Journal of Molecular Catalysis B: Enzymatic 66 (2010) 95–100

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Highly enantioselective hydrolysis of phenyl-1,2-ethanediol cyclic carbonates by newly isolated *Bacillus* sp. ECU0015

Lei Chang, Li-Ming Ouyang*, Yi Xu, Jiang Pan, Jian-He Xu*

Laboratory of Biocatalysis and Bioprocessing, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Meilong Road 130, Shanghai 200237, PR China

ARTICLE INFO

Article history:
Received 21 October 2009
Received in revised form 24 March 2010
Accepted 27 March 2010
Available online 7 April 2010

Keywords:
Cyclic carbonate
1-Phenyl-1,2-ethanediol
Enantioselective hydrolysis
Hydrolase
Bacillus sp.

ABSTRACT

A bacterial strain (No. ECU0015), which catalyzes the hydrolysis of phenyl-1,2-ethanediol cyclic carbonates (4-phenyl-1,3-dioxolan-2-one, **1**) to (*S*)-1-phenyl-1,2-ethanediol (**2**) with high enantioselectivity, was newly isolated from soil samples utilizing the cyclic carbonate as sole carbon and energy source. The bacterium was later identified as *Bacillus* species by its 16S rDNA sequence and phylogenetic analysis. The optimal reaction temperature and pH for the asymmetric hydrolysis of **1** using whole cells were 35 °C and pH 7.3, respectively. Partial bio-oxidation of the produced (*R*)-diol was observed, resulting in an increase in the ee_p (enantiomeric excess of product) of the main product (*S*)-diol. Under the improved reaction condition, the target product (*S*)-diol was prepared in gram scale, affording an excellent ee_p (>99%) with a moderate yield (27.8%) as compared to the maximum theoretical yield of 50% for kinetic resolution. This strain of *Bacillus* sp. also displayed fairly good activity and enantioselectivity towards some other compounds tested, such as 2-acetoxy-2-phenylacetic acid (**3**) and its derivatives.

1. Introduction

Because optically active diols and their cyclic carbonates are important intermediates for the synthesis of pharmaceutical and natural products, many efforts had already been devoted to effective preparation of such compounds with different methods [1–4]. However, the chemical synthesis of optically pure diols or cyclic carbonates is still rather difficult and thus needs to be further improved.

Enzymatic hydrolysis of cyclic carbonates is an attractive method for the preparation of optically active diols because of the unique advantages of bioreaction, such as no need of cofactors for the hydrolytic reaction [5,6] and the high selectivity of hydrolase. The carbonic acid produced in the reaction can be decomposed to carbon dioxide and quickly released from the system, which will facilitate the bioconversion to diol and the product purification as well. Over the past several years, the application of porcine pancreas lipase (PPL) or pig liver esterase (PLE) in the enantioselective hydrolysis of mono-substituted cyclic carbonates to obtain chiral 1,2-diols had received considerable attentions [7–11]. However, PPL or PLE is relative expensive and unstable as a free enzyme for the transformations of cyclic carbonates. On the contrary, enantioselective hydrolysis of racemic cyclic carbonate catalyzed by the microorganism whole-cell systems would be much cheaper and more convenient. To the best of our knowledge, only one microbial strain, *Pseudomonas diminuta*, has so far been reported and used for such a biocatalytic hydrolysis. However, it could only hydrolyze C2-symmetrical cyclic carbonates [12,13]. Therefore, the screening for new microorganisms with high activity and stereoselectivity for the hydrolysis of racemic cyclic carbonates become imperative.

The objective of the present study was to screen for a microbial strain that harbors a stereoselective cyclic carbonate hydrolase for the biocatalytic resolution of racemic cyclic carbonate (**1**) to (*S*)-diol (**2**) with high conversion and good selectivity (Scheme 1).

2. Experimental

2.1. Soil sample, reagents and medium

Soil samples used for the isolation of bacteria with hydrolytic activity were collected from different places of Shanghai, Jiangsu and Shandong Provinces, China. Styrene oxide was purchased from Sigma Chemical Co., USA. Cyclic carbonate **1**, racemic diol **2**, 2-acetoxy-2-phenylacetic acid **3** and its derivative (2-acetoxy-2-(3′-chlorophenyl) acetic acid) **4** were prepared by our lab with details described in Sections 2.2 and 2.3. All other chemicals were obtained from commercial suppliers as reagent grade.

The mineral salts medium (MSM) for enrichment culture consisted of the following components (per liter): $(NH_4)_2SO_4$ 1.0 g,

* Corresponding authors. Fax: +86 21 6425 0840.
E-mail addresses: ouyanglm@ecust.edu.cn (L.-M. Ouyang), jianhexu@ecust.edu.cn (J.-H. Xu).

