catalysts

MDPI

Communication

A Novel Oxidation of Salicyl Alcohols Catalyzed by Lipase

Ziyuan Zhao [1], Liu Zhang [1], Fengxi Li [1], Xuyong Tang [1], Yuwen Ma [1], Chunyu Wang [2], Zhi Wang [1], Rui Zhao [3,*] and Lei Wang [1,*]

1 Key Laboratory of Molecular Enzymology and Engineering of Ministry of Education, School of Life Sciences, Jilin University, 2699 Qianjin Street, Changchun 130000, China; zhaoziyuan2015@126.com (Z.Z.); liuzhangjlu@163.com (L.Z.); fengxili17@126.com (F.L.); Xuyongtangjlu@163.com (X.T.); yuwenmajlu@163.com (Y.M.); wangzhi@jlu.edu.cn (Z.W.)

2 State Key Laborarory of Supramolecular Structure and Materials, Jilin University, 2699 Qianjin Street, Changchun 130000, China; chunyu@jlu.edu.cn

3 China-Japan Union Hospital of Jilin University, 126 Xiantai Street, Changchun 130000, China

* Correspondence: zhaor@jlu.edu.cn (R.Z.); w_lei@jlu.edu.cn (L.W.)

Received: 26 October 2017; Accepted: 20 November 2017; Published: 23 November 2017

Abstract: A novel and efficient oxidation of salicyl alcohols to the corresponding salicylaldehydes catalyzed by lipase is reported for the first time. Under the optimal reaction conditions, the method exhibited high yields (81–95%) and selectivities for salicylaldehydes. Moreover, this study expands the application of enzyme catalytic promiscuity in organic synthesis.

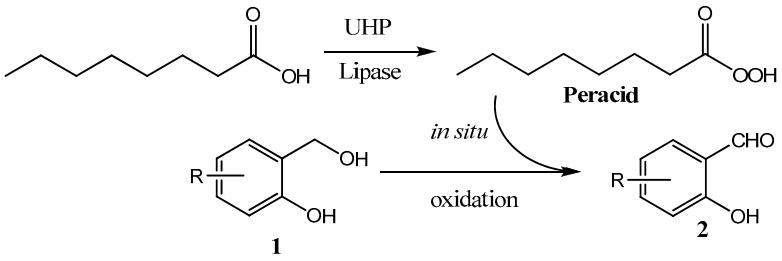
Keywords: promiscuity; lipase; TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy); oxidation; salicyl alcohol

1. Introduction

Enzyme catalytic promiscuity refers to the properties of enzymes which can catalyze different types of chemical transformations in addition to their main physiological ones [1]. Thus as a side benefit of the enzyme, it may afford a new synthesis pathway and widen the utilization of the enzyme [2]. Lipases have attracted wide attention in this area due to their broad promiscuous catalytic abilities in many organic reactions [3–7]. Recently, lipase-catalyzed perhydrolysis of carboxylic acids or esters, resulting in the formation of reactive peroxycarboxylic acids, have been studied as interesting cases of lipase catalytic promiscuity [8]. The in situ generated peroxycarboxylic acids by perhydrolysis have been directly used to oxidize ketones, alkenes, sulfides, enaminones, and amines [9–14]. However, lipase-catalysis of other oxidative reactions has been less explored, especially with alcohols as starting substrates.

Salicylaldehyde is one of the most fundamental and widely used aldehydes being an ingredient in agricultural pesticides, chemicals, perfumes, polymers, and fibers. Beside the Reimer Tiemann process [15,16], salicylaldehydes can be obtained by oxidation of salicylic alcohols over metal catalysts, such as Au, Pd, Pt, CuCo/C, and alkaline hydroxide [17–22]. However, certain drawbacks have always existed for most of the reported methods, such as the utilization of metals, hazardous or toxic solvents, high temperature, and undesirable byproducts. Therefore, the oxidation of salicylic alcohols is still a big challenge from the environmental and economic point of view.

As part of our studies on the development of lipase catalytic promiscuity, we focused on the lipase-catalyzed oxidation of salicyl alcohols to salicylaldehydes (Scheme 1). To the best of our knowledge, the lipase-catalyzed oxidation of salicyl alcohols to salicylaldehydes has not yet been reported.



Scheme 1. Lipase-catalyzed oxidation of salicyl alcohols to salicylaldehydes. UHP: urea-hydrogen peroxide complex.

