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Enantioselective benzylic hydroxylation of indan and tetralin with *Pseudomonas monteilii* TA-5

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ABSTRACT

A set of 22 toluene- and ethylbenzene-degrading strains were screened for the enantioselective benzylic hydroxylation of indan and tetralin, and *Pseudomonas monteilii* TA-5 was discovered as an active and selective biocatalyst for such hydroxylations. Cells of *P. monteilii* TA-5 can be easily grown to a high density and demonstrated a specific hydroxylation activity of 24 U/g cdw (cell dry weight). Conditions for the hydroxylation of indan **1a** and tetralin **1b** with resting cells of this strain were optimized, to give the corresponding (*R*)-1-indanol **2a** and (*R*)-1-tetralol **2b** in 99% ee and 62–67% yields, respectively. No significant product inhibition was observed, and biohydroxylation with cell-free extracts suggested that the responsible hydroxylase is a soluble enzyme depending on either NADH or NADPH. Preparative biohydroxylation was demonstrated with resting cells as biocatalysts, affording (*R*)-**2a** in 99% ee and 65% yield, and (*R*)-**2b** in 99% ee and in 63% yield, respectively.

1. Introduction

Regio- and stereoselective hydroxylations provide direct access to chiral alcohols that are important building blocks or intermediates for the syntheses of fine chemicals, pharmaceuticals, and agrochemicals.[1] However, these types of transformations, including the hydroxylation on activated or *non*-activated carbon atoms remain as a significant challenge in classic chemistry.[2] For instance, benzylic hydroxylations of benzocycloarenes were examined by using conventional chemical methods[3] as well as organocatalysts,[4] but the enantioselectivities were low. On the other hand, biohydroxylations have become a useful alternative. Successful examples include selective hydroxylation *non*-activated atom[5] as well as benzylic hydroxylation.[6–12] For example, enantioselective benzylic hydroxylation of methyl phenylacetate with engineered cytochrome P450 BM-3[6a] and *Helminthosporium* sp. CIOC3316,[6b] respectively, gave the corresponding (*S*)-methyl mandelate in 90% ee[6a] and 92% ee,[6b] respectively.

Enantioselective benzylic hydroxylations of indan and tetralin to enantiopure 1-indanol and 1-tetralol are difficult to perform. A number of biocatalysts have been examined for these hydroxylations,[7–12] but the enantioselectivity or the productivity are not satisfactory: *Pseudomonas putida* F1, *P. putida* F39/D, and purified naphthalene dioxygenase from *Pseudomonas* sp. NCIB9816-11 were found to catalyze the hydroxylation of indan, affording 1-indanol in 83% ee (*R*) and 96% ee (*R*), and 92% ee (*S*), respectively, with an unspecified yield;[7,8] Purified P450cam monooxygenase and mutant were reported for the hydroxylation of indan and tetralin to give (*R*)-1-indanol in 87% ee and (*R*)-1-tetralol in 93–95% ee with unspecified yields;[9] *Fusarium moniliforme* MS31 was able to catalyze the hydroxylation of indan and tetralin, but the corresponding (*R*)-1-indanol and (*R*)-1-tetralol were obtained in only 9% ee;[10] *P. putida* UV4 was used to catalyze the hydroxylation of indan, to afford (*R*)-1-indanol in 34% yield and 98% ee;[11] Hydroxylation of indan and tetralin with *Mortierella isabellina* for 2 and 4 days, respectively, resulted in (*R*)-1-indanol in 64% yield and 86% ee and (*R*)-1-tetralol in 38% yield and 92% ee, respectively.[12] To discover more efficient and selective biocatalysts for these hydroxylations, we have been working on the screening of toluene- and ethyl benzene-degrading microorganisms. Herein we report our recent success on the identification of *P. monteilii* TA-5 as a highly active and enantioselective biocatalyst for the desired benzylic hydroxylation and the development of whole cells-based biohydroxylation for the efficient preparation of enantiopure (*R*)-1-indanol and (*R*)-1-tetralol.

