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Soluble Expression of (+)-γ-Lactamase in *Bacillus subtilis* for the Enantioselective Preparation of Abacavir Precursor

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Abstract Chiral Vince lactam (γ-lactam) is an important precursor of many carbocyclic nucleoside analogues and pharmaceuticals. Here, a (+)-γ-lactamase encoding gene *delm* from *Delftia* sp. CGMCC 5755 was identified through genome hunting. To achieve its soluble and functional expression, *Escherichia coli* and *Bacillus subtilis* expression systems were introduced. Compared with *E. coli* system, recombinant (+)-γ-lactamase showed improved protein solubility and catalytic activity in *B. subtilis* 168. Reaction conditions for enantioselective resolution of γ-lactam were optimized to be at 30 °C, pH 9.0, and 300 rpm when employing the recombinant *B. subtilis* 168/pMA5-*delm* whole cells. Kinetic analysis showed that the apparent V_{max} and K_m were 0.595 mmol/(min·g_{DCW}) and 378 mmol/L, respectively. No obvious substrate inhibition was observed. In a 500-mL reaction system, enantioselective resolution of 100 g/L γ-lactam was achieved with 10 g/L dry cells, resulting in 55.2 % conversion and 99 % *ee* of (−)-γ-lactam. All above suggested that recombinant *B. subtilis* 168/pMA5-*delm* could potentially be applied in the preparation of optically pure (−)-γ-lactam.

Keywords γ-Lactamase · Heterologous expression · *Bacillus subtilis* · Whole-cell biocatalysis · Enantioselective resolution

Introduction

Optically active 2-azabicyclo [2.2.1] hept-5-en-3-one, γ-lactam or Vince lactam, is a compound with important pharmaceutical relevance [1]. Both enantiomers of γ-lactam are key chiral precursors of many carbocyclic nucleoside analogues [2]. Chemokine antagonist MK-0812, as well as anti-diabetic drug candidate Melogliptin can be synthesized from (+)-γ-lactam

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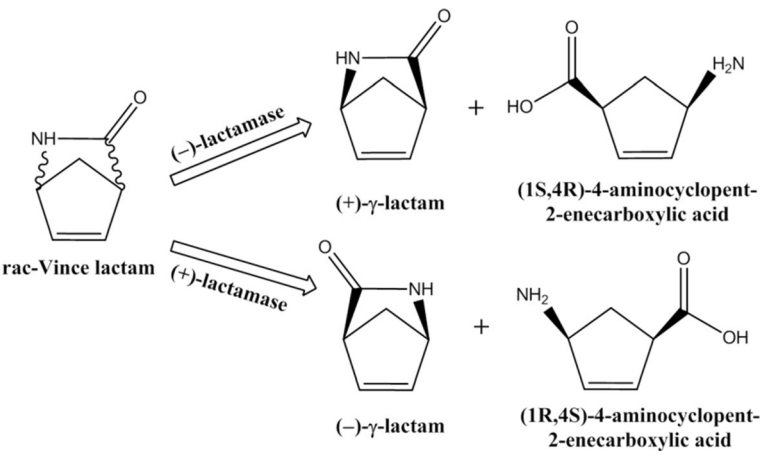
[3]. While (−)-γ-lactam can be applied in the synthesis of anti-viral drug Peramivir and anti-AIDS drug Abacavir, the most effective pharmaceutical against virus infection and having generated several billion dollars for GlaxoSmithKline. Compared with the intricate chemical synthesis, kinetic resolution of racemic Vince lactam employing enantioselective γ-lactamase is a preferable approach to obtain optically pure Vince lactam as depicted in Scheme 1, owing to its mild reaction conditions, high enantioselectivity and environmental friendliness.