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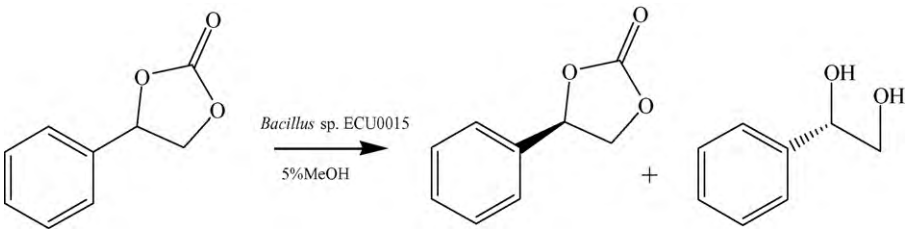
doi:10.1016/j.molcatb.2010.03.011



Scheme 1. Enantioselective hydrolysis of phenyl-1,2-ethanediol cyclic carbonate **1** to (*S*)-diol **2** by the resting cells of *Bacillus* sp. ECU0015.

$K_2HPO_4{\cdot}3H_2O$ 6.0 g, KH_2PO_4 3.0 g, NaCl 0.5 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, $CaCl_2$ 0.05 g, pH 7.0. The rich medium consisted of the following components (per liter): glycerol 15 g, yeast extract 5.0 g, peptone 5.0 g, $Na_2HPO_4{\cdot}12H_2O$ 2.7 g, KH_2PO_4 1.0 g, $MgSO_4$ 0.5 g. The medium was adjusted to pH 7.0 with NaOH before autoclaved at 121 °C for 20 min.

2.2. Preparation of 1-phenyl-1,2-ethanediol (PED) and the corresponding cyclic carbonate (PDC)

The racemic diol **2** was prepared from styrene oxide. Styrene oxide (1.5 g) was dissolved in 100 ml THF containing 10 ml water. Then 0.2 ml H_2SO_4 was added and the reaction mixture was stirred at 50 °C for 24 h. When the conversion reached 100%, saturated $NaHCO_3$ aqueous solution was added to neutralize the H_2SO_4 and then the product (diol **2**) was extracted with ethyl acetate. The organic phase was dried over anhydrous Na_2SO_4 and then most of the solvent was removed by vacuum distillation. The product was crystallized with ethyl acetate and petroleum ether. The purity of the product was tested by HPLC and the structure of product and substrate was confirmed by NMR.

The cyclic carbonate **1** was prepared from racemic diol **2** by transesterification with dimethyl carbonate catalyzed by heat and strong alkaline. The product, cyclic carbonate **1**, was separated by silica gel column chromatography with mobile phase of petroleum ether and ethyl acetate (3/1, v/v). The purity of the product was tested by HPLC and the structure of product was confirmed by NMR too.

2.3. Preparation of 2-acetoxy-2-phenylacetic acid ***3*** and its derivative ***4***

The organic acid **3** and its derivative **4** were prepared from the corresponding mandelic acid. The mandelic acid and equal equivalent of acetyl chloride were dissolved in 1,4-dioxane and refluxed for 12 h. When the conversion reached 100%, the reaction was stopped and the product mixture was vacuum distilled and the resultant white crystals were dried for NMR verification.

2.4. Isolation of hydrolase-producing microorganisms with high activity on cyclic carbonate ***1***

2.4.1. Primary screening for strains with high conversion rate

A tiny of each soil sample was suspended in test tubes containing 2 ml of mineral salts medium. Then the suspension was supplemented with 40 μl of 0.5 M cyclic carbonate (**1**) in methanol as the carbon source, giving a final substrate concentration of 10 mM. The enrichment culture was shaken aerobically at 160 rpm and 30 °C for 1–2 days. Then the reaction was stopped and the mixture was extracted by addition of 0.3 ml ethyl acetate. After centrifuge, the ethyl acetate phase was dried over anhydrous Na_2SO_4. Thin-layer chromatography (TLC) assay was performed using petroleum ether/ethyl acetate (3:1, v/v) as developing agent. And then, the soil culture samples with hydrolase-producing microorganisms, which were identified by the TLC, were inoculated onto the agar plate of the rich medium. The grown colonies were inoculated individually to the rich liquid medium (2 ml) in test tubes. After 1–2 days culture, the hydrolytic activity was measured by TLC again. Positive strains were plated onto the rich medium agar and preserved for future use.

2.4.2. Secondary screening for microbial strains with high enantioselectivity

In the second round of screening, about 0.5–1.0 g (wet weight) of washed cells were suspended in 9.5 ml of 50 mM potassium phosphate buffer (KPB, pH 7.0) and 0.5 ml of 200 mM cyclic carbonate **1** in methanol was added to give a final concentration of 10 mM. The mixture was incubated at 30 °C and 160 rpm for 36 h. After extraction with ethyl acetate and dehydration overnight with anhydrous Na_2SO_4, samples (0.5 ml each) of the reaction mixture were withdrawn for measuring the substrate conversion and product enantiomeric excess (ee_p) by HPLC.