2. Results and discussion

2.1. Screening of toluene- and ethylbenzene-degrading strains for enantioselective benzylic hydroxylation of indan and tetralin

In order to search for an efficient biocatalyst with high regio- and enantioselectivity for the benzylic hydroxylations, 22 strains were isolated from the sediment, and topsoils were collected in

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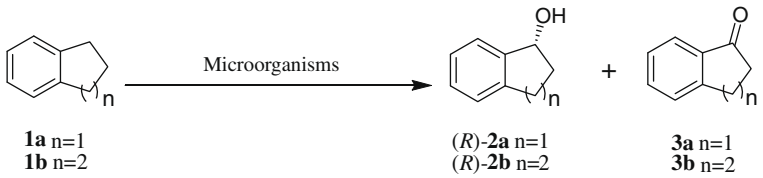
Table 1
Screening of efficient biocatalysts enantioselective benzylic hydroxylation of indan **1a** and tetralin **1b**



1a n=1
1b n=2

(*R*)-**2a** n=1
(*R*)-**2b** n=2

3a n=1
3b n=2

Entry	Strains	Sub[a]	(*R*)-**2a** Yield[b] (%)	(*R*)-**2a** ee[b] (%)	**3a** Yield[b] (%)	Sub[a]	(*R*)-**2b** Yield[b] (%)	(*R*)-**2b** ee[b] (%)	**3b** Yield[b] (%)
1	*Pseudomonas* sp. TA-1	**1a**	21	94	17	**1b**	64	90	30
2	*Pseudomonas monteilii* TA-2	**1a**	—	—	0	**1b**	89	96	2
3	*Pseudomonas monteilii* TA-4	**1a**	29	97	2	**1b**	67	91	1
4	*Pseudomonas monteilii* TA-5	**1a**	39	99	5	**1b**	92	96	2
5	*Pseudomonas monteilii* TB-1	**1a**	30	96	18	**1b**	59	89	25
6	*Pseudomonas monteilii* TB-2	**1a**	47	97	6	**1b**	99	95	3
7	*Pseudomonas monteilii* TB-3	**1a**	31	98	4	**1b**	94	94	4
8	*Pseudomonas monteilii* TB-4	**1a**	28	96	5	**1b**	99	94	3
9	*Pseudomonas monteilii* TB-5	**1a**	38	98	4	**1b**	94	93	5
10	*Pseudomonas monteilii* TC-1	**1a**	38	98	4	**1b**	57	99	18
11	*Rhodococcus coprophilus* TC-2	**1a**	7	22	4	**1b**	93	92	7
12	*Pseudomonas monteilii* TC-3	**1a**	49	98	5	**1b**	99	93	4
13	*Pseudomonas monteilii* TC-4	**1a**	54	97	8	**1b**	78	94	2
14	*Pseudomonas monteilii* TC-5	**1a**	48	98	5	**1b**	68	96	2
15	*Pseudomonas monteilii* TD-1	**1a**	48	75	0	**1b**	91	95	3
16	*Pseudomonas monteilii* TD-2	**1a**	49	98	5	**1b**	80	95	2
17	*Pseudomonas monteilii* TD-3	**1a**	—	—	5	**1b**	11	99	1
18	*Pseudomonas monteilii* TD-4	**1a**	34	98	4	**1b**	80	96	2
19	*Pseudomonas monteilii* EBV2-3	**1a**	26	35	9	**1b**	27	54	4
20	*Pseudomonas monteilii* EBC-3	**1a**	91	97	13	**1b**	97	99	4
21	*Pseudomonas monteilii* EBV2-1	**1a**	88	99	11	**1b**	79	99	2
22	*Cellulosimicrobium cellulans* EB8-4	**1a**	7	84	6	**1b**	40	94	3

[a] The reactions were run with 2 mM substrate in a 5-mL cell suspension (5 g cdw/L) of *P. monteilii* TA-5 in 50 mM KH_2PO_4–K_2HPO_4 buffer (pH 7.0) at 30 °C and 300 rpm for 45 min.
[b] Determined by HPLC analysis.