2. Results and Discussion

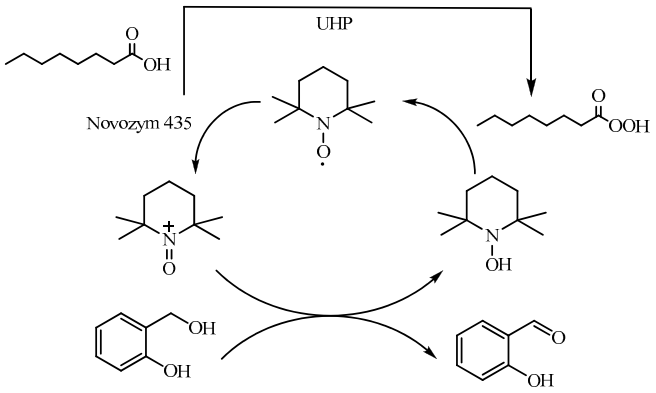
Based on previous reports [23], Novozym 435 (a commercial immobilized *Candida antarctica* lipase B) has usually displayed excellent activities in these lipase-catalyzed oxidations. Hydrogen peroxide, a useful oxidant in many biocatalytic reactions, can seriously inactivate enzymes. Many methods have been explored to avoid the enzyme inactivation [24,25]. In this study, urea-hydrogen peroxide complex (UHP) was adopted as oxidizing agent for its controllable liberation of hydrogen peroxide which could help to avoid the inactivation of the enzyme in lipase-catalyzed oxidations [26]. Initially, we selected salicyl alcohol (**1a**) as the substrate of the lipase-catalyzed oxidation and *n*-caprylic acid as the peracid precursor (in situ perhydrolysis catalyzed by lipase). As shown in Table 1, the yield of the lipase-catalyzed oxidation was poor (Entry 1), though salicylaldehyde (**2a**) was obtained as the only product. Only 5% yield of **2a** was achieved when mCPBA (meta-chloroperoxybenzoic acid, a commercial organic peracid, 1 equivalent) was used as the oxidant (Entry 2). TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) is always introduced for the oxidation of alcohols as a highly efficient oxidative catalyst or an additive combined with peracid [27–29]. It was observed that the reaction did not occur when TEMPO (1 equivalent) was used in this reaction (Entry 3). Meanwhile, the yield of salicylaldehyde was dramatically increased to 91% by using TEMPO (5 mol%) as the additive in the lipase-catalyzed oxidation (Entry 4). It is worth noting that the lipase-catalyzed oxidation presented a higher yield than that catalyzed by the combination of mCPBA and TEMPO (Entry 5). To certify the role of the active center of lipase in this oxidation, a serine-specific inhibitor (phenylmethanesulfonyl fluoride, PMSF) was utilized to inactivate Novozym 435 according to our previous report [9]. No oxidation of salicyl alcohol was observed when Novozym 435 inactivated by PMSF was used as catalyst (Entry 6). This result showed that the active center of lipase was directly involved in the reaction. Furthermore, the denatured Novozym 435 (Entry 7) and Bovine serum albumin (BSA, Entry 8) did not exhibit activity, which suggests that a specific conformation of lipase is necessary for the in situ generation of peracid.

Table 1. Oxidation of salicyl alcohol to salicylaldehyde catalyzed by lipase. [a] mCPBA: meta-chloroperoxybenzoic acid; TEMPO: 2,2,6,6-tetramethyl-1-piperidinyloxy; BSA: Bovine serum albumin.

Entry	Catalyst	Additive	Yield of 2a (%) [b]
1	Novozym 435	None	18
2	mCPBA	None	5
3	TEMPO	None	ND [c]
4	Novozym 435	TEMPO	91
5	mCPBA	TEMPO	52
6	Novozym 435 [d]	TEMPO	ND
7	Novozym 435 [e]	TEMPO	ND
8	BSA	TEMPO	ND

[a] Reaction conditions: **1a** (1 mmol), acetonitrile (5 mL), UHP (1.1 mmol), *n*-caprylic acid (0.2 mmol), additive (0.05 mmol), Novozym 435 (200 U), room temperature, 2 h; [b] isolated yield; [c] ND: not detected; [d] Inactivated by PMSF (phenylmethanesulfonyl fluoride). [e] Pretreated Novozym 435 by heating it for 1 h in boiling water.

Based on the experimental results, a possible reaction mechanism for this lipase-catalyzed oxidation of salicyl alcohol was proposed (Scheme 2). First, TEMPO bound to the oxyanion hole and was activated to form the active intermediate ($TEMPO^+$). Secondly, $TEMPO^+$ oxidized salicyl alcohol to salicylaldehyde. Finally, TEMPOH was formed along with the product, which was converted back to TEMPO by the peracid generated by the lipase-catalyzed perhydrolysis of *n*-caprylic acid. Then, the system was ready for another round of salicyl alcohol oxidation.