2.5. Identification of the bacterial strain ECU0015

Cell morphology of the bacterial strain ECU0015 was observed via an optical microscope. The bacterial genome DNA was extracted according to the procedure of Wilson [14]. The 16S rDNA was amplified by the polymerase chain reaction (PCR) with the universal primers (forward primer, 5′-AGAGTTTGATCCTGGCTCAG-3′; reverse primer, 5′-AAGGAGGTGATCCAGCCGCA-3′). The PCR reactions were performed with initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, extension at 72 °C for 1.5 min, and final extension at 72 °C for 5 min. PCR product was ligated into pMD18-T vector (Takara, Dalian, China) and sequenced. DNA analysis was completed using BLAST network services provided by the National Center for Biotechnology Information (NCBI), USA.

2.6. Monitoring of cell growth and enzyme production of Bacillus sp. ECU0015

The isolated strain, *Bacillus* sp. ECU0015, was grown aerobically in 250-ml Erlenmeyer flasks containing 50 ml of the rich medium at 30 °C and 160 rpm for 60 h. The sterilized rich medium was inoculated with 5% (v/v) of a 12 h pre-culture. At different time intervals, two flasks were withdrawn for the determination of enzyme activity, dry cell weight (DCW) and medium pH. The cells were harvested by centrifuge and used for hydrolytic activity assay. The pH value was determined by a pH meter and the dry cell weight was measured after drying the wet cells harvested from 10 ml of culture broth at 50 °C till a constant weight.

2.7. Enzyme assay

The hydrolytic activity of rest cells was measured by HPLC as follows. The cells harvested from 2 ml of the culture broth were washed with physiological saline solution (0.85% NaCl), then

resuspended in 0.48 ml of KPB (50 mM, pH 7.3). The mixture was preincubated on a mini-shaker (Thermomixer Compact, Eppendorf, Germany) at 30 °C and vortexed at 1100 rpm for 10 min. Then the reaction was started with the addition of 20 μl methanol solution containing 250 mM cyclic carbonate **1**. After 10 min of incubation, the reaction was stopped by extracted with 0.5 ml ethyl acetate. After centrifuge, the organic phase was withdrawn and dried over anhydrous Na_2SO_4, and then subjected to HPLC analysis to determine the quantity of (*S*)-diol (**2**) formed. One unit of hydrolytic activity was defined as the amount of enzyme catalyzing the formation of 1.0 μmol (*S*)-diol per minute under the above conditions.

2.8. Catalytic characteristics of Bacillus sp. ECU0015 resting cells

For the determination of optimal pH for the transformation by whole cells, 0.2 g wet resting cells and 10 mM cyclic carbonate **1** was added into 2 ml of buffer with pH varying from 5.0 to 9.0. Two kinds of buffers were used, viz. KPB for pH 5.0, 6.0, 7.0 and 8.0, and Na_2CO_3–$NaHCO_3$ buffer for pH 9.0. The reactions were carried out at 30 °C and 1000 rpm for 30 min.

To investigate the optimal reaction temperature, the reactions were set at different temperatures from 20 to 50 °C in buffer of pH 7.3 and the other reaction conditions were same as the above.

To investigate the thermal stability of the microbial enzyme, the cells were suspended (10%, w/v) in 20 ml KPB (100 mM, pH 7.3) and preserved at different temperature (4, 30 and 60 °C) for 5 days. Enzyme activity was determined each day by the standard method as described in Section 2.7.

To investigate the pH stability of the whole-cell biocatalyst, the cells were suspended (10%, w/v) in 20 ml 100 mM buffer with different pH (KPB for pH 5.0 and 7.3, Na_2CO_3–$NaHCO_3$ for pH 9.0) and preserved at 30 °C for 5 days. Enzyme activity was determined each day with the standard method as described in Section 2.7.

2.9. Bioconversion process of PDC with resting cells of Bacillus sp. ECU0015

The bioconversion of cyclic carbonate **1** with resting cells was performed in three-necked flask. Resting cells (1.0 g wet cell) was suspended in 9.6 ml of KPB (50 mM, pH 7.3), and 400 μl methanol solution of 250 mM cyclic carbonate **1** was added to give a final concentration of 10 mM. The reaction was carried out at 30 °C and 160 rpm. The bioconversion process was monitored with HPLC by withdrawing 500 μl each of samples at fixed time intervals. The reaction was stopped by adding 500 μl of ethyl acetate to 500 μl of the sample. The cells were removed by centrifugation and the organic phase was used for the determination of the product concentration and ee_p with HPLC.