Singapore through enrichment with toluene or ethylbenzene as sole carbon sources. Eighteen toluene-degrading strains (Table 1, entries 1–18) and four ethylbenzene-degrading strains (Table 1, entries 19–22) were tested for biohydroxylation of **1a** and **1b**. As shown in Table 1, nearly all 22 strains were able to catalyze the hydroxylation of **1a** and **1b** to give the corresponding *sec*-alcohol (*R*)-**2a** and (*R*)-**2b** as the major product, and the corresponding ketones **3a** and **3b** as by products. The ee values of the obtained (*R*)-**2a** and (*R*)-**2b** at 45 min are often higher than 90%. Strains *P. monteilii* EBV2-1, *P. monteilii* EBC-3, and *P. monteilii* TA-5 were in particular found to be able to hydroxylate **1a** and **1b** with considerably high activity and enantioselectivity. Unfortunately, the former two strains did not work well at higher substrate concentrations: only trace amounts of product were formed when substrate concentration of **1a** and **1b** was increased from 2 mM to 5 mM. On the other hand, *P. monteilii* TA-5 showed higher yields when substrate concentration was increased from 2 mM to 5 mM. This demonstrates that the *P. monteilii* TA-5 has a higher substrate tolerance. Therefore, the strain *P. monteilii* TA-5 was selected for further investigation on the enantioselective hydroxylation of indan **1a** and tetralin **1b**. The name of the strain TA-5 was suggested by 16S-rDNA sequence analysis by Microcheck Inc. (Northfield, USA): this strain showed a 99.77% sequence identity with *P. monteilii*.

2.2. Cell growth and hydroxylation activity of *P. monteilii* TA-5

The cells of *P. monteilii* TA-5 were grown in an M9 medium with the vapor of toluene. Samples were taken at different time points for the determination of optical density at 450 nm. At the same time, cells in the samples were harvested and resuspended in buffer for the hydroxylation of **1a** for 15 min to examine the specific activity. As shown in Figure 1, cells grew fast and reached 3.4 g cdw/L after 18 h with a hydroxylation activity of 24 U/g cdw. The cell growth reached a late exponential phase at 21 h, and after that time point the specific activity of the cells slightly decreased. Nevertheless, the cells after 24 h still retained an activity of 18 U/g cdw.

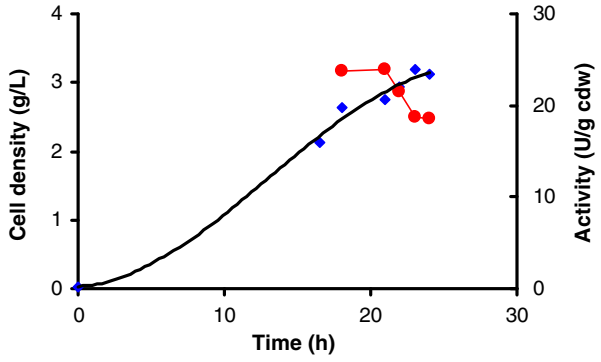


Figure 1. Growth curve and specific activity of *P. monteilii* TA-5 grown on toluene in M9 medium. ●: Activity, ◆: cell density.

2.3. Enatioselective benzylic hydroxylation of indan and tetralin with resting cells of *P. monteilii* TA-5

The effect of substrate concentration and different pH values on the productivity and enantioselectivity in hydroxylation of **1a** and

1b with resting cells of *P. monteilii* TA-5 was examined. As shown in Table 2 (entries 1–5), hydroxylation of 6–10 mM **1a** at a cell density of 10 g cdw/L and pH 7.0 for 4 h gave 35–67% of (*R*)-**2a** in 97–99% ee and 10–14 % of **3a**. The best results are obtained with 8 mM of **1a**, leading to the formation of 67% of (*R*)-**2a** in 99% ee. pH of 7.0 was shown to be better than pH of 6.0 or 8.0 from entries 2, 6, and 7. On the other hand, hydroxylation of 7 mM tetralin **1b** afforded the (*R*)-**2b** with 62% yield and 99% ee (entry 8). In addition, the biohydroxylations of indan or tetralin were compared with or without glucose (2%) in the reaction medium, but no effect on the product yield or ee was observed.