For the efficient biocatalytic synthesis of chiral γ-lactam, absolute enantioselectivity and prominent catalytic efficiency are two primary properties of potential γ-lactamases. Various γ-lactamases-producing microorganisms have been identified, such as *Sulfolobus solfataricus*, *Comamonas acidovorans*, *Aureobacterium*, *Microbacterium hydrocarbonoxydans*, and *Aeropyrum pernix* species [4–8]. However, wild-type strains often exhibited unsatisfied enantioselectivity and (or) catalytic efficiency because of their complicated enzymes system, genetic background, and regulation system in vivo [9, 10]. Consequently, heterologous expression of enantioselective γ-lactamases is a more efficient approach to obtain robust biocatalysts for the synthesis of chiral γ-lactams. One thermophilic (+)-γ-lactamase from *Sulfolobus solfataricus* was heterologously expressed and characterized in *Escherichia coli* [11, 12]. Using a novel anchoring scaffold to increase the reusability of the recombinant (+)-γ-lactamase, 100 g/L racemic γ-lactam was enantioselectively hydrolyzed within 4 h [9]. Recently, a (+)-γ-lactamases from *E. coli*, RutB, was identified via silicon screening. The thermostability and substrate inhibition threshold of the crude enzyme were greatly improved after cross-linking immobilization. In a 100-mL reaction, 49.9 % conversion rate and 99.5 % enantiomeric excess (*ee*) were achieved at 2 M substrate (218 g/L) catalyzed by 250 g/L immobilized enzyme [13], giving a calculated substrate to catalyst ratio of 0.872. A robust γ-lactamase coding gene had been identified by Taylor using a novel screening method and expressed in a fusion form with the downstream putative fmdB family regulatory protein [5]. This recombinant γ-lactamase could tolerate as much as 500 g/L racemic γ-lactam with >98 % *ee* employing the partially purified and immobilized forms, however with little information on the expression level and form in the recombinant *E. coli*.



Scheme 1 Kinetic resolution of racemic Vince lactam using enantioselective γ-lactamases

Previously, a *Delftia* sp. CGMCC 5755 strain with high (+)-γ-lactamase activity was enriched and isolated from soil samples, yielding (−)-γ-lactam with 53.1 % conversion rate and 99.9 % *ee* at 100 g/L racemic γ-lactam [14]. In this study, one candidate (+)-γ-lactamase gene (*delm*) from *Delftia* sp. was identified, cloned and heterologously expressed in *E. coli* BL21(DE3), however mostly as inclusion body. Various strategies including alternative promoters, molecular chaperones, fusion expression, and expression hosts were attempted to improve its solubility. Interestingly, soluble expression of (+)-γ-lactamase was achieved in *Bacillus subtilis* host. Before this report, there has been actually no report on the enantioselective resolution of γ-lactam by recombinant *B. subtilis* whole cells. Further efforts were committed on the application of whole-cell biocatalysis in enantioselective hydrolytic preparation of (−)-γ-lactam.

Material and Methods

Materials

Delftia sp. CGMCC 5755 strain was previously isolated and preserved in our laboratory. All strains and plasmids used in this study are shown in Table 1. Restriction endonucleases and T4 DNA ligase were purchased from Takara (Dalian, China). Primers were synthesized by Generay Biotech Co. Ltd (Shanghai, China). Sequencing service was provided by Saiyin Biological Co. Ltd (Shanghai, China). 2-Azabicyclo [2.2.1] hept-5-en-3-one (γ-lactam) was

Table 1 Plasmids and strains used in this study

Strains/plasmids	Characteristics	Source
pET43a	NusA fusion protein, T7 promoter, Ampicillin^R	Invitrogen
pET24a	T7 promoter, Kanamycine^R	Invitrogen
pQE-80 L	T5 promoter, Kan^R	Qiagen
pMA5	Hpa II promoter, colE1 ori, *repB*, *E. coli* (Amp^R), *B. subtilis* (Kan^R)	Laboratory stock
Escherichia coli JM109	*rec*A1, *end*A1, *gyr*A96, *thi*-1, *hsd*R17, *sup*E44, *rel*A1, Δ (*lac-pro*AB) / F' [*tra*D36, *pro*AB$^+$, *lac*Iq, *lac*ZΔM15]	Invitrogen
Escherichia coli BL21(DE3)	*E. coli*:F-, *omp*T, *hsd*S_B($r_B^-m_B^-$), *gal*, *dcm*, *lon*, λ(DE3[lacUV5-T7 gene 1 ind1 sam7 nin5])	Invitrogen
Bacillus subtilis 168	trpC2	Laboratory stock
E. coli JM109/pQE-80 L-*delm*	*E. coli* JM109 strain expressing (+)-γ-lactamase under T5 promoter, Kan^R	This study
E. coli BL21(DE3)/ pET43-*delm*	*E. coli* BL21(DE3) strain expressing NusA-*De*Lm fusion protein, Amp^R	This study
E. coli BL21(DE3)/ pGro7/pET24-*delm*	*E. coli* BL21(DE3) strain coexpressing pGro7 and pET24-*delm*, Cm^R and Kan^R	This study
B. subtilis 168/pMA5-*delm*	*B. subtilis* 168 strain expressing (+)-γ-lactamase under Hpa II promoter, Kan^R	This study
E. coli BL21(DE3)/pGro7	*E. coli* BL21(DE3) strain containing chaperone plasmid pGro7, araB promoter, Chloromycetin^R, encoding GroES and GroEL chaperone proteins.	Takara

purchased from Weidu Chemical Co. Ltd (Jinan, China). Acetonitrile and isopropanol of HPLC grade were purchased from Oceanpak (Suzhou, China). All other chemicals were commercially available reagents of analytical grade.