2.10. Preparation of enantiopure (S)-diol **2** by resting cells of Bacillus sp. ECU0015 in gram scale

Fifteen grams of resting wet cells of *Bacillus* sp. ECU0015 were suspended in 96 ml of KPB (50 mM, pH 7.3), and 4.0 ml cyclic carbonate **1** solution (250 mM, dissolved in methanol) was added to give a final substrate concentration of 10 mM. The mixture was incubated at 30 °C and 160 rpm with rotated shaker. After incubation for 36 h, the mixture was saturated with NaCl and then extracted for four times with 50 ml ethyl acetate each time. After dried over anhydrous Na_2SO_4, the ethyl acetate was evaporated under reduced pressure. The crude product of (*S*)-diol **2** was purified by silica gel column chromatography with petroleum ether/ethyl acetate (4:1, v/v) as eluent. The product structure was confirmed by ^{1}H NMR. The absolute configuration was determined by comparing the optical rotation measured with that of literature.

2.11. Bioconversion of other ester substrates with resting cells of Bacillus sp. ECU0015

We also examined the hydrolytic reactions of other ester substrates, viz. **3**, **4** and dimethyl carbonate (DMC) **5** with resting cells of *Bacillus* sp. ECU0015. The reaction condition was the same as cyclic carbonate **1**. As for substrates of **3** and **4**, after 36 h of bioconversion, the cells were removed by centrifugation and the supernatant was acidified with concentrated H_2SO_4 to pH 1.0 and extracted with ethyl acetate. The ethyl acetate layer was collected, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain a crude crystal of (*R*)-mandelic acid or (*R*)-3-chloro-mandelic acid. The acids were purified by silica gel column chromatography. The eluent components are toluene–ethyl acetate–formic acid (5:1:0.2, v/v/v) for mandelic acid and 3-chloromandelic acid.

As for the substrate of DMC, the residual substrate was detected by gas chromatography (SHIMADZU GC-14C, β-cyclodextrin capillary column, length = 30 m, diameter = 0.5 mm, column temperature 150 °C, injector temperature 280 °C, detector temperature 320 °C) after 36 h of transformation.

2.12. HPLC method

The concentrations and enantiomeric excess (*ee*) of the diol **2** and its cyclic carbonate **1** were determined by HPLC (LC-10AT, Shimadzu, Japan) using a chiral column (Chiralcel OD, Ø4.6 mm × 250 mm, Daicel, Japan). The mobile phase was hexane/2-propanol (95:5, v/v) and the flow rate was 1.0 ml/min. Detection was made at 254 nm. The retention times for (*R*)-, (*S*)-**2** and (*R*)-, (*S*)-**1** were 20.8, 22.1, 38.9 and 44.9 min, respectively. The *ee* of (*S*)-**2** was calculated as follows, where [*S*] and [*R*] denote the concentrations of (*S*)-**2** and (*R*)-**2**, respectively:

$$ee = \frac{[S] - [R]}{[S] + [R]} \times 100\%$$

2.13. Compound characterization

1: ^{1}H NMR (500 MHz, MeOH), δ/ppm: 4.37 (t, *J* = 8.7 Hz, 1H), 4.82 (t, *J* = 8.2 Hz, 1H), 5.77 (t, *J* = 7.9 Hz, 1H), 7.41 (m, 5H).

(*S*)-**2**: $[\alpha]_D^{25} + 56.5$ (*c* 0.5, MeOH). ^{1}H NMR (500 MHz, MeOH), δ/ppm: 3.60 (t, *J* = 5.48 Hz, 2H), 4.67 (t, *J* = 6.03 Hz, 1H), 7.20–7.40 (m, 5H) [11].

(*R*)-Mandelic acid: $[\alpha]_D^{25} - 147.8$ (*c* 0.5, MeOH). ^{1}H NMR (500 MHz, MeOH), δ/ppm: 5.19 (s, *J* = 5.17 Hz, 1H), 7.33 (m, 5H).

(*R*)-3-Chloro-mandelic acid: $[\alpha]_D^{25} - 124.1$ (*c* 0.5, MeOH). ^{1}H NMR (500 MHz, MeOH), δ/ppm: 5.18 (s, *J* = 8.86 Hz, 1H), 7.29 (m, 3H), 7.44 (m, 1H) [15].

3. Results and discussion

3.1. Isolation and identification of microorganisms for biohydrolysis of PDC

In the first round of screening, many kinds of microorganisms were found to have hydrolytic activity on cyclic carbonate (**1**). Using different rich media, 500 strains were isolated from over 200 soil samples by enrichment culture and used for the analysis of hydrolytic activity on cyclic carbonate **1**. The amount of product formed was determined by TLC. Then 66 strains with obvious product (diol, **2**) spots on TLC plates were subjected to next round of screening.

