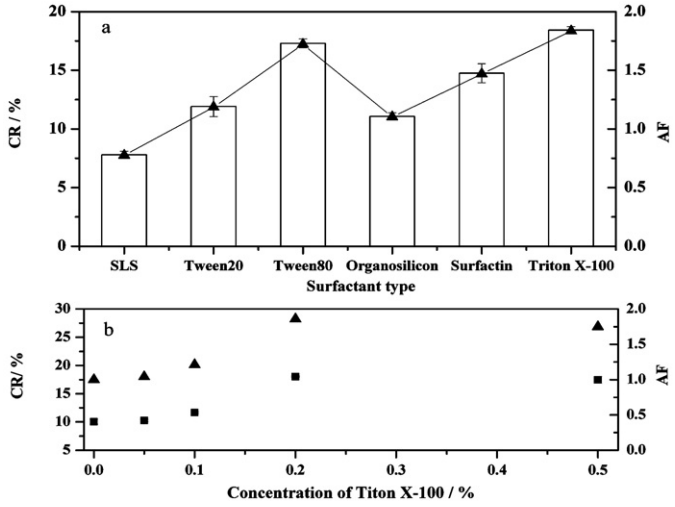

Fig. 5. Effect of surfactant on the biocatalytic activity of tannase. (a) With 90 mM pH 6.0 citric acid buffer as solvent, 16 IU tannase were imprinted with six types of surfactants at 0.2%. (b) With 90 mM pH 6.0 citric acid buffer as solvent, 16 IU tannase were imprinted with 0–0.5% Triton X-100. The imprinted tannases were applied to catalyze the synthesis of propyl gallate from TA by transesterification. The effect of surfactant on the biocatalytic activity of tannase was estimated by comparing the CRs or AF values. The catalytic reaction conditions were the same as those in Section 2, and the control CR was 9.7%.

Table 4
Effect of the immobilization on the catalytic activity of bio-imprinted tannase.

Treatment	Solvent	Immobilization	CR (mean ± SD)/%	AF
BE	Buffer	F	7.4 ± 0.33	8.22
Ew	Distilled water	F	0.9 ± 0.01	1.00
SE	Distilled water	F	1.26 ± 0.07	1.35
BTSE	Buffer	F	18.03 ± 0.1	19.39
Es	Distilled water	F	0.93 ± 0.04	1.00
BEi	Buffer	T	20.86 ± 1.36	52.15
BTSEi	Buffer	T	35.87 ± 0.31	89.68
BSTEi	Buffer	T	40.16 ± 0.14	100.40
Ei	Distilled water	T	0.40 ± 0.01	1.00

BE: Buffer imprinted tannase, 16 IU tannase imprinted with buffer. SE: Surfactant imprinted tannase, 16 IU tannase imprinted with 0.2% Triton X-100 with distilled water as solvent. BTSE: Combinational imprinted tannase in the order BTS; with buffer as solvent 16 IU tannase complexly imprinted with 0.05 g TA and 0.2% Triton X-100. BSTE: Combinational imprinted tannase in the order of BST; with buffer as solvent 16 IU tannase complexly imprinted with 0.2% Triton X-100 and 0.05 g TA. Es: The control of SE, a mixture of the tannase lyophilization powder with 0.2% Triton X-100. BEi, BSTEi, BTSEi, and Ei refer to the immobilized BE, the immobilized BSTE, the immobilized BTSE, and the immobilized Es, respectively. The imprinted tannases catalyzed the synthesis of propyl gallate from TA by transesterification. The effect of immobilization on the biocatalytic activity of tannase was estimated by comparing the CRs or AFs. The catalytic reaction conditions were the same as those in Section 2, and all buffers were 90 mM pH 5.5–6.0 citric acid buffer. "F" and "T" in the "Immobilization" column refer to free enzyme and immobilized enzyme on 0.5 g celite, respectively.

3.4. Effect of surfactant on the activity of imprinted tannase

The effect of surfactant on the AF of tannase is shown in Fig. 5. In Fig. 5a, five nonionic surfactants have a bigger effect on CR than the ionic surfactant sodium lauryl sulfate (SLS). In particular, Triton X-100, as the optimal nonionic surfactant, makes an interfacial imprinted tannase with a CR of approximately 18% and an AF of 1.8. Fig. 5b shows that the AF rises with increasing Triton X-100 concentration below 0.2%, at which the AF reaches its maximum. It can be seen from Fig. 5 that nonionic surfactants have a positive effect on the activation of tannase, and just a small quantity of surfactant, generally less than the critical micelle concentration (CMC) [21,22], can activate the enzyme. On the contrary, too much surfactant reduces the catalytic activity of the enzyme, likely by irreversible covalent inhibition [22]. Therefore, the appropriate amount of surfactant is beneficial to the activation of the enzyme. Previous studies [4,21,22,30] have reported that a few surfactants can improve the catalytic performance of enzymes by enhancing the rigidity of protein dynamics and substrate accessibility to the active site and/or by inducing a more competent catalytic center in anhydrous media [21]. In addition, surfactant can also enhance the catalytic activity of the enzyme by diminishing the aggregation of imprinted enzymes in anhydrous media.