The time course of biohydroxylations of 8 mM indan **1a** with resting cells of *P. monteilii* TA-5 at a density of 10 g cdw/L is shown in Figure 2. The reaction was very fast at the first 1 h; 4.6 mM (*R*)-**2a** and 0.3 mM (*S*)-**2a** were formed after 1 h. Both (*R*)-**2a** and (*S*)-**2a** were found to be oxidized to **3a** at very slow but nearly the same rates. There was no reduction of **3a** to **1a.** The ee of product (*R*)-**2a** was increased to 99% after 4 h, and 5.4 mM (*R*)-**2a** was obtained (entry 3). Hydroxylation of tetralin **1b** afforded (*R*)-**2b** in 81% ee at 1 h, and similar to the above observation, the product ee was increased to 99% at 7 h and 4.4 mM of (*R*)-**2b** was produced (entry 8).

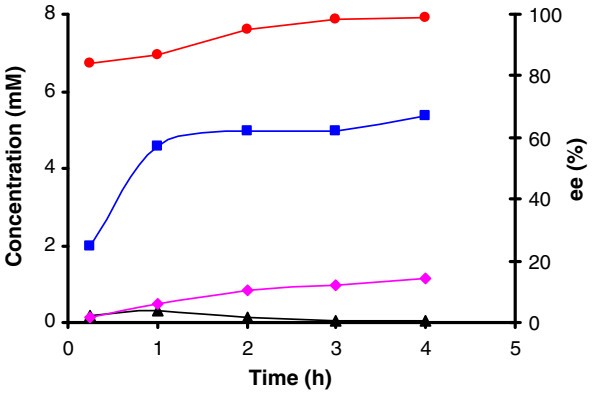


Figure 2. Biohydroxylation of indan **1a** (8.0 mM) with resting cells of *P. monteilii* TA-5 (10 g cdw/L) in 10 mL 50 mM KH_2PO_4–K_2HPO_4 (pH 7.0). ● ee of **2a**, ■ concentration of (*R*)-**2a**, ▲ concentration of (*S*)-**2a**, ♦ concentration of **3a**.

2.4. Inhibition of indanol on the hydroxylation of indan with resting cells of *P. monteilii* TA-5

To investigate whether there are significant product inhibitions for the hydroxylation of indan **1a**, (*R*)-**2a** at different concentrations was added to a resting cell suspension (10 g cdw/L) in

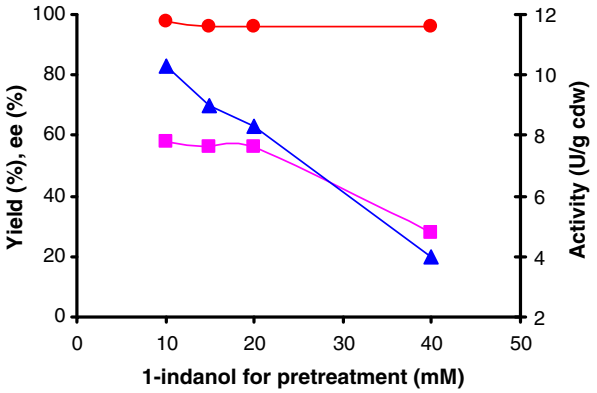


Figure 3. Effect of the concentration of (*R*)-1-indanol **2a** on the enantioselective hydroxylation of 7 mM indan **1a** with a resting cell (10 g cdw/L) of *P. monteilii* TA-5 (●: ee, ▲: activity, ■: yield).

KH_2PO_4–K_2HPO_4 buffer (pH 7.0); the mixture was incubated at 30 °C and 300 rpm for 1 h; after centrifugation, the cells were washed twice with KH_2PO_4–K_2HPO_4 buffer (50 mM, pH 7.0), and then resuspended in KH_2PO_4–K_2HPO_4 buffer (pH 7.0) to examine the hydroxylation of **1a**. As shown in Figure 3, there was no significant influence on the final product yield when cells were pretreated with (*R*)-**2a** at lower than 20 mM, although the specific activity was reduced. Thus, it was possible to use as high as 20 mM indan for the enzymatic hydroxylation without indanol inhibition. This indicates that the stopping of the reaction after 1 h shown in Figure 2 was not caused by the product inhibition. Increasing (*R*)-**2a** concentration to 40 mM for the pretreatment of the cells resulted in decrease of 60% activity and 50% of final product yields.