During the secondary round of screening, the activities and enantioselectivities of the 66 strains in the bioconversion of cyclic carbonate (**1**) were investigated with HPLC analysis, and the

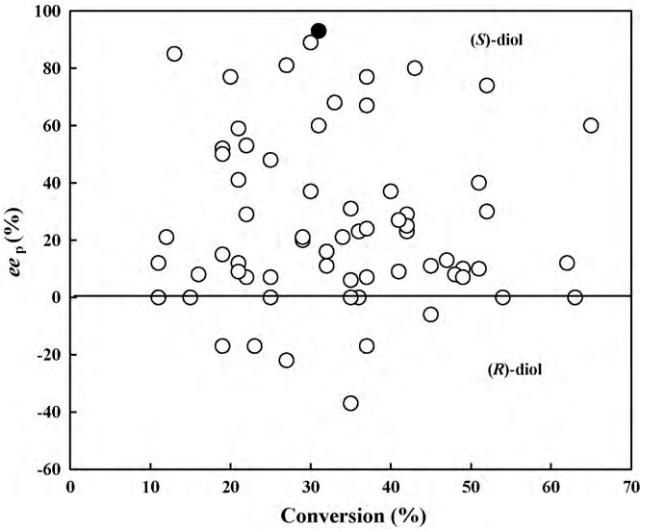


Fig. 1. The results of secondary round screening. Sixty-six strains of microorganisms with obvious carbonate hydrolase activity were found, which could enantioselectively hydrolyze substrate **1** into product **2**. Symbols: (●) the strain of No. ECU0015; (○) the other strains.

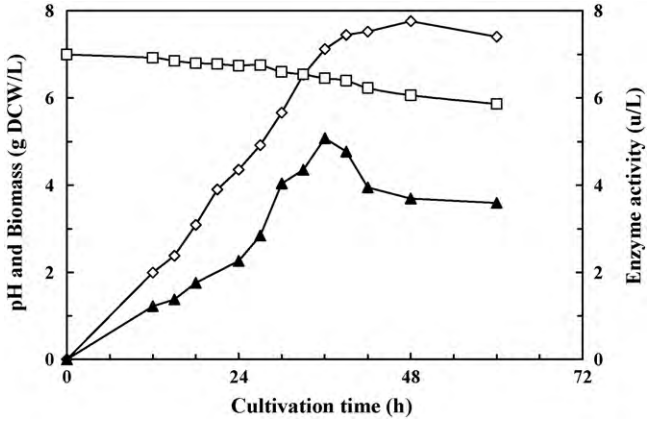


Fig. 2. Profiles of cell growth and enzyme production of *Bacillus* sp. ECU0015. Symbols: (▲) cyclic carbonate hydrolase activity; (◊) biomass; (□) pH.

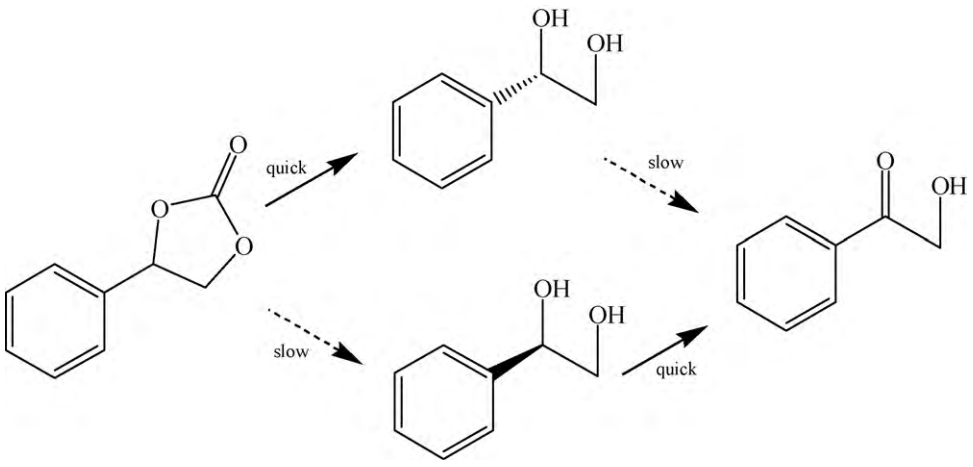
results were shown in Fig. 1. Among the strains preferentially producing (*S*)-isomer of the diol (**2**), 7 strains showed relatively high enantioselectivities (ee_p > 70%). This result indicates that the microorganisms preferentially producing (*S*)-**2** were widespread in nature, while those preferentially producing (*R*)-**2** were relatively rare.