Construction of Recombinant Strains

Five potential lactamases as shown in Table S1 were cloned, and protocol for the construction of recombinant *E. coli* and *Bacillus subtilis* was exemplified with *delm* gene as follows:

For recombinant *E. coli* construction, the primers with different restriction sites were designed as listed in Table S2. The *delm* gene was amplified by PCR using the forward primer 5′-CGC*CATATG*GCCGAAACCCTGATCAAGGTCG-3′ and reverse primer 5′-AATC*CTCGAG*TTACTTGTCCTGGGCGATG-3′ (restriction endonucleases *Nde*I and *Xho*I were underlined) with genome of *Delftia* sp. CGMCC 5755 as template. The purified PCR product and pET24a vector were simultaneously double-digested with *Nde*I and *Xho*I. After ligation, the resultant plasmid pET24a-*delm* were transformed into *E. coli* JM109 competent cells and spread on Luria-Broth (LB) agar plates supplemented with kanamycin (50 μg/mL) for the positive clone selection. Recombinant plasmids pET43a-*delm* and pQE-80L-*delm* were constructed in a similar way. For the expression of *De*Lm, the plasmids pET24a-*delm* and pET43a-*delm* were extracted and further transformed into *E. coli* BL21(DE3).

The recombinant plasmid pMA5-*delm* was constructed for expression of *De*Lm in *B. subtilis* [15]. The *delm* gene was amplified, digested with *Nde* I and *Hind* III, and ligated into shuttle plasmid pMA5 [16]. The recombinant plasmid pMA5-*delm* was transformed into *E. coli* JM109 and selected on LB agar plates with ampicillin (100 μg/mL). Positive clones were confirmed by sequencing verification. Afterward, the recombinant plasmid was transformed into *B. subtilis* 168 and screened against kanamycin (50 μg/mL) resistance.

Fermentation Conditions

The recombinant *B. subtilis* 168/pMA5-*delm* was cultured at 37 °C overnight in 100 mL LB. The seed culture was inoculated into a 3-L bioreactor to a final OD_{600} of 0.1~0.15. The fermentation medium consisted of (g/L): peptone 20, yeast extract 16, NaCl 5, glucose 4, Na_2HPO_4 4, KH_2PO_4 0.5, $(NH_4)_2SO_4$ 3. During the fermentation, pH and temperature were maintained at 7.0 and 37 °C. The dissolved oxygen was controlled at about 15 % of air saturation by altering the agitation speed and air flow. Samples were removed at intervals to determine the cell density and enzyme activity.

After fermentation, *B. subtilis* 168/pMA5-*delm* cells were collected by centrifuging at 6000×*g* for 10 min. The pellets were washed with 100 mmol/L sodium phosphate buffer (pH 7.0) for two times and then subjected to lyophilization. The lyophilized cells were stored at 4 °C and used for further studies.

Optimization of Whole-Cell Catalysis

To determine the optimal temperature for the enantioselective hydrolysis of racemic γ-lactam, 1 g/L lyophilized cells were dissolved in sodium phosphate buffer (pH 7.0, 100 mmol/L) to a final volume of 1 mL, and then incubated at different temperatures over range of 10–80 °C at 10 °C intervals. The reactions were initiated after addition of 100 μL γ-lactam (50 g/L) and proceeded for 30 min at specific temperature. Initial reaction velocity was then assayed to calculate the (+)-γ-lactamase activity.

To evaluate the effect of pH on the whole-cell biocatalysis, the reaction was conducted at various pH, covering 100 mmol/L sodium phosphate buffer (pH 6.0–8.0), 100 mmol/L glycine-NaOH buffer (pH 9.0–11.0). In a 1-mL reaction mixture as mentioned above, 1 g/L lyophilized cells were used to hydrolyze 5 g/L γ-lactam at the optimum temperature. The conversion and *ee* were assayed after 5-h reaction.

The agitation speed optimization was carried out in a 100-mL reactor containing 50-mL reaction mixture as described above at 200, 300, and 400 rpm using optimized pH and temperature.