2.5. Enatioselective benzylic hydroxylation of indan and tetralin with soluble cell-free extracts of *P. monteilii* TA-5

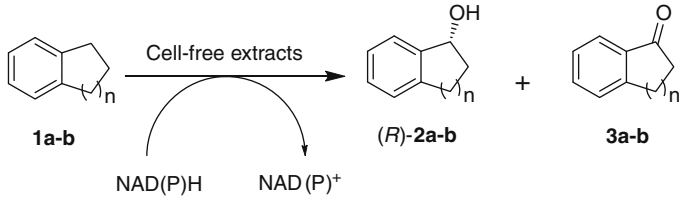
To obtain some information on the enzyme in *P. monteilii* TA-5 that is responsible for the benzylic hydroxylation, hydroxylation with cell-free extracts of strain TA-5 was investigated. The soluble cell-free extracts were prepared by passage of the cell suspension (20 g cdw/L in 50 mM KH_2PO_4–K_2HPO_4 buffer at pH 7.0) through a French press, followed by removal of the cell debris and membranes by centrifugation at 16,000 rpm for 30 min. As shown in Table 3, incubation of **1a** or **1b** and the equivalent NADH or NADPH with the soluble cell-free extracts gave the same products and same ee of (*R*)-**2a**–**b** as those generated with resting cells. In comparison, biohydroxylation of **1a–b** with the soluble cell-free extracts without NADH or NADPH gave only a small amount of

Table 2
Optimization of the reaction conditions for the hydroxylation of indan and tetralin with *P. monteilii* TA-5

Entry	Substrate[a]	Concentration (mM)	Time (h)	pH	Activity[b] (U/g cdw)	(*R*)-**2a**		**3a** Yield[c] (%)	*R*)-**2b**		**3b** Yield[c] (%)
						Yield[c] (%)	ee[c] (%)		Yield[c] (%)	ee[c] (%)	
1	**1a**	6	4	7.0	15	35	99	11			
2	**1a**	7	4	7.0	12	63	99	12			
3	**1a**	8	4	7.0	13	67	99	14			
4	**1a**	9	4	7.0	13	57	98	12			
5	**1a**	10	4	7.0	13	47	97	10			
6	**1a**	7	4	6.0	20	58	99	19			
7	**1a**	7	4.5	8.0	21	21	99	14			
8	**1b**	7	7	7.0	13				62	99	9
9	**1b**	8	4	7.0	10				56	92	6
10	**1b**	10	4	7.0	10				45	91	5

[a] The reactions were run with a 10-mL cell suspension (10 g cdw/L) of *P. monteilii* TA-5 in 50 mM KH_2PO_4–K_2HPO_4 buffer (pH 7.0) at 30 °C and 300 rpm.
[b] Activity was determined over the first 15 min.
[c] Determined by HPLC analysis.

Table 3
Hyroxylation of indan and tetralin with soluble cell-free extracts of *P. monteilii* TA-5



Entry	Substrate[a]	Cofactor	(*R*)-**2a**		**3a** Yield[b] (%)	(*R*)-**2a**		**3b** Yield[b] (%)
			Yield[b] (%)	ee[b] (%)		Yield[b] (%)	ee[b] (%)	
1	**1a**	—	19	97	3			
2	**1a**	NADH	44	98	9			
3	**1a**	NADPH	37	99	13			
4	**1b**	—				2	99	0.2
5	**1b**	NADH				10	98	2
6	**1b**	NADPH				5	99	1

[a] The reactions were run with 2 mM substrate in a 2-mL solution (protein concentration 11.19 g cdw/L, 50 mM KH_2PO_4–K_2HPO_4 buffer), at 30 °C and 300 rpm for 1 h.
[b] Determined by HPLC analysis.

product. These results indicated that *P. monteilii* TA-5 contained a soluble NADH- or NADPH-dependent monooxygenase for the benzylic hydroxylations. Obviously, NADH is the more preferred cofactor than NADPH.

