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Biocatalytic Properties of a Recombinant *Fusarium proliferatum* Lactonase with Significantly Enhanced Production by Optimal Expression in *Escherichia coli*

Bing Chen · Li-Qiang Fan · Jian-He Xu · Jian Zhao · Xian Zhang · Li-Ming Ouyang

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Abstract The *levo*-lactonase gene of *Fusarium proliferatum* ECU2002 (EC3.1.1.25) was cloned and expressed in *Escherichia coli* JM109 (DE3) for biocatalytic resolution of industrially important chiral lactones, including DL-pantoyl lactone which was a key precursor to calcium D-pantothenate. By increasing the biomass concentration and lowering the inducer (isopropyl-β-D-thiogalactoside) concentration and induction temperature, the lactonase production was significantly enhanced up to 20 kU/L, which was 20 times higher than that of wild-type strain *F. proliferatum* ECU2002. The recombinant *Fusarium* lactonase was purified using immobilized metal affinity chromatography, and its SDS-PAGE revealed a molecular mass of 50 kDa for the recombinant protein, suggesting that the enzyme was a simplex protein. Furthermore, biocatalytic properties of the recombinant lactonase were investigated, including kinetic parameters, additive's effect, and substrate specificity. The results reported in this paper provide a feasible method to make the whole cells of *E. coli* JM109 (DE3) expressing lactonase gene to be a highly efficient and easy-to-make biocatalyst for asymmetric synthesis of chiral compounds.

Keywords *Levo*-lactonase · DL-pantoyl lactone · Recombinant protein · Immobilized metal affinity chromatography · Biocatalytic property · Substrate specificity

Introduction

Chiral butyrolactones are a vast family of compounds with great importance due to their wide occurrence in natural products [1] and their utility as key precursors to HIV-protease inhibitors [2]. Different methods to prepare these optically active lactones have been developed, and enzymatic or chemo-enzymatic routes have played more and more important

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roles [3–8]. For example, about 30% of the world production of calcium D-pantothenate occurs through chemo-enzymatic process, in which *Fusarium* lactonases were widely used biocatalysts due to its excellent catalytic properties [9].

To make this biocatalyst more efficient and readily available, different procedures have been developed via gene engineering, as reported by different researchers [9–13]. The amino acid sequences of *Fusarium* lactonases were firstly reported by Shimizu et al. [10], who tried the expression of *Fusarium* lactonase genes in *Aspergillus oryzae* [9] when high-level production of recombinant enzyme was unsuccessful in *E. coli* system. Encountered with the same problem, Sun et al. reported the route of directed evolution of *Fusarium* lactonase, through which the specific activity was increased to 10.5-fold [11]. All these works pave the way for further understanding and application of this industrially relevant biocatalyst.

In our group [5], a fungal strain *Fusarium proliferatum* ECU2002 capable of asymmetrically hydrolyzing chiral butyrolactones with high optical purity has been isolated. The cloning and expression of this lactonase gene was studied in *E. coli* JM109 (DE3) system, resulting in a majority of inactive inclusion bodies. However, to our surprise, significant improvement of specific activity (U/g wet cells) could also be achieved by lowering the induction temperature. Encouraged by this, we further increased the concentration of biomass before induction and then lowered the inducing temperature, resulting in a 20-fold increase in volumetric activity (U/L) as compared with that of wild-type strain *F. proliferatum* ECU2002. With this easy-to-make biocatalyst in hand, we reported the method of purification of the recombinant enzyme using affinity chromatography and its biocatalytic properties, indicating the promising application in asymmetric bioresolution.

Materials and Methods

Reagents and Plasmids

Ampicillin, kanamycin, isopropyl-β-D-thiogalactoside (IPTG) and agarose were purchased from Genebase (Beijing, China). Classical total RNA isolation kit and AMV first-strand cDNA synthesis kit were from Sangon (Shanghai, China). Plasmid pMD18-T and Ex-Taq DNA polymerase were obtained from TaKaRa (Dalian, China). Plasmid pET-28a(+) was from Merck Biosciences, Novagen, Darmstadt, Germany. Tryptone and yeast extract were from Oxoid Ltd. (Cambridge, UK). Pantolactone, γ-butyrolactone, methyl 2-chloropropanoate, 4-nitrophenyl butyrate, 4-nitrophenyl acetate, and methyl 2-chloroacetate were from Sigma–Aldrich (St. Louis, MO, USA), and 2-hydroxy-γ-butyrolactone was from Fluka (Riedel-deHaën, Germany).

Cloning of *F. proliferatum lactonase* (FPL) Gene

F. proliferatum strain ECU2002 was grown aerobically at 30 °C for 48 h at 160 rpm in an optimized medium with the following composition (per liter): glycerol, 30.0 g; peptone, 10.0 g; yeast extract, 10.0 g; $NaNO_3$, 3.0 g; NaCl, 1.0 g; $MgSO_4{\cdot}7H_2O$, 1.0 g; $FeSO_4{\cdot}7H_2O$, 0.02 g; $ZnSO_4{\cdot}7H_2O$, 0.03 g; and $CuSO_4{\cdot}5H_2O$, 0.005 g; pH 7.0.

