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Escherichia coli BioH: a highly enantioselective and organic solvent tolerant esterase for kinetic resolution of *sec*-alcohols

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ABSTRACT

Escherichia coli BioH, which is obligatory for biotin synthesis, was found to be an organic solvent tolerant esterase with high enantioselectivity for the kinetic resolution of *sec*-alcohols using free enzyme powder. With this esterase, a variety of racemic *sec*-alcohols were efficiently resolved with *ee* values of up to 99%.

Esterases are able to be produced from prokaryote sources, which is much easier and economic in cloning and expression compared with lipases which are mainly from eukaryotic microorganisms. In addition, the source of esterases is broader than lipases. Despite the progress made in the genetic area, the supply of synthetically useful esterases remains inadequate. Therefore, the search for new esterases exhibiting high activity and enantioselectivity, especially stability in organic solvents, and meanwhile those capable of accepting a wide variety of non-natural substrates is always highly desirable.

Enantiomerically pure secondary alcohols are very important as they are pivotal compounds in organic synthesis.[1] Of the conventional methods, enzyme-catalyzed kinetic resolution (KR) of secondary alcohols is a standard procedure and is still the most practical.[2] To date, a large variety of enzymes have been reported. Nevertheless, the availability of true esterase for kinetic resolution in organic solvents is limited only as they get inactivated or give a very low reaction rate in non-aqueous media. For example, Pig Liver Esterase (PLE) showed no activity in organic solvents with vinyl acetate, in order to confer activity to PLE, the addition of an organic polymer is required.[3] Therefore, an organic solvent tolerant biocatalyst is being applied in wide applications for the synthesis of important products.[4]

Herein, we wish to report a new organic solvent tolerant esterase-*Escherichia coli* BioH (lotus tag **b3412** from gene bank), which showed high activity and enantioselectivity in the acylation of secondary alcohols with vinyl acetate in organic solvents using free enzyme powder. Applying this esterase, a variety of *sec*-alcohols were well resolved and the selectively acylated alcohols were afforded with ee values of up to 99% under mild conditions.

In our previous work,[5] we have demonstrated *E. coli* BioH as a hydrolase with high enantioselectivity and activity in the hydrolysis of 1-phenylethyl acetate. Coincidently, we found this esterase exhibiting good performance in the kinetic resolution of racemic 1-phenylethanol in organic solvents and (*R*)-1-phenylethyl acetate was obtained in 47% yield with ee values of 93%. The result

Table 1
Evaluation of reaction conditions for the KR of racemic 1-phenylethanol applying *E. coli* BioH as the biocatalyst[a]

Entry	Solvent	Ratio[b]	Temp (°C)	Time (h)	Conversion[c] (%)	ee[d] (%)
1	—	—	30	24	46	92
2	Toluene	1:3	30	24	39	93
3	*n*-Hexane	1:1	30	24	47	92
4	*n*-Hexane	1:3	35	24	48	93
5	*n*-Hexane	1:3	35	32	51	91
6	*n*-Hexane	1:3	35	28	>49	93
7	*iso*-Octane	1:5	35	28	44	94
8	Ethyl acetate	1:3	35	28	42	90
9	Acetone	1:1	35	28	33	89

[a] Reactions were performed on a 6 mL scale: 1-phenylethanol, 0.4 mmol, organic solvent, and vinyl acetate 6 mL, crude enzyme powder of *E. coli* BioH, 12 mg (the powder contents 12% of *E. coli* BioH esterase).
[b] VA/Sol (v/v), 'VA' represents vinyl acetate, and 'Sol', organic solvent.
[c, d] Conversions and ee values of (*R*)-1-phenylethyl acetate were determined by GC analysis.