2.6. Preparation of (*R*)-1-indanol and (*R*)-1-tetralinol by enantioselective benzylic biohydroxylation with resting cells of *P. monteilii* TA-5

Preparative biotransformation of indan **1a** and tetralin **1b** was carried out in a 500-mL bioreactor containing substrate **1a** (41.3 mg, 0.35 m mol) or **1b** (46.2 mg, 0.35 m mol) in a 50-mL suspension of resting cells (10 g cdw/L) in 50 mM of KH_2PO_4–K_2HPO_4 buffer (pH 7.0). The reactions were performed for 7 h. After work up with centrifugation and extraction with ethylacetate (EtOAc), the products were purified by flash chromatography on a silica gel column (10% EtOAc: 90% *n*-hexane). This gave 65% yield (30.5 mg) of (*R*)-**2a** in 99% ee and 63% yield (32.0 mg) of (*R*)-**2b** in 99% ee.

3. Conclusion

Twenty two toluene or ethylbenzene degrading strains were found to catalyze the enantioselective benzylic hydroxylation of indane **1a** and tetralin **1b**. *P. monteilii* TA-5 is among the best strains with high activity, high conversion, and excellent regio- and enantioselectivity. The strain grows fast, high cell density is easy to achieve; the resting cells show a high hydroxylation activity of 24 U/g cdw. Optimal reaction conditions with the resting cells as biocatalysts have been established and preparative biohydroxylation has been demonstrated. Hydroxylation of indan **1a** and tetralin **1b** with resting cells of *P. monteilii* TA-5, respectively, afforded (*R*)-**2a** in 99% ee and 65% yield, and (*R*)-**2b** in 99% ee and 63% yield, respectively. Thus, we have discovered and developed *P. monteilii* TA-5 as the best catalyst known thus far for the highly active and enantioselective benzylic hydroxylation of indan **1a** and tetralin **1b**.

4. Experimental

4.1. General

^1H and ^{13}C NMR spectra were determined at 500 (^1H) and 75 (^{13}C) MHz, all in $CDCl_3$, with chemical shifts in ppm relative to TMS and coupling constants *J* in hertz. The purity of product and ee were determined with ShimadzuTM Prominence HPLC on a Daicel™ OB-H chiral column (250 × 4.6 mm, 5 μm) at 25 °C. Optical rotations were determined in chloroform at 22 °C with a JascoTM spectropolarimeter. Indan (>99%), tetralin (>99%), (*R*)-1-indanol (>97%), (*R*)-1-tetralol (>97%), (*S*)-1-indanol (>97%), (*S*)-1-tetralol (>97%), indanone (>99%), and tetralone (>99%) were purchased from Sigma–Aldrich. 22 microorganisms used for screening were isolated and are available in our laboratory at Department of Chemical and Biomolecular Engineering, National University of Singapore.

4.2. Analytical method

The ee values of bioproducts (*R*)-**2a** and (*R*)-**2b** were determined with a Shimadzu™ Prominence HPLC on a Daicel™ OB-H chiral column (250 × 4.6 mm, 5 μm) at 25 °C. The mobile phase used was hexane/isopropanol (95:5), 1 mL/min monitored at 210 nm. Retention time: 4.1 min for **1a**, 8.4 min for (*R*)-**2a**, 13.1 min for (*S*)-**2a**, 19.9 min for **3a**.; 3.8 min for **1b**, 7.3 min for (*R*)-**2b**, 10.9 min for (*S*)-**2b**, 13.4 min for **3b**, respectively. The absolute configuration of the biohydroxylation products **2a** and **2b** was assigned by comparison of the retention time in HPLC analysis with those of the standard sample of (*R*)-1-indanol and (*R*)-1-tetralol.