Reaction velocity at different substrate concentrations (ranging from 0.05 to 250 g/L) was measured under the optimized temperature and pH conditions. Conversion rate was controlled at less than 10 % for accurate calculation of the initial velocity.

Preparation of (−)-Lactam

For the preparation of (−)-lactam, a 500-mL reaction was conducted in a 1-L reactor at 30 °C, composed of 10 g/L dry cells and 100 g/L γ-lactam in glycine-NaOH buffer (pH 9.0, 100 mmol/L). Samples were withdrawn and analyzed at regular time intervals.

After the reaction was complete, the reaction mixture was centrifuged to force the separation of cell pellets and the supernatant were extracted with 500 mL dichloromethane for three times. The organic solutions were combined and dried overnight with sodium sulfate before vacuum evaporation. The crystallized (−)-lactam was analyzed by HPLC and ^{1}H NMR.

General Analytical Protocols

The dry cell weight (DCW) was determined as follows: 1 mL culture broth was centrifuged at 12,000×*g* for 10 min, and the pellet was collected and dried to constant weight at 80 °C.

The γ-lactamase activity of the *B. subtilis* 168/pMA5-*delm* lyophilized cells was defined as the amount of cells required to hydrolyze 1 μmol of γ-lactam per minute.

The reaction samples with 0.1 g/L nicotinamide as inner standard were extracted with *n*-butanol. After centrifugation, the supernatant was analyzed at 230 nm on a Daicel Chiralpak AS-H column (Tokyo, Japan) using an Agilent 1100 HPLC system (Palo Alto, CA). The mobile phase was composed of acetonitrile/isopropanol (8:2) at a flow rate of 0.5 mL/min and 30 °C.

The conversion, enantiomeric excess (*ee*), and enantiomeric ratio (E value [17]) were calculated as follows:

$$Conv. = \frac{C0 - Ct}{C0} \times 100\%$$

$$\mathrm{ee} = \frac{C(-) - C(+)}{C(-) + C(+)} \times 100\%$$

$$E = \frac{Ln[(1 - Conv.)(1 - \mathrm{ee})]}{Ln[(1 - Conv.)(1 + \mathrm{ee})]}$$

C_0 and C_t were the concentration of γ-lactam at the initial and different time point of the reaction, while $C_{(-)}$ and $C_{(+)}$ were the concentration of (−)-γ-lactam and (+)-γ-lactam.

Results and Discussion

Identification of the Active γ-Lactamase from *Delftia* sp. CGMCC5755

As the isolated *Delftia* sp. CGMCC 5755 showed potential in preparation of (−)-lactam, we attempted to identify the active (+)-γ-lactamase encoding genes in *Delftia* sp. CGMCC 5755 by genome hunting strategy. Analysis of 16s rDNA revealed that *Delftia* sp. CGMCC 5755 was highly similar to *Delftia acidovorans SPH-1*. Based on genome search of *D. acidovorans SPH-1* (accession no. CP000884), five genes coding for potential lactamase and amidase/formamidase proteins (GenBank nos. ABX32871, ABX32825, ABX34805, ABX37591, and ABX37592) were cloned into pET24a for further expression in *E. coli* BL21(DE3). The resultant recombinant plasmids were verified by sequencing. After induction at 16 °C for 12 h, the recombinant proteins were tested for lactamase activity employing racemic γ-lactam as substrate. However, little activity was detected because heterologous proteins were mostly expressed as inclusion bodies in *E. coli*. One γ-lactamase (GenBank no. ABX32871), denoted as formamidase, was selected for further study due to its relatively better expression and high enantioselectivity. This *De*Lm displayed the 100 % sequence identity with γ-lactamase from *Comamonas acidovorans* (also known as *Delftia acidovorans*) except a frameshift mutation at site 302 possibly due to sequencing mistake [5], and the semipurified γ-lactamase had great potentiality in industrial application for the synthesis of chiral γ-lactam [5].

Soluble Expression of γ-Lactamase in *E. coli* and *B. subtilis*

Inclusion body (IB) is a common phenomenon in heterologous protein expression in *E. coli* [18]. Occurrence of IB often hinders the production of biologically active polypeptides and limits their industrial applications. A number of methods have been developed to be effective in improving soluble expression of heterologous protein [19, 20]. Zhu and coworkers have raised and exemplified a simple strategy of His-tag deletion to improve the soluble expression of γ-lactamase from *Bradyrhizobium japonicum* USDA 6 [21]. Despite numerous successful reports on heterologous protein expression, it is virtually impossible to predict a suitable expression pattern of a specific protein [22].