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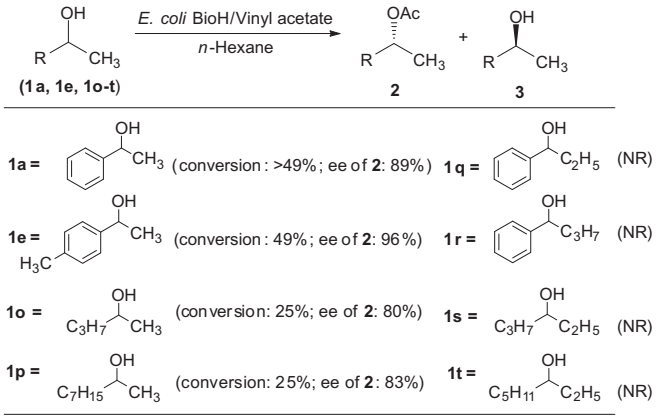


Fig. 1. The type screening of *sec*-alcohols catalyzed by *E. coli* BioH (here, yield and ee values were of esters, 'NR' in brackets represents no reaction)

intrigued us to make a further exploration of substrate scope of *E. coli* BioH. Different organic solvents, reaction times, ratios (vinyl acetate: organic solvent), and temperatures were investigated to evaluate the effect of different reaction conditions on yields and ee values catalyzed by *E. coli* BioH, and the results were summarized in Table 1. It was indicated that under the conditions: *n*-hexane/vinyl acetate, 1:3, temperature 35 °C, and reaction time, 28 h. (*R*)-1-Phenylethyl acetate was afforded in the yield of >49% with ee values of 89% (Table 1, entry 6).

Various types of *sec*-alcohols were then further investigated under the optimized conditions. The results indicated that among these eight substrates, the acylation of only four of them could be catalyzed to form the corresponding (*R*)-esters. All of these four substrates bore an α-hydroxyethyl group (Fig. 1, the group was in blue dashed frame) at the end of the chain. For substrates with other hydroxyalkyl groups like **1q, 1r, 1s**, and **1t** no acylation was observed.

Based on the above results, it was concluded that *E. coli* BioH could only accept alcohols bearing an α-hydroxyethyl group. Substrates bearing α-hydroxyalkyl groups of more than two carbon atoms could not be accepted by this esterase.

As was indicated in Table 1, 1-phenylethanol was well resolved in several organic solvents with *E. coli* BioH, thus we could draw a conclusion that *E. coli* BioH was an organic solvent tolerant esterase. We assumed that it is the property of organic solvent tolerance mainly due to the scattered hydrophobic areas on the molecular surface and around the entrance to an active pocket (Fig. 2). Meanwhile, the presence of disulfide bonds close to the surface also conferred its organic solvent tolerance (Supplementary data, Fig. S1).

E. coli BioH was reported as a carboxylesterase.[7] Hitherto, its application in biocatalysts has been very limited. The function of the enzyme is derived from its molecular structure. *E. coli* BioH esterase has a catalytic triad composed of Ser^{82}, His^{235}, and Asp^{207}. The active site contains a small pocket and a large pocket (Fig. 3), which is buried between two domains and is sufficiently large to accommodate big groups like long linear aliphatic moieties, aromatic rings, or biphenyl rings within the large pocket. Nevertheless, only small groups whose sizes are not bigger than a methyl group could be accommodated in the small pocket, for groups whose sizes are bigger than a methyl group could not or partly be fitted in the small pocket, leading to its hydroxyl group not being accessible to the catalytic site and hence could not be acylated. With its three hydrogen atoms in the hydroxymethyl group being fully replaced by fluorine atoms, no acylation was