Among the active strains, three bacterial strains showing good enantioselectivity, with ee_p higher than 90%, were selected for further study. In the subsequent experiments, it was found surprisingly that with one of them the *ee* of (*S*)-**2** could be improved up to higher than 99% when the reaction time was lengthened. This result indicates that the enzymatic hydrolysis may afford the optically pure (*S*)-diol. On the other hand, the increase of *ee* of (*S*)-**2** during the reaction may be due to the further oxidation of the opposite isomer (*R*)-**2**. It was confirmed that the bacterium could preferentially oxidize the (*R*)-**2** and the formation of α-hydroxyacetophenone was also demonstrated in an independent biodegradation experiment of *rac*-**2**. And then the hydroxy ketone could be transformed to some other substances (data not shown) and it was difficult to quantify the amount of hydroxy ketone. Therefore, a reaction mechanism is proposed for the biosynthesis of (*S*)-**2** from the racemic cyclic carbonate (**1**) by the bacterial strain ECU0015, as shown in Scheme 2.

This newly isolated microbial strain (ECU0015) was a rod-shape, gram positive bacterium by observation via an optical microscope. The 16S rDNA sequence of our strain was deposited in the GenBank with an accession number of GQ304942. The sequence was 100% identical with the 16S rDNA sequence of *Bacillus* sp. N6 in the Genbank, so the strain ECU0015 was identified as *Bacillus* species and subsequently marked as *Bacillus* sp. ECU0015. This strain has been deposited in China General Microbiological Cultures Center (CGMCC), with an accession number of CGMCC 2874.

3.2. Cell growth and enzyme production of Bacillus sp. ECU0015

To further characterize the new bacterial isolate, time course of the enzyme production was monitored by cultivating *Bacillus* sp. ECU0015 in 250-ml flasks containing 50 ml fermentation medium. As shown in Fig. 2, the maximum cyclic carbonate hydrolase activity (5.08 U/l) was observed at about 36 h and the hydrolase activity increased in parallel with the cell growth during the first 36 h. Then the enzyme activity began to decline whereas the cell growth continued until 48 h. The change of pH during the cultivation was little, with a trend of slight decline.



Scheme 2. The proposed reaction mechanism for *Bacillus* sp. ECU0015 whole-cells catalyzed bioconversion of phenyl-1,2-ethanediol cyclic carbonate to α-hydroxyacetophenone.

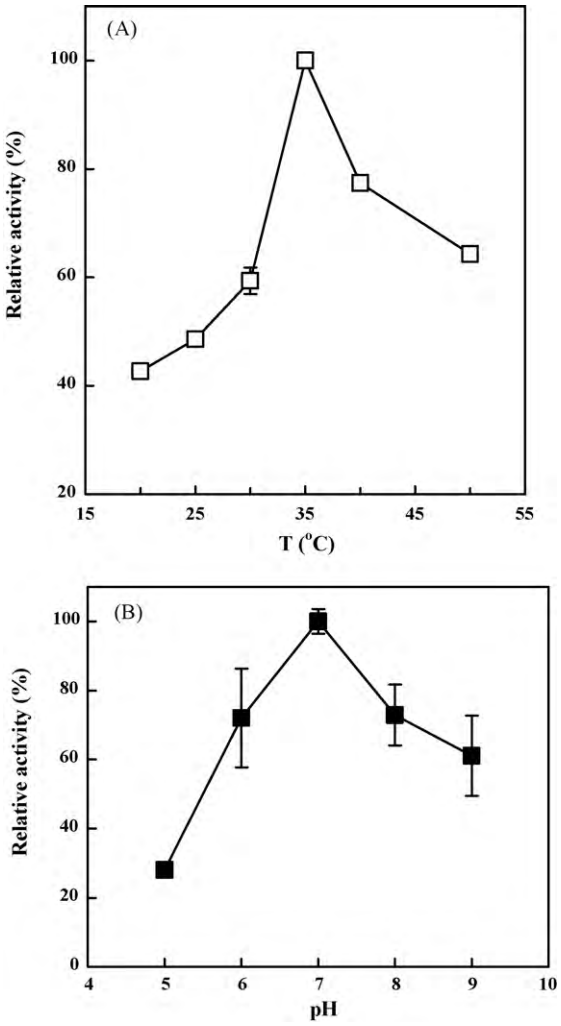


Fig. 3. Effects of reaction temperature (A) and pH (B) on the cyclic carbonate hydrolase activity of *Bacillus* sp. ECU0015 resting cells. Experimental methods were described in Section 2.8.