In this study, different strategies including alternative promoter and host cells, fusion proteins, and molecular chaperones were adopted to improve the soluble expression of *De*Lm. Firstly, *E. coli* JM109/pQE-80L-*delm* was constructed for reduced expression level of *De*Lm under a T5 promoter. Compared with the *E. coli* BL21(DE3)/pET24a-*delm* showing merely detectable hydrolysis activity towards γ-lactam, recombinant *E. coli* JM109/pQE-80L-*delm* exhibited improved (+)-γ-lactamase activity of 0.66 U/g_{DCW}, whereas no obvious improvement in soluble expression was observed according to SDS-PAGE. Transcription termination/anti-termination factor NusA from *E. coli* has been proved to be successful in alleviation the inclusion body formation as a fusion partner [23, 24]. In our test, recombinant *E. coli* BL21(DE3)/pET43a-*delm* was constructed, and the expressed fusion protein *De*Lm with NusA tag (NusA-*De*Lm) also showed improved solubility as confirmed by SDS-PAGE (Fig. 1a), and a slightly enhanced specific activity of 1.02 U/g_{DCW} was observed. Although the soluble expression of *De*Lm was improved after introduction of NusA tag, the specific activity was still not desirable for the synthesis of chiral γ-lactam. A number of studies have proven that the molecular chaperone could stabilize or improve soluble expression of protein [25–28]. Strain *E. coli* BL21(DE3) harboring a chaperone plasmid pGro7 (co-expressing molecular

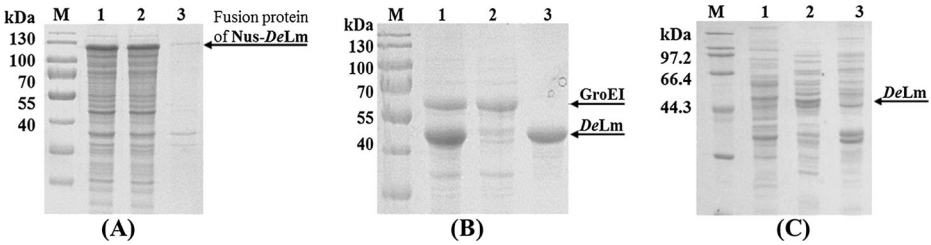


Fig. 1 SDS-PAGE analysis of recombinant *De*Lm from *Delftia* sp. CGMCC 5575. **a** *E. coli* BL21(DE3)/pET43a-*delm*, *lanes 1–3*: whole cell, supernatant, and precipitant parts; **b** *E. coli* BL21(DE3)/pGro7/pET24a-*delm*, *lanes 1–3*: whole cell, supernatant, and precipitant parts; **c** *B. subtilis*/pMA5-*delm*, *lane 1*: negative control (*B. subtilis* 168/pMA5), *lanes 2–3*: supernatant and precipitant parts; *lanes M*: protein molecular marker

chaperones GroES and GroEL) and pET24a-*delm* was also constructed. However, the solubility of (+)-γ-lactamase was barely improved (Fig. 1b).

B. subtilis, known for its huge capacity for secreting proteins and nonbiased codon usage, has been successfully applied in the production of various industrial enzymes [29–31]. Therefore, *B. subtilis* was considered as an alternative expression host to improve the solubility of recombinant *De*Lm. Recombinant strain *B. subtilis* 168 expressing *De*Lm was constructed by a shuttle plasmid pMA5. Although the expression level was significantly lower than that of the recombinant *E. coli* (Fig. 1b, c), an enhanced specific (+)-γ-lactamase activity of 1.62 U/g_{DCW} was detected, indicating the recombinant *B. subtilis* 168 was an efficient host strain for (+)-γ-lactamase production. Consequently, the *De*Lm-producing strain *B. subtilis*/pMA5-*delm* was further investigated for the enantioselective hydrolysis of γ-lactam.