4.3. Isolation of microorganisms and general culture conditions

Thirty soil samples, five sea water samples, and five sewage sludge samples were collected from Singapore. Luria Bertani (LB) broth and minimal M9 medium[13] supplemented with trace element[14] and different carbon (0.1% v/v) sources were used throughout this study. Agar plates of the above-mentioned media were prepared by adding 20 g of bacto™ agar per 1 L of medium. All cultures in liquid medium were incubated in a shaker at 30 °C and 250–300 rpm unless otherwise stated. All cultures on agar plate were grown aerobically at room temperature with carbon source supplied in vapor phase in desiccators unless otherwise stated. The procedure for the isolation of the microorganisms is as follows: enrichment cultures were prepared by adding 1 g of soil or 1 mL liquid of collected samples to 10 mL M9 medium and then supplying toluene or ethylbenzene as carbon source in a tube within a 28-mL closed glass culture bottle with a metal screw cap. After incubation for 2 weeks at 30 °C and 250 rpm, 0.1 mL diluted enrichment cul-

ture (1000–100,000 dilution) was dispensed onto M9 agar plates. These plates were incubated in desiccators with the vapor of toluene or ethylbenzene as the carbon source. The generated colonies were isolated and transferred onto a new agar plate. Pure strain was obtained by repeatedly transferring single colonies of the strain onto a new agar plate.

4.4. General screening procedure of microorganisms for enantioselective benzylic hydroxylation of indan and tetralin

At first, 1 μL inoculated strain was transferred from M9 agar plate into LB liquid medium. The culture was allowed to grow for 24 h at 30 °C and 300 rpm. One milli liter of LB seed culture was then transferred into 50-mL M9 medium supplemented with trace element in 125-mL conical flask, a plastic tube with length of 7.8 cm containing 0.5 mL toluene or ethylbenzene was put into the flask and the vapor of toluene or ethylbenzene was used as carbon source. The cells were grown at 30 °C and 250 rpm for 2 days and harvested by centrifugation at 8000 rpm for 10 min. The cells of microorganism were suspended in 5 mL 50 mM KH_2PO_4–K_2HPO_4 buffer (pH 7.0) to a density of 5 g cdw/L. Two milli molar of indan **1a** or tetralin **1b** was added and the mixtures were shaken at 30 °C and 300 rpm for 45 min. 1-mL samples were taken, cells were removed by centrifugation, and the product was extracted with 1 mL ethyl acetate containing 1 mM benzyl alcohol as internal standard. The organic phase was analyzed by HPLC.

4.5. Cell growth and hydroxylation activity of *P. monteilii* TA-5

At first, 1 μL inoculated strain was transferred from M9 agar plate into 5 mL LB medium in 28 mL glass bottle with screw cap. The culture was shaken at 30 °C and 300 rpm for 7 h and then added into 100 mL medium M9 liquid containing trace element in a 250-mL shaking flask with ventilated plastic stopper to reach initial cell density of 0.1 g cdw/L. Fifteen milli liters of plastic tube with length of 9 cm containing 1 mL toluene were put into the flask, and the vapor of toluene was used as carbon source. The culture was incubated at 30 °C and 250 rpm. After 18 h, 10-mL samples were taken after one- or two-hour intervals, the cell density was measured by OD at 450 nm. The cells were then harvested and resuspended in buffer for biohydroxylation of 2 mM indan **1a** for 15 min to get the specific hydroxylation activity. The cell growth reached the late exponential stage at 21 h with highest hydroxylation activity of 24 U/g cdw. The results are given in Figure 1.

4.6. General procedure for enantioselective benzylic hydroxylation of indan and tetralin with resting cells of *P. monteilii* TA-5

Cells of *P. monteilii* TA-5 were suspended in 10 mL of 50 mM KH_2PO_4–K_2HPO_4 buffer (pH 6.0–8.0) to a density of 10 g cdw/L, **1a** and **1b** (6–10 mM) was added and the mixture was shaken at 300 rpm and 30 °C. The reaction was followed by HPLC analysis: samples (0.5 mL) were taken at predetermined time points, cells were removed by centrifugation, products were extracted with 1 mL ethyl acetate containing 1 mM benzyl alcohol as internal standard, and organic phase was analyzed. Hydroxylation of **1a** and **1b** afforded (*R*)-**2a** and (*R*)-**2b** in high ee and good yields, shown in Table 2.