3.3. Catalytic performance of Bacillus sp. ECU0015 resting cells and the preparation of enantiopure (S)-**2** in gram scale

The strain of *Bacillus* sp. ECU0015 could catalyze the asymmetric hydrolysis of substrate **1** with high enantioselectivity. To further optimize the catalytic conditions for the biohydrolysis of ester **1**, the initial rate of enzymatic hydrolysis (10 mM) catalyzed by the resting cells was measured at different pHs and temperatures by the method described in Section 2.8. As shown in Fig. 3A, the enzyme showed the maximum activity at 35 °C. However, considering the thermo stability of enzymes, we chose 30 °C as the optimal reaction temperature. As shown in Fig. 3B, the optimum pH was about 7.0. When the pH was over 7.0, the whole-cell activity decreased and the spontaneous hydrolysis of substrate increased, and when the pH was below pH 6.0 the activity became very low. Finally we chose 7.3 as the pH optimum to reduce the inhibition caused by the by-product CO_2.

As for the stability of microbial cells under different storage conditions, low temperature and mild pH were helpful for the maintenance of enzyme activity. When preserved at 4 °C, the enzyme activity was maintained over 70% even at the 5th day. The residual enzyme activities were about 70 and 50%, respectively, when preserved at 30 °C for 3 and 5 days. However, the enzyme lost 90% of its initial activity when the cells were preserved at 60 °C for 3 days (Fig. 4A).

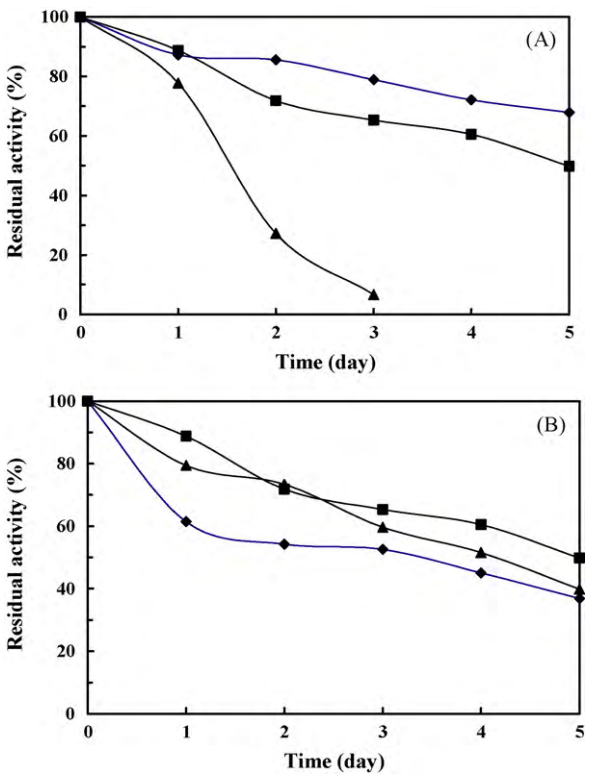


Fig. 4. Effects of preservation temperature (A) and pH (B) on the stability of the enzyme. Symbols: A: thermal stability. The cells were suspended (10%, w/v) in 20 ml KPB (100 mM, pH 7.3) and preserved at different temperature for 5 days. (♦) 4 °C; (■) 30 °C; (▲) 60 °C. B: pH stability. The cells were suspended (10%, w/v) in 20 ml 100 mM buffer with different pH and preserved at 30 °C for 5 days. The initial enzyme activity was regarded as 100%. Under certain conditions, the ratio of enzyme activity measured after preservation and the initial value was defined as the residual activity. (♦) pH 5.0; (■) pH 7.3; (▲) pH 9.0.

The cells kept similar enzyme activity when preserved at different pHs for 5 days, varying from 40 to 50%, while the neutral pH of 7.3 is the best, followed by pH 9.0 and 5.0 (Fig. 4B).

Based on the results above, the hydrolysis of cyclic carbonate **1** by resting cells of *Bacillus* sp. ECU0015 was performed under the optimal reaction conditions, 30 °C and pH 7.3, with an initial substrate concentration of 10 mM.

As shown in Fig. 5, an *ee* of 97.3% for the product was achieved after 40 h in reaction volume of 10 ml, at 36.5% conversion. How-

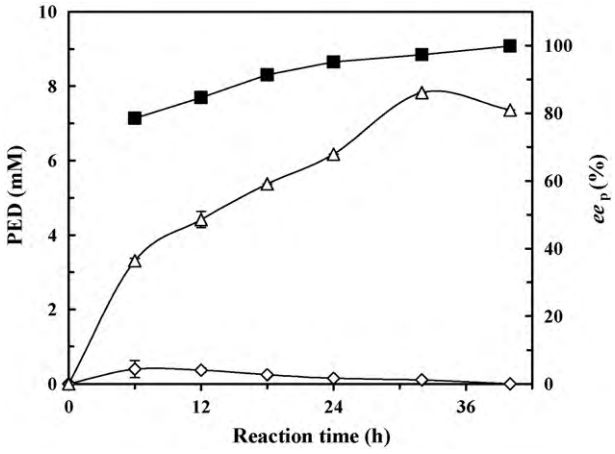


Fig. 5. Time course of enantioselective hydrolysis of phenyl-1,2-ethanediol cyclic carbonate **1** (20 mM) into diol **2** by *Bacillus* sp. ECU0015. Symbols: (△) concentration of (*S*)-diol; (◊) concentration of (*R*)-diol; (■) enantiomeric excess (*ee*) of (*S*)-diol.