Cell Growth and Enzyme Production of Recombinant *B. subtilis*

Fermentation of *B. subtilis* 168/pMA5-*delm* was carried out in a 3-L bioreactor (Fig. 2). The enzyme production was increased in accordance with the cell growth and reached a maximum yield of 137 U/L after cultivation for 12 h. Thereafter, *B. subtilis* cells started sporulation

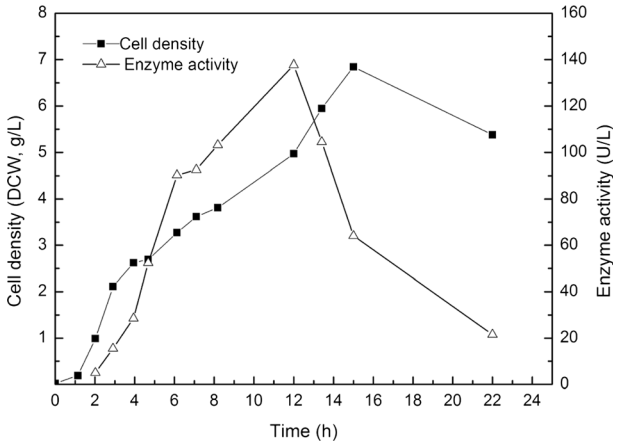


Fig. 2 Cell growth and *De*Lm production by recombinant *B. subtilis*/pMA5-*delm* in a 3-L bioreactor

which might account for the decreased enzyme production. The correlation between the enzyme production and cell growth was as expected because *delm* was expressed under the control of a constitutive promoter Hpa II. The increased (+)-γ-lactamase activity, compared with that in *E. coli*, demonstrated that the *B. subtilis* 168 is an efficient host for *De*Lm production.

Effect of Temperature and pH on Enantioselective Hydrolysis Catalyzed by Recombinant *B. subtilis*

The reaction temperature may affect the catalytic efficiency and stability of the enzyme [32]. The influence of temperature on the enantioselective resolution of γ-lactam by recombinant *B. subtilis* whole cells was investigated over a temperature range of 10–80 °C. As shown in Fig. 3, the catalytic activity increased with the temperature until it reached 40 °C but decreased dramatically at much higher temperatures. Less than 50 % of the hydrolytic activity was remained after 1-h incubation at 40 °C (data not shown). Considering the poor thermostability of the recombinant *De*Lm at over 40 °C, 30 °C was chosen for the enantioselective hydrolysis. The mesophilic feature of the recombinant *B. subtilis* was advantageous for the reduced energy cost to initiate reaction compared with the thermophilic enzymes originated from archaeal organisms because of their dependence on high temperature [9].

To determine the pH profile of recombinant *B. subtilis*/pMA5-*delm* whole cells, reactions under different pH were performed. Under the acidic conditions (pH<7.0) as depicted in Fig. 4, the catalyst retained little activity and resulted in less than 5 % conversion after reaction for 5 h. The recombinant *De*Lm was more efficient over a pH range of 8.0–10.0. Under pH 9.0, 55 % conversion and 99 % *ee* of (−)-γ-lactam were achieved. Therefore, 100 mmol/L glycine-NaOH buffer (pH 9.0) was employed in subsequent studies. The preference of alkaline reaction condition was similar to RutB from *E. coli* as well as Mhp and Mhpg enzymes from *Microbacterium hydrocarbonoxydans* in hydrolysis of γ-lactam [9, 13].

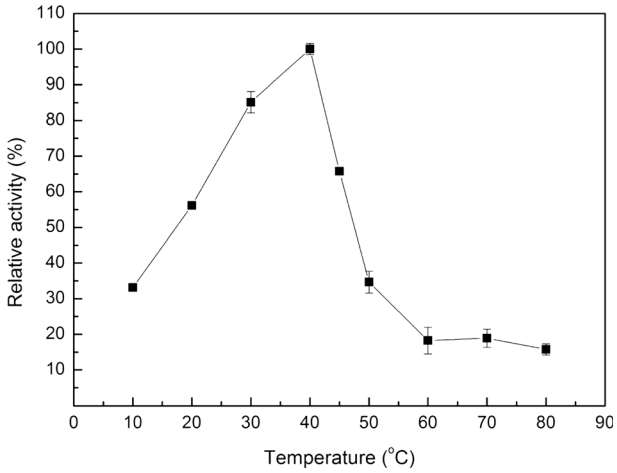


Fig. 3 Effect of temperature on the activity of recombinant *De*Lm

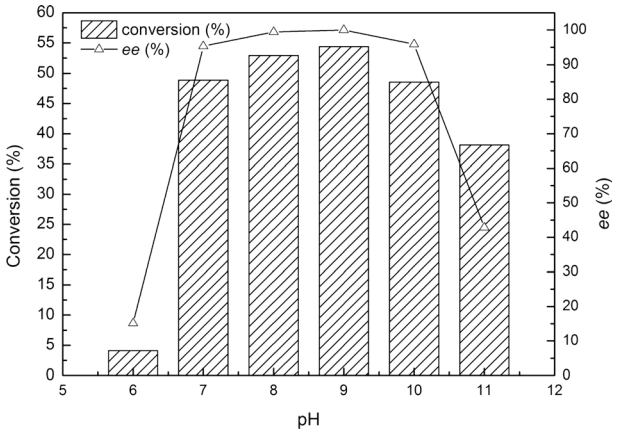


Fig. 4 Effect of pH on conversion and enantioselectivity of recombinant *B. subtilis*/pMA5-*delm* whole cells