Scheme 2. The proposed mechanism for the lipase-catalyzed oxidation of salicyl alcohol.

In this lipase-catalyzed system, two competitive reactions were involved in the conversion of salicyl alcohol. One reaction was the perhydrolysis of carboxylic acid catalyzed by lipase to generate peracid, where the in situ generated peracid oxidized salicyl alcohol to produce salicylaldehyde. The other reaction was the esterification of salicyl alcohol with the carboxylic acid catalyzed by lipase. Therefore, the carboxylic acid used as the peracid precursor plays a key role in the lipase-catalyzed reaction. As shown in Table 2, the yield of **2a** was gradually increased when the alkyl chain of the carboxylic acid was elongated, and the carboxylic acids with shorter alkyl chains were preferred as acylation agents rather than the peracid precursors (Entries 1–4). In principle, water can inhibit the acylation in lipase catalyzed esterification. However, the conversion of **1a** decreased gradually, and no apparent effect on the oxidative selectivity of **1a** could be observed in this reaction system even when the water content varied from 0 to 50% *v/v* in acetonitrile (data not shown). Furthermore, the optimal amount of *n*-caprylic acid was also screened (Entries 4–7). When the amount of *n*-caprylic acid was 2 mmol, a satisfactory yield of **2a** could be obtained. Further increasing the amount of *n*-caprylic acid did not affect the reaction obviously. More importantly, no esterification occurred when the amount of *n*-caprylic acid varied from 0.1 mmol to 0.4 mmol. Therefore, 2 mmol was selected as the optimal amount of *n*-caprylic acid in this study.

Table 2. The effect of carboxylic acid on the oxidation of salicyl alcohol [a].

Entry	Carboxylic Acid	Yield of 2a (%) [b]	Yield of Ester (%) [b]
1	Acetic acid (0.2 mmol)	16	17
2	*n*-Butyric acid (0.2 mmol)	42	12
3	*n*-Hexanoic acid (0.2 mmol)	63	9
4	*n*-Caprylic acid (0.2 mmol)	91	ND [c]
5	*n*-Caprylic acid (0.1 mmol)	69	ND
6	*n*-Caprylic acid (0.3 mmol)	92	ND
7	*n*-Caprylic acid (0.4 mmol)	94	ND

[a] Reaction conditions: **1a** (1 mmol), acetonitrile (5 mL), UHP (1.1 mmol), TEMPO (0.05 mmol), Novozym 435 (200 U), room temperature, 2 h; [b] isolated yield; [c] ND: not detected.

Generally, the reaction medium can influence the catalytic performance of an enzyme. In this study, we used several organic solvents and the results in Figure 1 indicated that acetonitrile was the best choice between dichloromethane, tetrahydrofuran, dimethyl sulfoxide, dioxane, and water. Some literature references reported that ethyl acetate could be adopted as reaction medium and peracid precursor in a lipase-catalyzed oxidation [26,30]. However, it was disappointing that only the acetate of **1a** was obtained instead of **2a** when ethyl acetate was used as the reaction medium. Therefore, acetonitrile was selected as the reaction medium for this lipase-catalyzed oxidation.