One milliliter of culture was harvested by centrifugation at 10,000×*g* for 10 min, then frozen in liquid N_2, grounded, and transferred into a fresh tube. Further steps to prepare mRNA were carried out according to the manufacturer's protocol (Classical Total RNA Isolation Kit, Sangon, Shanghai, China).

First-strand cDNA was synthesized by RT-PCR with Oligo (dT) 15 using mRNA as template according to the manufacturer's protocol (AMV First-Strand cDNA Synthesis Kit, Sangon, Shanghai, China). The first-strand cDNA synthesized was then used as the template of PCR. Primers were designed as follows:

Forward: 5′ GGAACATATGCCTTCTTCCATTTCTGT 3′ (underlined bases indicate the N*de* I digestion site)
Reverse: 5′AAGGGGATCCCTAATCATAGAGCTTGGGAC 3′ (underlined bases indicate the B*am*H I digestion site)

One thermal cycle consisted of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 1 min. A total of 30 cycles were performed. The gel-purified PCR-synthesized product (1,200 bp) was cloned into the pMD18-T plasmid. The resultant plasmid was designated as pMD18-T-FPL, and nucleotides were sequenced by Generay Biotech Co., Ltd. (Shanghai, China).

Expression of Recombinant *F. proliferatum lactonase*

FPL gene, cut from plasmid pMD18-T-FPL by N*de* I and B*am*H I, was ligated to pET-28a (+) which was cut by the same enzymes. The ligation mixture was transformed into *E. coli* JM109 (DE3), and the resultant plasmids were extracted and digested by N*de* I and B*am*H I. The digestion product containing the fragment of 1.2 kb in agarose gel electrophoresis was chosen out, and its corresponding plasmid was sequenced, which leads to the identification of positive clones. The positive clone was then designated as pET-28a(+)-FPL. The recombinant *E. coli* JM109 (DE3) containing pET-28a(+)-FPL was cultured aerobically in 50 ml LB medium (tryptone, 10 g/L; yeast extract, 5 g/L; NaCl, 10 g/L) containing 100 μg/ml ampicillin in 250-ml shaking flasks at 37 °C until OD_{600} reached 1.6, and then the cultures were immediately shifted to 30 °C. IPTG was added to a final concentration of 0.17 mM to induce the *lac* promoter. Cells from overnight-grown culture were harvested by centrifugation (10,000×*g* for 10 min), washed thoroughly with physiological saline, and then used for further studies.

Optimization of Recombinant *F. proliferatum lactonase* Production

One-at-a-time method was used for optimization of the enzyme production. One factor at three to five different levels was tested without changing the other components [14]. The optimization process started from the expression conditions as described in Expression of Recombinant *F. proliferatum* lactonase. The significant factors of expression process at different levels were examined, including temperature (15, 23, and 30 °C), medium load (15, 50, 90, and 150 ml in 250-ml shaking flask), IPTG concentration (0.09, 0.17, 0.33, and 0.50 mM), and induction timing (OD_{600} 0.7, 1.6, 2.0, and 3.0). Preliminary results [15] showed that higher density of *E. coli* JM109 (DE3) and enhanced productivity of recombinant protein than that in LB medium were obtained in MLB medium (tryptone, 10 g/L; yeast extract, 15 g/L; NaC1, 5 g/L; and K_2HPO_4, 5 g/L) supplemented with glucose as additional carbon source. Thus, major factors of the MLB medium composition at different levels, including glucose (1.5, 2.5, and 3.5 g/L) and the ratio of tryptone to yeast extract (0, 1:9, 1:6, and 1:1.5), were examined by using 250-ml shaking flasks in triplicate.

Optical density was measured by spectrophotometer (Unico UV-2100, Shanghai, China). One OD unit was found to be equivalent to 0.316 g of dry cell weight.

Purification and Biocatalytic Properties of Recombinant *F. proliferatum lactonase*

The N-terminal 6× His-tagged recombinant *F. proliferatum* lactonase (reFPL) was purified in one step through immobilized metal affinity chromatography on a nickel-nitrilotriacetic acid column from Qiagen (Germany) according to the instruction manual. Standard SDS-PAGE method was applied for the fusion protein assay. Protein concentration was determined according to Bradford method using bovine serum albumin as a standard. Biocatalytic properties of the purified lactonase (reFPL) were further characterized by examining the kinetic parameters, additives effect and substrate specificity.