Effect of Agitation on Enantioselective Hydrolysis Catalyzed by Recombinant *B. subtilis*

As shown in Fig. 5, hydrolysis reactions were conducted by recombinant *B. subtilis* at 200, 300, and 400 rpm. The highest conversion of 55 % at 300 rpm was slightly higher than that of 200 and 400 rpm. Agitation can accelerate reaction by promoting mass transfer, while it also has negative effect on the reaction through compromising membrane rigidity or decreasing enzyme stability caused by sheering stress [33, 34]. Most importantly, the agitation may have an influence on the enantioselectivity of enzymatic hydrolysis by affecting the mass transfer and the activities toward (+)-γ-lactam and (−)-γ-lactam [35]. Optimum agitation speed could therefore ensure excellent catalytic efficiency while maintaining high enzymatic stability. As a result, 300 rpm was regarded as the optimum speed for the enantioselective hydrolysis of γ-lactam catalyzed by recombinant *B. subtilis*.

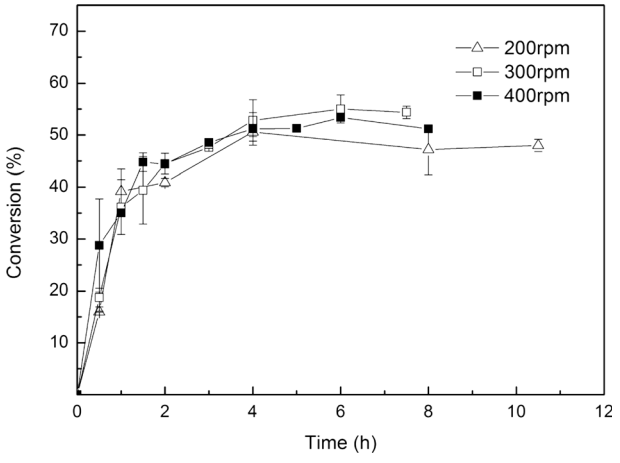


Fig. 5 Effect of agitation speed on enantioselective hydrolysis catalyzed by recombinant *B. subtilis*/pMA5-*delm* whole cells

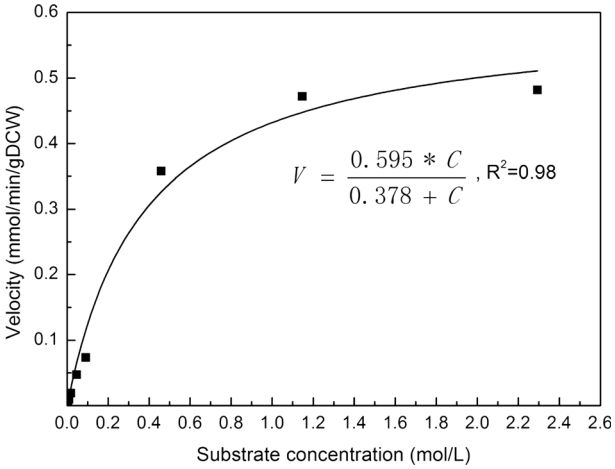


Fig. 6 Effect of substrate concentration on reaction velocity of enantioselective hydrolysis by recombinant *B. subtilis*/pMA5-*delm* whole cells

Apparent Kinetic Parameters of the Recombinant *B. subtilis*

Substrate concentration is a critical parameter in whole-cell biocatalysis [36]. In our study, reactions at different substrate concentrations were evaluated. As shown in Fig. 6, the initial velocity increased steadily with the substrate concentration. No substrate inhibition was observed alongside the increase of substrate concentration, which was different from the previous reports [12, 13]. This recombinant *De*Lm is a promising biocatalyst for the synthesis of chiral (−)-γ-lactam with high substrate tolerance. Non-linear curve fitting analysis using Origin 8.5 (Northampton, USA) showed that the apparent maximal reaction velocity (V_{max}) and affinity constant (K_m) were 0.595 mmol/(min g_{DCW}) and 378 mmol/L (about 41 g/L) to γ-lactam, respectively. Compared with *E. coli* rosetta (DE3)/pDxcc_est-*gla* [9], the V_{max} and K_m