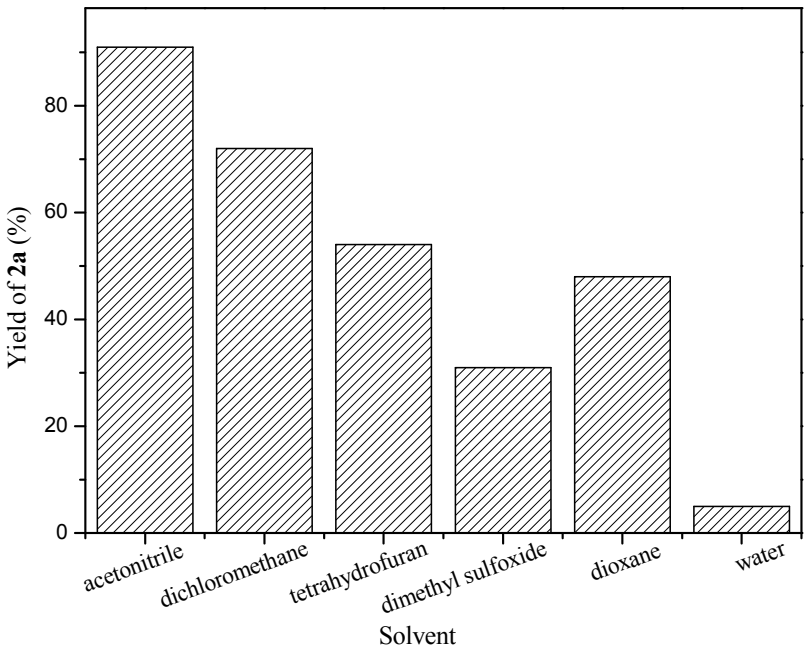


Figure 1. The effect of solvent on the oxidation of salicyl alcohol. Reaction conditions: **1a** (1 mmol), solvent (5 mL), UHP (1.1 mmol), *n*-Caprylic acid (0.2 mmol), TEMPO (0.05 mmol), Novozym 435 (200 U), room temperature, 2 h; isolated yield.

The dosage of TEMPO and lipase was also investigated in this study. The effect of the dosage of TEMPO is outlined in Entries 1–4 of Table 3. The yield of **2a** was increased by increasing the dosage of TEMPO from 1 mol% to 5 mol%. However, the yield did not obviously improve by further increasing the dosage of TEMPO. In addition, the yield of **2a** increased as the dosage of lipase was elevated from 50 to 200 U, followed by a slight change of yield at higher dosage of lipase. As for the oxidant, we fixed the amount of UHP at 1.1 mmol due to the instability of salicylaldehyde exposed to excessive peracid [31].

Table 3. The effect of dosage of TEMPO and lipase on the oxidation of salicyl alcohol. [a]

Entry	Dosage of TEMPO (mol%)	Dosage of Enzyme (U)	Yield of 2a (%) [b]
1	1	200	57
2	3	200	76
3	5	200	91
4	7	200	92
5	5	50	32
6	5	100	53
7	5	150	84
8	5	250	92

[a] Reaction conditions: **1a** (1 mmol), acetonitrile (5 mL), UHP (1.1 mmol), *n*-caprylic acid (0.2 mmol), room temperature, 2 h; [b] isolated yield.

Generally, the immobilized enzyme is easy to recover and reuse, which makes the enzymatic process economically viable [32–34]. In this work, Novozym 435 can be easily recovered after each run by filtration. The recovered Novozym 435 was then reused directly for the oxidation of **1a** under the same conditions. It is demonstrated in Figure 2 that 82% oxidation yield of **2a** could be obtained even after six reaction cycles. Furthermore, with the optimized reaction conditions to hand, we scaled up the lipase-catalyzed oxidation 50-fold (**1a** (50 mmol), acetonitrile (250 mL), Novozym 435 (10,000 U), TEMPO (2.5 mmol), and UHP (55 mmol)) and the yield of **2a** was up at 93% after 2 h. These results demonstrated that this lipase-catalyzed method has high potential for industrial production.

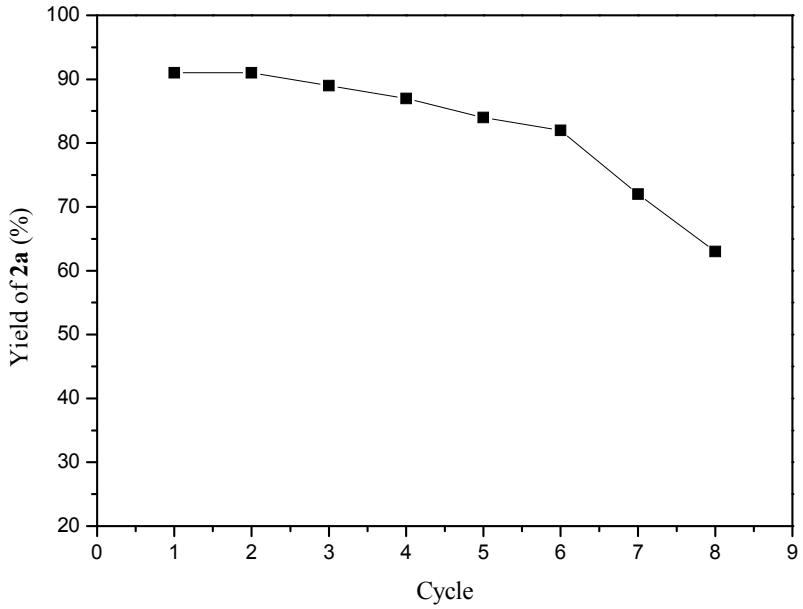
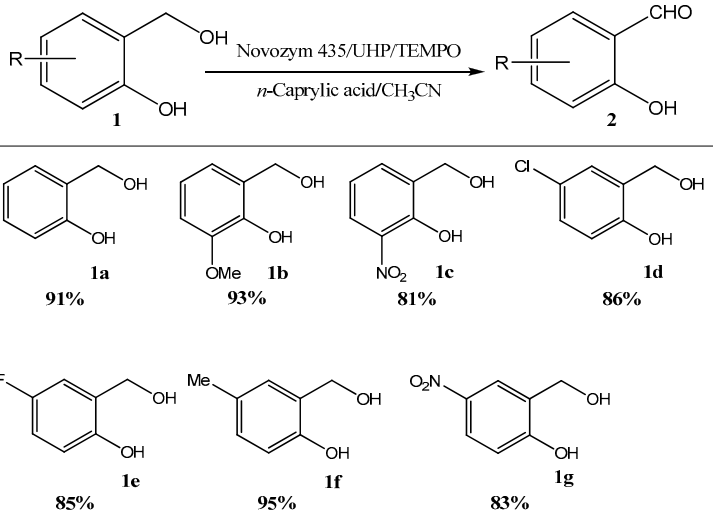