A demonstration test was designed to investigate the role of Triton X-100 in this work. Table 4 shows that the CR of the Triton X-100-imprinted tannase (SE) is higher than that of the control (the non-imprinted tannase with the surfactant, Es), and Es also has a slight advantage over Ew in CR. According to the difference in CR between SE and Es, it is believed that Triton X-100 can activate tannase by bio-imprinting due to its interaction with tannase, which favors a molecular conformation of tannase that is more stable and similar to that of a highly active enzyme (shown in step 2 in Fig. 3). By comparing the CR of Es with that of Ew, it is inferred that Triton X-100 slightly enhances substrate accessibility to the active site of the enzyme.

3.5. Effect of imprinting order on the activity of imprinted tannase

The effect of the imprinting order on the AF of tannase was investigated by analyzing the following series: (1) first pH imprinting, second substrate (TA) imprinting, third interfacial imprinting (BTS); (2) first pH imprinting, second interfacial imprinting, third substrate (TA) imprinting (BST). Table 4 shows that the immobilized tannase imprinted in the BST order has a slight advantage in CR over the immobilized tannase imprinted in the BTS order. This may be due to the fact that TA as the imprinting template is hydrolyzed to GA by tannase, and thus the improvement in AF caused by TA imprinting may be reduced. Therefore, once substrate imprinting is combined with other imprinting methods (i.e. interfacial imprinting and pH imprinting), the imprinting order has to be carefully considered to achieve amplification of the AF.

3.6. Effect of enzyme immobilization on the activity of imprinted tannase

The effect of enzyme immobilization on CR is shown in Table 4. It is expected that all forms of the immobilized imprinted tannase are more active than their free counterparts. In particular, immobilized tannase imprinted in the BST order exhibits a surprising CR of 40.16%, 100.4-fold that of the original control (the immobilized Ew). This indicates that just a simple immobilization method can boost the apparent AF of bio-imprinted tannase remarkably due to the surface extension of the immobilized enzyme.

Fig. 1 and Table 4 show that immobilized TE, immobilized BE, and immobilized tannase imprinted in the BTS order Exhibit 4.86-fold, 2.82-fold, and 1.99-fold higher CR, respectively, than their free counterparts. From this it is inferred that the viscosity of the imprinted enzyme is higher, and the enzyme immobilization improves the apparent activity of the imprinted enzyme by reducing the viscosity. On the other hand, it verifies once again that TA in TE can make TE so sticky that mutual agglomeration occurs, whereas surfactant linked with protein can improve the dispersibility of the enzyme sufficiently so as to decrease this effect of immobilization on the activation of the enzyme.

Furthermore, the imprinted enzyme, unlike natural enzyme, is usually used in organic media, in which an immobilized enzyme formed by a simple method such as adsorptive immobilization can avoid a loss of enzyme activity. In addition, celite has been frequently used as an economical support. Therefore, it is easy to manufacture an immobilized imprinted tannase with specific and high biocatalytic activity in an industrial process.

In the present work, a bio-imprinting technique combined with a simple adsorptive immobilization has been shown to improve the CR of natural tannase by 40%. As expected, the CR can be further enhanced by some other optimizations in the reaction system and in the reaction conditions. This promising biocatalyst can be applied to catalyze the transesterification reaction of TA to propyl gallate. TA is a hydrolysable, water-soluble gallotannin, and is found in a variety of agro-forestry residues such as wood, bark, leaves, fruits, and galls [31]. Thus, it is of great importance to the economy and in environmental protection to synthesize propyl gallate in a way that couples it with making high-value use of TA. In addition, the promising biocatalyst is only used in organic media, where the transesterification reaction occurs more easily than the hydrolysis reaction. The differences in hydrophobicities of propyl gallate, substrate, and biocatalyst allow us to simply isolate propyl gallate and to recycle the biocatalyst from the organic media. All of the above are required for industrial production of propyl gallate.

4. Conclusions

In summary, bio-imprinting can improve the activity of tannase remarkably. The modification of tannase by the combined use of various bio-imprinting methods created a promising biocatalyst with a surprising 40% enhancement of CR, 100-fold that of the original control. Furthermore, joint application of a simple

immobilization method with the transesterification reaction in organic media diminishes the production costs of propyl gallate. Therefore, the synthesis of propyl gallate coupled with the exploitation of TA on a large scale is a promising approach. This work not only presents an effective way to make high-value use of various TA-rich agro-forestry residues, but also broadens the applications of bio-imprinting.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2012.01.007.

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