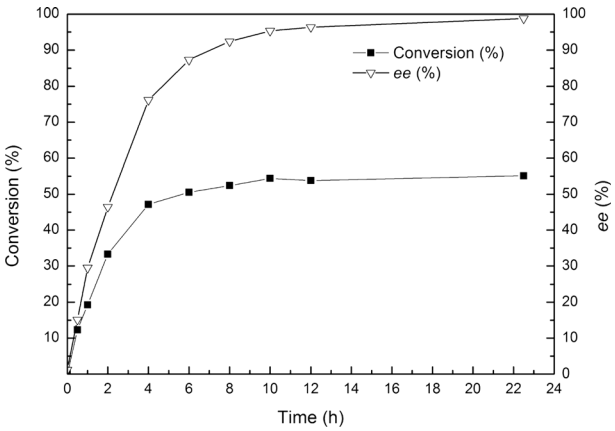


Fig. 7 Time course of enantioselective hydrolysis catalyzed by recombinant *B. subtilis*/pMA5-*delm* in a 500-mL reaction system

of recombinant *B. subtilis*/pMA5-*delm* were 1-fold and 6-fold higher, respectively, indicating the superiority of recombinant *De*Lm especially at high substrate loading.

Enantioselective Hydrolysis Catalyzed by Recombinant *B. subtilis*

Enantioselective preparation of (−)-γ-lactam at larger scale was performed at 100 g/L by recombinant *B. subtilis*/pMA5-*delm* under optimum conditions (pH 9.0, 300 rpm, 30 °C) in a 500-mL reaction mixture (Fig. 7). In the initial stage, the conversion and *ee* increased dramatically, while after 6 h, the increasing tendency flattened out. To obtain optically pure (−)-γ-lactam, the reaction was prolonged for 22.5 h and the conversion and *ee* reached 55.2 and 99 %, respectively, with enantiomeric ratio (E-value) of 47 (Fig. S1). Finally, 14 g (−)-γ-lactam was obtained after purification with a recovery rate of 56 %. In this study, the substrate to catalyst ratio (S/C) was calculated to be 10 which was superior to those of *E. coli* RutB (S/C, 0.872) as well as the recombinant *E. coli* rosetta (DE3)/pDxcc_est-*gla* (S/C, 2.5), indicating recombinant *B. subtilis* is highly efficient in the preparation of (−)-γ-lactam.

(−)-γ-Lactam was purified and characterized with ^{1}H NMR (400 MHz, D_2O) as follows: δ=2.33 (1H, d, J=7.6 Hz, −C*H*H–), δ=2.46 (1H, d, J=8 Hz, −CH*H*–), δ=3.35(1H, s, −COC*H*CH=), δ=4.55 (1H, s, −NHC*H*CH=), δ=6.83 (1H, t, J=3.2 Hz, −C*H*=CH–), δ=7.03(1H, d, J=5.2 Hz, −C*H*=CH–).

Conclusion

In summary, a *delm* gene coding for (+)-γ-lactamase was identified from *Delftia* sp. CGMCC 5755 through genome hunting. Various strategies were adopted to improve the soluble expression of *De*Lm. *Bacillus subtilis* was proved to be an effective expressing host for the soluble expression of *De*Lm. The specific activity of *B. subtilis*/pMA5-*delm* is 1.62 U/g_{DCW}, much higher than that of *E. coli*. The pH and temperature were optimized to be at pH 9.0 and 30 °C, respectively. No substrate inhibition was detected with the recombinant *De*Lm. Preparation of (−)-γ-lactam was achieved at 100 g/L, with 55.2 % conversion and 99 % *ee* in a 500 mL enlarged reaction. Our results suggest that recombinant *B. subtilis*/pMA5-*delm* is a promising biocatalyst for the synthesis of Abacavir precursor.

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Conflict of Interest All the authors certify that this manuscript is original and has not been published and will not be submitted elsewhere for publication while being considered by Applied Microbiology and Biotechnology. And, the study is not split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time. No data have been fabricated or manipulated (including images) to support your conclusions. No data, text, or theories by others are presented as if they were our own. The submission has been received explicitly from all co-authors. And, authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results. The authors declare that they have no conflict of interest. This article does not contain any studies with human participants or animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

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