Figure 2. The reusability of Novozym 435 on the oxidation of salicyl alcohol. Reaction conditions: **1a** (1 mmol), acetonitrile (5 mL), UHP (1.1 mmol), *n*-Caprylic acid (0.2 mmol), TEMPO (0.05 mmol), Novozym 435 (200 U), room temperature, 2 h; isolated yield.

To explore the scope and limitation of the method, we used a panel of substituted salicylic alcohols as the substrates in the lipase-catalyzed oxidation (Table 4). It was found that all the substituted salicylic alcohols could be oxidized to the desired salicylaldehydes in high yields (81–95%), and the yields of salicylaldehydes were higher when salicyl alcohols bearing electron donating groups were adopted as the substrates (**1b** and **1f**). It is noteworthy that no ester of salicyl alcohol was observed in the oxidation. Therefore, this method is a mild, efficient, and highly selective means for the synthesis of salicylaldehydes. Furthermore, some substituted benzyl alcohols were also investigated as substrates at the same reaction conditions. However, only benzyl alcohol could be totally converted to benzaldehyde, and other substituted benzyl alcohols afforded both corresponding benzaldehydes and esters (data not shown). Further investigation on the selective oxidation of other benzyl alcohols is currently ongoing in our laboratory.

Table 4. The oxidation of substituted salicylic alcohols catalyzed by lipase.



Reaction conditions: **1** (1 mmol), acetonitrile (5 mL), UHP (1.1 mmol), *n*-caprylic acid (0.2 mmol), TEMPO (0.05 mmol), Novozym 435 (200 U), room temperature, 2 h; isolated yield.

3. Materials and Methods

3.1. Materials

Novozym 435 (15,000 U/g) and Bovine serum albumin were purchased from Sigma (Beijing, China). One unit (U) of enzyme activity was defined as the amount of enzyme that hydrolyzes 1 μmol of 4-nitrophenyl acetate per min at 30 °C. Silica gel was purchased from Qing Dao Hai Yang Chemical Industry Co. (Qingdao, China). The chemical reagents were of analytical reagent grade and purchased from J & K Scientific Ltd. (Beijing, China). NMR spectra were taken with an Inova 500 (500 MHz) spectrometer (Vernon Hills, IL, USA).

3.2. A Typical Lipase-Catalyzed Oxidation of Salicyl Alcohol

A mixture of UHP (1.1 mmol), *n*-caprylic acid (0.2 mmol), TEMPO (0.05 mmol), Novozym 435 (200 U), in acetonitrile (5 mL) was mixed for 5 min in a 25 mL round-bottom flask, and then salicyl alcohol (1 mmol) was added to the reaction system. The mixture was stirred for 2 h at room temperature. Then the reaction mixture was filtered and the residue was washed with acetonitrile. The combined organic phases were concentrated under vacuum, and the resulting residue was purified by flash column chromatography on silica gel with ethyl acetate /hexane (1/20) to afford the desired salicylaldehyde. All the isolated products were well characterized by the ^{1}H-NMR spectral analysis. The experiments were performed in triplicate, and all data were obtained based on the average values.

3.3. ^{1}H-NMR of Products

The spectra of products **2a**–**2g** were displayed in the Supplementary Materials.

2a: ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.97–7.02 (m, 2H), 7.50–7.55 (m, 2H), 9.87 (s, 1H), 11.03 (s, 1H).