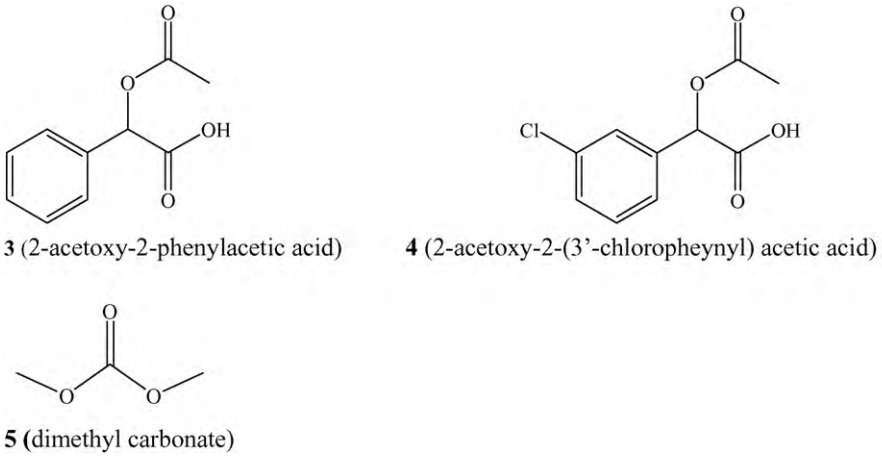


Fig. 6. Structures of substrates **3**, **4** and **5**.

ever, the *ee* and yield of residual carbonate 1 was 81.1% and 22.4%, as determined by HPLC.

In the preparation of diol **2** in gram scale, 15 grams of resting cells of *Bacillus* sp. ECU0015 were suspended in 96 ml of KPB (50 mM, pH 7.3), and 4.0 ml cyclic carbonate **1** solution (250 mM, dissolved in methanol) was added to give a final substrate concentration of 10 mM. After incubation at 30 °C, 160 rpm for 36 h, the reaction was stopped and the product of (*S*)-**2** was extracted as described in section 2.10, with an excellent enantiomeric excess (> 99%, after refined) and a moderate yield (27.8%).

3.4. Substrate spectrum of Bacillus sp. ECU0015

To examin the substrate spectrum of *Bacillus* sp. ECU0015, substrates **1**, **3**, **4** and **5** (Fig. 6) were tested. The strain displayed pretty high activity and stereoselectivity for majority of the substrates (10 mM) examined. In the case of **3** and **4**, high yields (31.5% and 26.5%, respectively) and almost enantiopure (*R*)-mandelic acid and (*R*)-3-chloro-mandelic acid were obtained. While non-cyclic dimethyl carbonate **5** could be hydrolyzed to methanol and carbon dioxide completely.

Cyclic carbonate **1** was enzymatically hydrolyzed to afford (*S*)-**2** with an excellent enantiomeric excess (>99%) and a moderate yield (27.8%).

4. Conclusions

We have succeeded in isolating a new bacterial strain with high activity and ideal stereoselectivity for the transformation of cyclic carbonate **1** from soil samples and it was identified as *Bacillus* sp. ECU0015. As we known, this is the first example of microbial strain which catalyze the stereoselective hydrolysis of cyclic carbonate **1**. The maximum production of the cyclic carbonate hydrolase (5.08 U/L) by *Bacillus* sp. ECU0015 was reached at 36 h of cultivation, when the biomass (DCW) was 7.12 g/L. The optimal reaction temperature and pH were 35 °C and pH 7.3, respectively. The resting cells of *Bacillus* sp. ECU0015 catalyzed the enantioselective hydrolysis of cyclic carbonates, producing (*S*)-1-phenyl-1,2-ethanediol in high ee value (>99%) and moderate yield. It can also catalyze the enantioselective hydrolysis of other chiral esters, such as substrate **3** and **4**, affording (*R*)-mandelic acid (97.8% ee) and (*R*)-3-chloro-mandelic acid (>99% ee) in satisfactory optical purities.

Acknowledgements

This research was financially supported by the National Natural Science Foundation of China (Nos. 20773038 and 20902023), Ministry of Science and Technology, PR China (Nos. 2007AA02Z225, 2009CB724706 & 2009ZX09501-016), China National Special Fund for State Key Laboratory of Bioreactor Engineering (No. 2060204), Shanghai Municipal Fund for Natural Science (No. 07ZR14030) and Shanghai Leading Academic Discipline Project (No. B505).

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