4.7. Investigation of product inhibition on enantioselective benzylic hydroxylation of idan with resting cell of *P. monteilii* TA-5

At first, 10–40 mM (*R*)-**2a** was added to a resting cell suspension (10 g cdw/L) in 10 mL KH_2PO_4–K_2HPO_4 buffer (pH 7.0), the mixture was incubated at 30 °C and 300 rpm for 1 h. After centrifugation, the cells were washed twice with 25 mL KH_2PO_4–K_2HPO_4 buffer (50 mM, pH 7.0), then resuspended in 10 mL KH_2PO_4–K_2HPO_4 buffer (pH 7.0). The substrate indan (7 mM) was added and the mixture was incubated at 30 °C and 300 rpm for 7 h. The results are shown in Figure 3.

4.8. Enantioselective benzylic hydroxylation of indan and tetralin with soluble cell-free extracts of *P. monteilii* TA-5

Cells of *P. monteilii* TA-5 were prepared from a 21-h culture as described above. The cells were resuspended in KH_2PO_4–K_2HPO_4 buffer (50 mM pH 7.0) to a density of 20 g cdw/L. The cell suspension was disrupted using cell disrupter at 30 kPa for 5 min. The cell debris was removed by centrifugation at 16,099*g* at 4 °C for 40 min and the supernatant was collected. The protein concentration of the cell-free extract was 11 g/L determined by using the Bradford protein content assay. Three parallel reactions were performed for 1 h: 5-mL cell-free extract and 2 mM **1a**; cell-free extract and 2 mM **1a** and 2 mM NADH; cell free extract and 2 mM **1a** and NADPH in three different flasks. Samples were taken and analyzed by HPLC, and the results are listed in Table 3.

4.9. Preparation of 1-indanol (*R*)-2a and 1-tetralol (*R*)-2b by biohydroxylation

The cells of *P. monteilii* TA-5 were suspended in 50 mL of 50 mM KH_2PO_4–K_2HPO_4 buffer (pH 7.0) to a density of 10 g cdw/L, substrate **1a** (41.3 mg, 0.35 mmol) or **1b** (46.2 mg, 0.35 mmol) was added, and the mixture was incubated in a shaker at 30 °C and 300 rpm for 7 h. The product was extracted with EtOAc, dried over $MgSO_4$, and concentrated by evaporation at reduced pressure. The crude product was purified by flash chromatography (10% EtOAc in *n*-hexane, R_f = 0.2). This gave 30.5 mg of (*R*)-**2a** and 32.0 mg of (*R*)-**2b**, corresponding to 65% and 63% yield, respectively. (*R*)-1-indanol **2a**: $[\alpha]_D^{22} = -35.2$ (*c* 1.05, $CHCl_3$) {lit.[15]: $[\alpha]_D^{23} = -36.1$ (c 0.05 $CHCl_3$)}; ^{1}H NMR ($CDCl_3$, 500 Hz): δ 7.44–7.42 (d, 1H, Ph), 7.29–7.22 (m, 3H, Ph), 5.26 (t, 1H, *J* = 6 Hz, CH), 3.10–3.04 (m, 1H, CH), 2.86–2.80 (m, 1H, CH), 2.53–2.46 (m, 1H, CH), 1.99–1.92 (m, 1H, CH), 1.76 (br s, 1H, -OH); ^{13}C NMR ($CDCl_3$, 75 Hz): δ 145.0, 143.3, 128.3, 126.7, 124.9, 124.2, 76.5, 36.0, 29.8. (*R*)-1-tetralol-**2b**: $[\alpha]_D^{22} = -34.9$ (*c* 1.12, $CHCl_3$) {lit.[15]: $[\alpha]_D^{23} = -33.2$ (c 0. 31 $CHCl_3$)}; ^{1}H NMR ($CDCl_3$, 500 Hz): δ 7.42–7.27 (m, 1H, Ph), 7.26–7.23 (m, 2H, Ph), 7.19–7.15 (t, 1H, Ph), 4.80 (t, *J* = 6 Hz, 1H, CH), 2.87–2.84 m, 1H, CH), 2.74–2.72 (m, 1H, CH), 2.04–1.75 (m, 5H, CH_2, CH_2, OH); ^{13}C NMR (CCl_3, 75 Hz):138.8, 137.1, 128.9, 128.6, 127.5, 126.1, 68.1, 32.5, 29.2, 18.8.

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