2b: ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 3.92 (s, 3H), 6.97 (t, *J* = 6.5 Hz, 1H), 7.12 (dd, *J* = 1.0 Hz, 6.5 Hz, 1H), 7.18 (dd, *J* = 1.0 Hz, 6.5 Hz, 1H), 9.91 (s, 1H), 11.1 (s, 1H).

2c: ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.97 (t, *J* = 6.5 Hz, 1H), 7.17 (dd, *J* = 1.0 Hz, 6.5 Hz, 1H), 7.21 (m, 1H), 9.91 (s, 1H), 11.11 (s, 1H).

2d: ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.98 (d, *J* = 7.5 Hz, 1H), 7.49 (dd, *J* = 2.0 Hz, 7.5 Hz, 1H), 7.55 (d, *J* = 7.5 Hz, 1H), 9.86 (s, 1H), 10.93 (s, 1H).

2e: ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.00 (dd, *J* = 3.5 Hz, 7.5 Hz, 1H), 7.26–7.31 (m, 2H), 9.88 (s, 1H), 10.8 (s, 1H).

2f: ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.36 (s, 3H), 6.91 (d, *J* = 7.5 Hz, 1H), 7.35 (s, 1H), 7.35–7.37 (m, 2H), 9.87 (s, 1H), 10.85 (s, 1H).

2g: ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.15 (d, *J* = 7.5 Hz, 1H), 8.44 (dd, *J* = 2.0 Hz, 7.5 Hz, 1H), 8.59 (d, *J* = 2.0 Hz, 1H), 10.03 (s, 1H), 11.63 (s, 1H).

4. Conclusions

In summary, this is a first report on the efficient lipase-catalyzed oxidation of salicylic alcohols to salicylaldehydes under mild conditions. Under the optimal reaction conditions, the salicylic alcohols could be selectively converted to the salicylaldehydes in high yields (81–95%). Meanwhile, Novozym 435 could be reused six times without significant loss of activity which indicated that the method has great potential for the preparation of salicylaldehydes. Generally, immobilization is a powerful technique to enhance the reusability and stability of enzyme [35–38]. Further study to improve the enzyme properties (mainly stability) of this lipase-catalyzed oxidation by using other immobilization strategies or even coupling immobilization and chemical modification is underway and will be reported in due course. Moreover, this study provides a new case of lipase-catalyzed oxidation and expands the utilization of lipase in organic synthesis.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4344/7/12/354/s1.

Acknowledgments: We gratefully acknowledge the Foundation of Graduate Innovation Fund of Jilin University for the financial support (No. 2016057) and Changchun BC & HC Pharmaceutical Technology Co., Ltd. (No. 3R116V401465).

Author Contributions: Ziyuan Zhao, Liu Zhang, Fengxi Li, Xuyong Tang, and Yuwen Ma performed the experiments; Chunyu Wang and Zhi Wang analyzed the data; Rui Zhao and Lei Wang wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hult, K.; Berglund, P. Enzyme promiscuity: Mechanism and applications. *Trends Biotechnol.* **2007**, *25*, 231–238. [CrossRef] [PubMed]
2. Busto, E.; Gotor-Fernández, V.; Gotor, V. Hydrolases: Catalytically promiscuous enzymes for non-conventional reactions in organic synthesis. *Chem. Soc. Rev.* **2010**, *39*, 4504–4523. [CrossRef] [PubMed]
3. Branneby, C.; Carlqvist, P.; Magnusson, A.; Hult, K.; Brinck, T.; Berglund, P. Carbon-carbon bonds by hydrolytic enzymes. *J. Am. Chem. Soc.* **2003**, *125*, 874–875. [CrossRef] [PubMed]
4. Yang, F.J.; Wang, Z.; Wang, H.R.; Zhang, H.; Yue, H.; Wang, L. Enzyme catalytic promiscuity: Lipase catalyzed synthesis of substituted 2H-chromenes by a three-component reaction. *RSC Adv.* **2014**, *4*, 25633–25636. [CrossRef]
5. Guan, Z.; Fu, J.P.; He, Y.H. Biocatalytic promiscuity: Lipase-catalyzed asymmetric aldol reaction of heterocyclic ketones with aldehydes. *Tetrahedron Lett.* **2012**, *53*, 4959–4961. [CrossRef]
6. Lou, F.W.; Liu, B.K.; Wu, Q.; Lv, D.S.; Lin, X.F. *Candida antarctica* Lipase B (CAL-B)-Catalyzed Carbon-Sulfur Bond Addition and Controllable Selectivity in Organic Media. *Adv. Synth. Catal.* **2008**, *350*, 1959–1962. [CrossRef]
7. Carboni-Oerlemans, C.; María, P.D.; Tuin, B.; Bargeman, G.; Meer, A.; Gemert, R. Hydrolase-catalysed synthesis of peroxycarboxylic acids: Biocatalytic promiscuity for practical applications. *J. Biotechnol.* **2006**, *126*, 140–151. [CrossRef] [PubMed]
8. Björkling, F.; Frykman, H.; Godtfredsen, S.E.; Kirk, O. Lipase catalyzed synthesis of peroxycarboxylic acids and lipase mediated oxidations. *Tetrahedron* **1992**, *48*, 4587–4592. [CrossRef]
9. Yang, F.J.; Wang, Z.; Zhang, X.W.; Jiang, L.Y.; Li, Y.Z.; Wang, L. A Green Chemoenzymatic Process for the Synthesis of Azoxybenzenes. *ChemCatChem* **2015**, *7*, 3450–3453. [CrossRef]