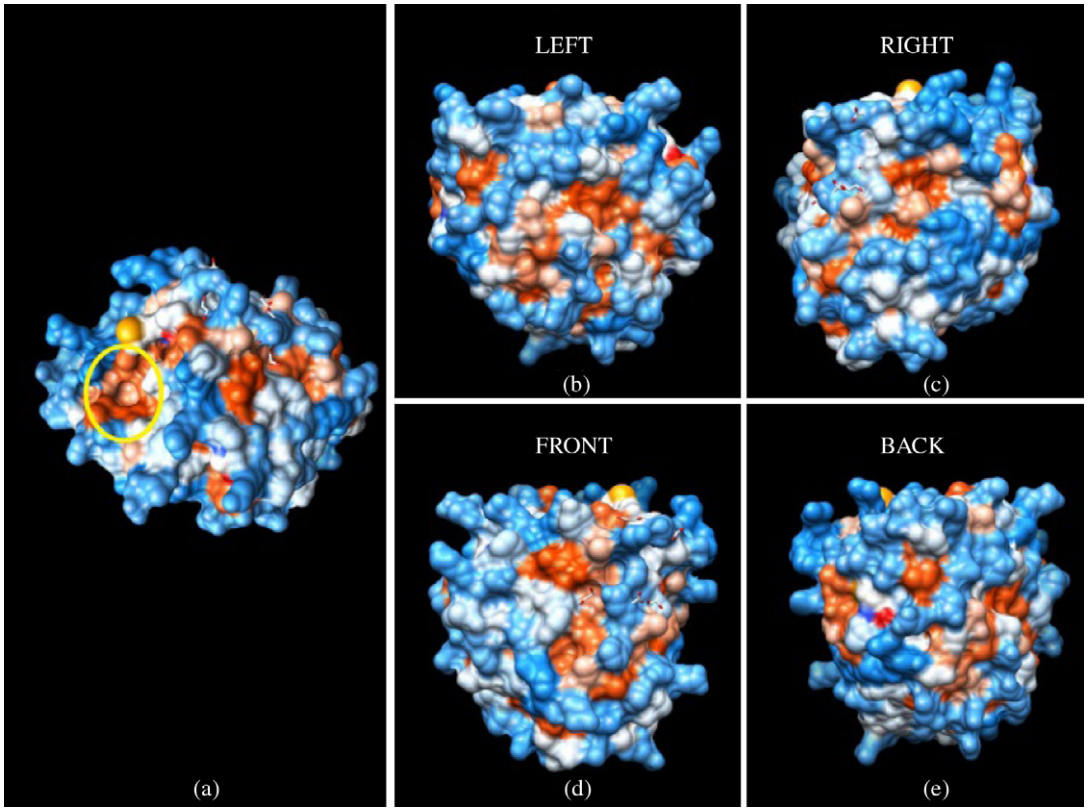


Fig. 2. Hydrophobic areas distributed on the molecular surface of BioH in the interval. (a) The selected side contains the active pocket (marked by oval in yellow). (b–e) Side views from different directions, respectively (the hydrophobic areas were in red).