10. Kotlewska, A.J.; Rantwijk, F.; Sheldon, R.A.; Arends, I.W.C.E. Epoxidation and Baeyer-Villiger oxidation using hydrogen peroxide and a lipase dissolved in ionic liquids. *Green Chem.* **2011**, *13*, 2154–2160. [CrossRef]
11. Yang, F.J.; Zhang, X.W.; Li, F.X.; Wang, Z.; Wang, L. A lipase-glucose oxidase system for the efficient oxidation of *N*-heteroaromatic compounds and tertiary amines. *Green Chem.* **2016**, *18*, 3518–3521. [CrossRef]
12. Xu, Y.; Khaw, N.R.B.J.; Li, Z. Efficient epoxidation of alkenes with hydrogen peroxide, lactone, and lipase. *Green Chem.* **2009**, *11*, 2047–2051. [CrossRef]
13. Zhang, L.; Li, F.X.; Wang, C.Y.; Zheng, L.; Wang, Z.; Zhao, R.; Wang, L. Lipase-Mediated Amidation of Anilines with 1,3-Diketones via C-C Bond Cleavage. *Catalysts* **2017**, *7*, 115. [CrossRef]
14. Lau, R.M.; van Rantwijk, F.; Seddon, K.R.; Sheldon, R.A. Lipase-catalyzed reactions in ionic liquids. *Org. Lett.* **2000**, *2*, 4189–4191.
15. Theror, A.; Denis, G.; Delmas, M.; Gaset, A. The Reimer-Tiemann reaction in slightly hydrated solid-liquid medium: A new method for the synthesis of formyl and diformyl phenols. *Synth. Commun.* **1988**, *18*, 2095–2101. [CrossRef]
16. Hamada, K.; Suzukamo, G.; Masuko, F.; Nakamura, M. Process for the Preparation of Hydroxybenzaldehydes. U.S. Patent 4,584,410, 16 March 1986.
17. Milone, C.; Ingoglia, R.; Pistone, A.; Neri, G.; Galvagno, S. Activity of gold catalysts in the liquid-phase oxidation of o-hydroxybenzyl alcohol. *Catal. Lett.* **2003**, *87*, 201–209. [CrossRef]
18. Ludec, J.L. Process for the Preparation of Hydroxybenzaldehydes. U.S. Patent 4,026,950, 31 May 1977.
19. Tsunoyama, H.; Sakurai, H.; Negishi, Y.; Tsukuda, T. Size-specific catalytic activity of polymer-stabilized gold nanoclusters for aerobic alcohol oxidation in water. *J. Am. Chem. Soc.* **2005**, *127*, 9374–9375. [CrossRef] [PubMed]
20. Lefranc, H. Preparation of Hydroxybenzaldehydes. U.S. Patent 5,689,009, 18 November 1997.
21. Wang, F.; Xu, J.; Liao, S.J. One-step heterogeneously catalytic oxidation of *o*-cresol by oxygen to salicylaldehyde. *Chem. Commun.* **2002**, *6*, 626–627. [CrossRef]
22. Christidis, Y.; Vallejos, J.C. Process for the Preparation of Hydroxybenzaldehydes. U.S. Patent 4,481,374, 6 November 1984.
23. Klaas, M.R.; Warwel, S. Lipase-catalyzed preparation of peroxy acids and their use for epoxidation. *J. Mol. Catal. A Chem.* **1997**, *117*, 311–319. [CrossRef]
24. Hernandez, K.; Berenguer-Murcia, A.; Rodrigues, R.C.; Fernandez-Lafuente, R. Hydrogen peroxide in biocatalysis. A dangerous liaison. *Curr. Org. Chem.* **2012**, *16*, 2652–2672. [CrossRef]
25. Hernandez, K.; Fernandez-Lafuente, R. Lipase B from *Candida antarctica* immobilized on octadecyl Sepabeads: A very stable biocatalyst in the presence of hydrogen peroxide. *Process Biochem.* **2011**, *46*, 873–878. [CrossRef]
26. Ríos, M.Y.; Salazar, E.; Olivo, H.F. Baeyer-Villiger oxidation of substituted cyclohexanones via lipase-mediated perhydrolysis utilizing urea-hydrogen peroxide in ethyl acetate. *Green Chem.* **2007**, *9*, 459–462. [CrossRef]
27. Duan, R.F.; Cheng, L.; Zhang, Q.C.; Ma, L.S.; Ma, H.Y.; Yang, J.C. Mechanistic insight into the aerobic oxidation of benzyl alcohol catalyzed by the Cu II-TEMPO catalyst in alkaline water solution. *RSC Adv.* **2015**, *5*, 83976–83984. [CrossRef]
28. Ganern, B. Biological spin labels as organic reagents. Oxidation of alcohols to carbonyl compounds using nitroxyls. *J. Org. Chem.* **1975**, *40*, 1998–2000.
29. Rychnovsky, S.D.; Vaidyanathan, R. TEMPO-catalyzed oxidations of alcohols using m-CPBA: The role of halide ions. *J. Org. Chem.* **1999**, *64*, 310–312. [CrossRef] [PubMed]
30. Ankudey, E.G.; Olivo, H.F.; Peeples, T.L. Lipase-mediated epoxidation utilizing urea-hydrogen peroxide in ethyl acetate. *Green Chem.* **2006**, *8*, 923–926. [CrossRef]
31. Wang, Z.; Chen, X.; Wang, C.Y.; Zhang, L.; Li, F.X.; Zhang, W.A.; Chen, P.; Wang, L. A mild and efficient Dakin reaction mediated by lipase. *Green Chem. Lett. Rev.* **2017**, *10*, 269–273. [CrossRef]
32. Rodrigues, R.C.; Ortiz, C.; Berenguer-Murcia, Á.; Torres, R.; Fernández-Lafuente, R. Modifying enzyme activity and selectivity by immobilization. *Chem. Soc. Rev.* **2013**, *42*, 6290–6307. [CrossRef] [PubMed]
33. Barbosa, O.; Ortiz, C.; Berenguer-Murcia, Á.; Torres, R.; Rodrigues, R.C.; Fernandez-Lafuente, R. Strategies for the one-step immobilization–purification of enzymes as industrial biocatalysts. *Biotechnol. Adv.* **2015**, *33*, 435–456. [CrossRef] [PubMed]

34. Rueda, N.; Santos, J.C.S.; Ortiz, C.; Torres, R.; Barbosa, O.; Rodrigues, R.C.; Berenguer-Murcia, Á.; Fernandez-Lafuente, R. Chemical modification in the design of immobilized enzyme biocatalysts: Drawbacks and opportunities. *Chem. Rec.* **2016**, *16*, 1436–1455. [CrossRef] [PubMed]
35. Izquierdo, D.F.; Barbosa, O.; Burguete, M.I.; Lozano, P.; Luis, S.V.; Fernandez-Lafuente, R.; García-Verdugo, E. Tuning lipase B from *Candida antarctica* C-C bond promiscuous activity by immobilization on poly-styrene-divinylbenzene beads. *RSC Adv.* **2014**, *12*, 6219–6225. [CrossRef]
36. Barbosa, O.; Torres, R.; Ortiz, C.; Berenguer-Murcia, A.; Rodrigues, R.C.; Fernandez-Lafuente, R. Heterofunctional supports in enzyme immobilization: From traditional immobilization protocols to opportunities in tuning enzyme properties. *Biomacromolecules* **2013**, *14*, 2433–2462. [CrossRef] [PubMed]
37. Barbosa, O.; Ortiz, C.; Berenguer-Murcia, Á.; Torres, R.; Rodrigues, R.C.; Fernandez-Lafuente, R. Glutaraldehyde in bio-catalysts design: A useful crosslinker and a versatile tool in enzyme immobilization. *RSC Adv.* **2014**, *4*, 1583–1600. [CrossRef]
38. Garcia-Galan, C.; Berenguer-Murcia, Á.; Fernandez-Lafuente, R.; Rodrigues, R.C. Potential of different enzyme immobilization strategies to improve enzyme performance. *Adv. Synth. Catal.* **2011**, *353*, 2885–2904. [CrossRef]