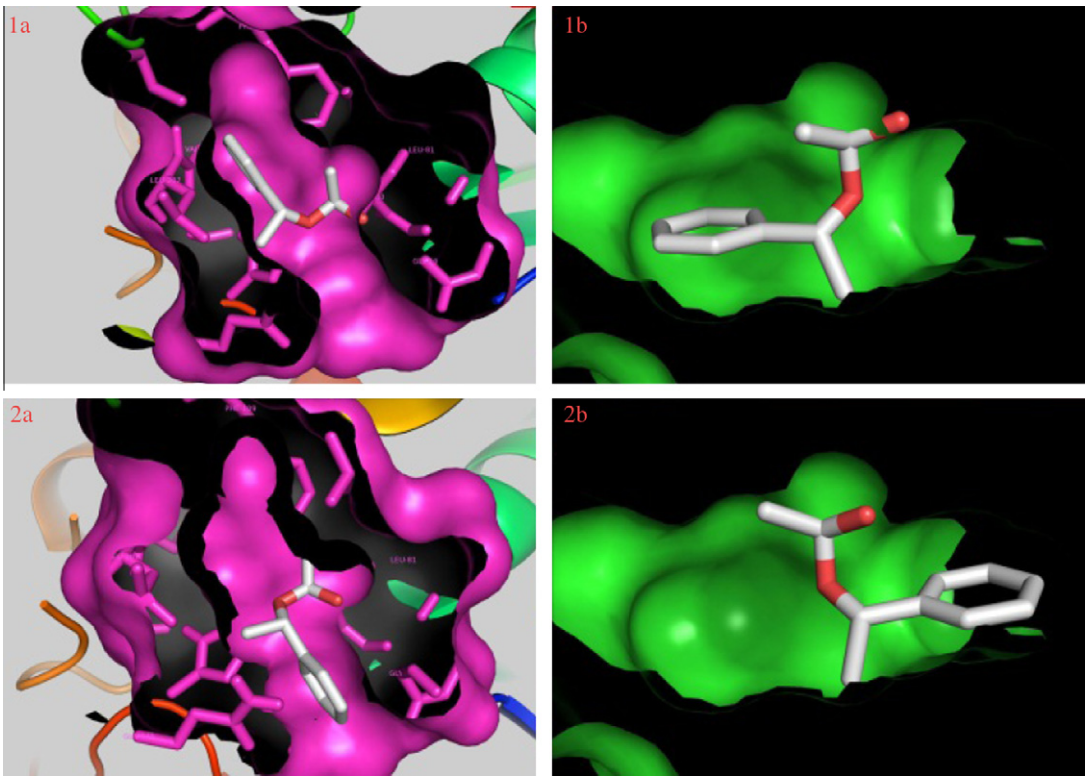


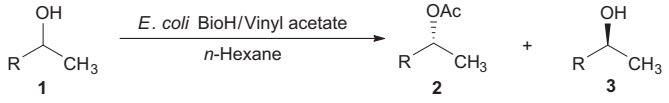
Fig. 3. Microview of the active pocket of *E. coli* BioH binding with a molecular of (*R*)-1-phenylethyl acetate (**1a**, **1b**), and (*S*)-1-phenylethyl acetate (**2a**, **2b**). (a) Seen from the right side of the entire active binding pocket. (b) Local view from the right side of the active pocket.

observed. It was assumed that the formed F–H bonds by fluorine atoms from substrate **1b** and hydrogen atoms from the amino acids in the wall of active pocket were more stable, which resulted in the occupation of the active pocket or the entrance to the active pocket, leading to no other molecules of substrate **1b** going in and out of the active pocket. This might be the reason why substrates like **1b, 1q, 1r, 1s**, and **1t** could not be accepted by *E. coli* BioH.

Kinetic resolution of a series of racemic 1-arylethanols and two 2-alkylalcohols were conducted by the enzyme and the results were summarized in Table 2. When group R were bulky aromatic rings or long linear aliphatic chains, (*R*)-esters were obtained in low yields (Table 2, entries **5**–**8**, **11**, and **12**), to obtain the desired products in high yields, longer reaction times, or higher temperatures were required.

Enantiomeric excess of (*R*)-esters from the corresponding alcohols with aliphatic chains especially short aliphatic chains were lower than those with aryl rings and long chains, respectively. This was probably due to the size difference between R and methyl

Table 2
Results of kinetic resolution of *sec*-alcohols[a]



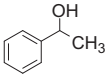
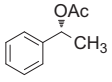
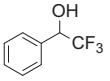
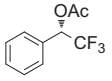
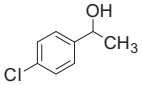
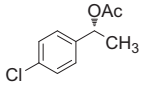
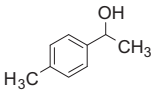
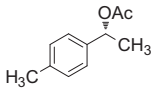
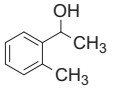
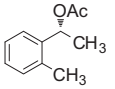
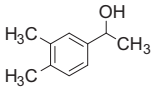
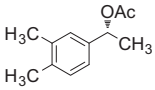
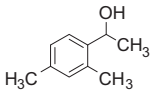
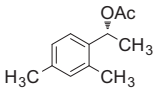
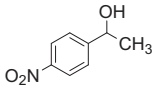
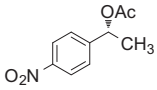
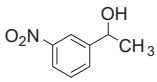
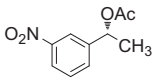
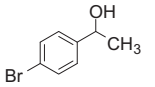
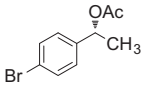
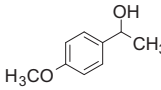
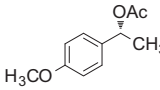
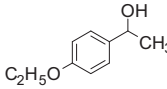
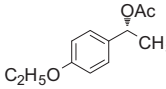
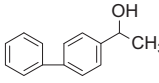
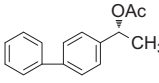
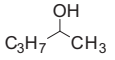
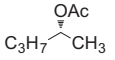
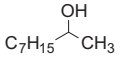
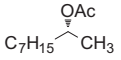
Entry	Substrate	1	Ester	2	Yield [b] (%, **2**/**3**)	ee_p (%)	ee_s (%)	Abs. config.[c] of **2c**	*E*[d]
1		**1a**		**2a**	45/47	89	97	*R*	72
2		**1b**		**2b**	0/93	—	0	—	—
3		**1c**		**2c**	45/48	96	93	*R*	>150
4		**1d**		**2d**	45/49	96	94	*R*	>180

Table 2 (*continued*)

Entry	Substrate	1	Ester	2	Yield [b] (%, **2**/**3**)	ee_p (%)	ee_s (%)	Abs. config.[c] of **2c**	*E*[d]
5		**1e**		**2e**	37/55	99	66	*R*	>700
6		**1f**		**2f**	39/47	96	82	*R*	>120
7		**1g**		**2g**	37/52	96	63	*R*	93
8		**1h**		**2h**	43/55	95	75	*R*	80
9		**1i**		**2i**	44/48	99	99	*R*	>1000
10		**1j**		**2j**	43/47	95	92	*R*	>100
11		**1k**		**2k**	44/48	92	96	*R*	>100
12		**1l**		**2l**	39/47	93	65	*R*	54
13[e]		**1m**[e]		**2m**	37/55	99	66	*R*	>400
14		**1n**		**2n**	21/69	80	28	*R*	11
15		**1o**		**2o**	23/68	83	22	*R*	13

[a] Reactions were performed on a 6 mL scale (*sec*-alcohols, 0.4 mmol; crude BioH esterase powder, 12 mg; vinyl acetate, 1.5 mL; *n*-hexane, 4.5 mL; reaction time, 28 h at 35 °C). Unless otherwise stated, ee values were determined by GC-analysis with a chiral column (Cyclodex-B, 30 m × 0.32 mm × 0.25 μm, Agilent Technologies).
[b] Isolated yields
[c] Absolute configuration of esters were determined by comparison of the sign of their optical rotations with the data published in the literature.[6]
[d] *E* values were calculated from ee_s and ee_p. $E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$, $c = ee_s/ee_s + ee_p$.
[e] ee values were determined by HPLC analysis with a chiral AD-H column.

group which was not so bigger according to Kazlauskas' rule.[8] Small size R group enabled (*S*)-alcohols to be fitted into the active pocket leading to its hydroxyl group close to the catalytic site forming (*S*)-esters. Figure 2 (**2a**, **2b**) showed the active site binding with a (*S*)-1-phenylethyl acetate molecular by molecular docking method, part of the active pocket entrance was utilized as the large pocket. Due to small size of the entrance aperture, only short linear aliphatic alcohols or no substituted aryl rings could be accommodated within it. For those with groups in big size, the chance of forming (*S*)-configuration esters was less. For example, when substrate **1n** was applied in this reaction, the acylated **2n** was obtained with ee value of 93%, however, *E* value decreased to 53. Nevertheless, when substrate **1m** was employed, ee value of **2m** was 99% and *E* value was >300 (Table 2, entries 13 and 14). With the sizes of R groups becoming bigger, *ee* values of the corresponding esters became higher (Table 2).

In conclusion, we have found a new organic solvent tolerant esterase with high enantioselectivity for the kinetic resolution of *sec*-alcohols simply by free enzyme powder, no immobilization, or addition of organic polymers (like PLE) were required to exhibit its activity. And the corresponding esters were efficiently resolved with ee values of up to 99%. We believe that *E. coli* BioH has potential applications to take the place of lipases for producing enantiomerically pure chemicals.

Acknowledgments

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Supplementary data

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References and notes

1. (a) Koga, N.; Kishimoto, J.; Haze, S. I.; Ifuku, O. *J. Ferment. Bioeng.* **1996**, *81*, 482–487; (b) Entcheva, P.; Phillips, D. A.; Streit, W. R. *Appl. Environ. Microbiol.* **2002**, *68*, 2843–2848; (c) Farina, V.; Reeves, J. T.; Senanayake, C. H.; Song, J. J. *Chem. Rev.* **2006**, *106*, 2734–2793; (d) Lee, J. H.; Han, K.; Mahn-Joo Kim, M. J.; Park, J. *Eur. J. Org. Chem.* **2010**, 999–1015; (e) France, S.; Guerin, D. J.; Miller, S. J.; Lectka, T. *Chem. Rev.* **2003**, *103*, 2985–3012.

2. (a) Lee, J. H.; Han, K.; Kim, M. J.; Park, J. *Eur. J. Org. Chem.* **2010**, *8*, 999–1015; (b) Liljeblad, A.; Kanerva, L. T. *Tetrahedron* **2006**, *62*, 5831–5854; (c) Ghanem, A. *Tetrahedron* **2007**, *63*, 1721–1754; (d) Breuer, M.; Ditrich, K.; Habicher, T.; Hauer, B.; Keßeler, M.; Stürmer, R.; Zelinski, T. *Angew. Chem., Int. Ed.* **2004**, *43*, 788–824; (f) Theil, F. *Chem. Rev.* **1995**, *95*, 2203–2227.
3. Gais, H. J.; Jungen, M.; Jadhav, V. *J. Org. Chem.* **2001**, *66*, 3384–3396.
4. Gupta1, A.; Khare, S. K. *Crit. Rev. Biotech.* **2009**, *29*, 44–54.
5. (a) Wang, B.; Tang, X. L.; Ren, G. F.; Liu, J.; Yu, H. W. *Biochem. Eng. J.* **2009**, *46*, 345–349; (b) Wang, B.; Liu, J.; Tang, X. L.; Cheng, C.; Gu, J. L.; Dai, L. Y.; Yu, H. W. *Tetrahedron Lett.* **2010**, *51*, 309–312; (c) Tang, X. L.; Liu, J.; Wang, B.; Yu, H. W. *World J. Microbiol. Biotechnol*, 2010. doi:10.1007/s11274-010-0437-0.
6. (a) Mavrynsky, D.; Päiviö, M.; Lundell, K.; Sillanpää, R.; Kanerva, L. T.; Leino, R. *Eur. J. Org. Chem.* **2009**, *9*, 1317–1320; (b) Arita, S.; Koike, T.; Kayaki, Y.; Ikariya, T. *Angew. Chem., Int. Ed.* **2008**, *47*, 2447–2449; (c) Zhu, Y. Z.; Fow, K. L.; Chuah, G. K.; Jaenicke, S. *Chem. Eur. J.* **2007**, 541–547.
7. (a) Sanishvili, R.; Yakunin, A. F.; Laskowski, R. A.; Skarina, T.; Evdokimova, E.; Kirby, A. D.; Lajoie, G. A.; Thornton, J. M.; Arrowsmith, C. H.; Savchenko, A.; Joachimiak, A.; Edwards, A. M. *J. Biol. Chem.* **2003**, *278*, 26039–26045; (b) Kwon; Min, A.; Hyun, S. K.; Joon, Y. O.; Bong, K. S.; Jae, K. S. *J. Microbiol. Biotechnol.* **2009**, *19*, 147–154.
8. Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656–2665.