Enzyme Assay

The recombinant *E. coli* JM109 (DE3) cells were harvested by centrifugation (10,000×*g* for 10 min) and washed thoroughly with physiological saline. The standard assay mixture comprised of 2.5% (*w/v*) racemic pantolactone and appropriate amount of the cells or protein with a final volume of 10.0 ml. The reaction was performed at 30 °C, and the pH was automatically controlled at 6.7–6.9 with 0.1 M NaOH. The hydrolytic rate of each lactone substrate was calculated based on the initial rate of NaOH titration [5]. One unit of lactonase was defined as the amount of the enzyme that converts 1.0 μmol of pantolactone into pantoic acid per minute under the above conditions.

Resolution of Racemic Pantolactone by Purified Recombinant *F. proliferatum lactonase*

Purified reFPL (15.0 IU) was added into a 10-ml reaction system containing 200 mM of racemic pantolactone. After 4 h, the supernatant was extracted by ethyl acetate for eight times to remove residual (*S*)-lactone completely. The aqueous layer containing the product (*R*)-hydroxyl acid was acidified with diluted HCl to pH 1–2, and the corresponding (*R*)-lactone was afforded by heating for 0.5 h and extracted by ethyl acetate. The ethyl acetate layer was collected, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a crude crystal of chiral lactones. These lactones were further purified by recrystallization in isopropyl ether. The conversion of substrate was calculated according to the consumed amount of alkali by an automatic potential titrimeter (ZD-2, Cany Precision Instruments, Shanghai, China). The *ee* values were determined by gas chromatography (Shimadzu GC-14C, Japan) with a flame ionization detector, using a chiral column (Beta Dex™ 120, Supelco, Φ 0.25 mm×30 m) and N_2 as the carrier gas. The column temperature was 110 °C. Both the injector and detector were set at 280 °C. The optical rotation $\left([\alpha]_D^{20}\right)$ of pantolactone was measured using a digital polarimeter (WZZ-1, Cany Precision Instruments).

Results and Discussion

Cloning and Sequence Analysis of FPL Gene

The gene sequences of *Fusarium* lactonases have been reported by different groups. Primers were designed according to their similarity, and a fragment of about 1.2 kb was obtained and sequenced. Blasted against the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi), our sequence (GenBank accession number EU596535) exhibited similarity of 94% with *Fusarium oxysporum* lactonohydrolase and 97% with *Gibberella fujikuroi* lactonohydrolase.

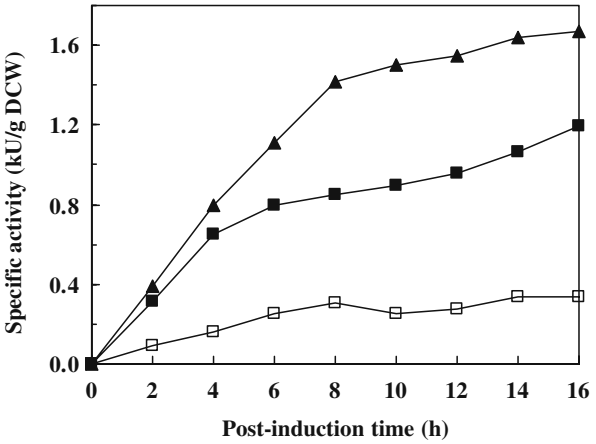


Fig. 1 Effects of inducing temperature on production of *levo*-lactonase in *E. coli* JM109 (DE3). *Filled triangles* 15 °C; *filled squares* 23 °C; *empty squares* 30 °C; the experiments were carried out in 250-ml flask, and the culture volume was 50 ml. Firstly, the *E. coli* JM109 (DE3) was cultured at 37 °C until OD_{600} reaching 1.6 and then IPTG was added to a final concentration of 0.17 mM when the temperature was shifted immediately to different temperatures

Optimal Expression of Recombinant *F. proliferatum lactonase* in *E. coli* JM109 (DE3)

In our case, *E. coli* JM109 (DE3) and plasmid pET-28a(+) were used in transformation experiments as the recipient strain and the expression vector, respectively. When the recombinant *E. coli* JM109 (DE3) containing plasmid pET-28a(+)-FPL was cultured in LB medium at 37 °C to OD_{600} 0.7, IPTG was added to a final concentration of 0.5 mM to initiate the expression of recombinant protein. The expression was performed at 30 °C, 180 rpm for another 12 h. The enzyme activity of reFPL was 37.2 U/g wet cells, which was consistent with the result (37–41 U/g) reported by Sun et al.

Based on these results, the expression conditions were optimized in LB medium by firstly changing the induction temperature (Fig. 1), then changing the medium load and the IPTG concentration, and increasing the cell density up to OD_{600} 1.6 before adding IPTG.

Table 1 Effects of different culture conditions on the expression of target fusion protein.

	Medium load (ml)[a]					IPTG concentration (mM)[b]				Induction timing (OD_{600})[c]			
	15	30	50	90	150	0.09	0.17	0.33	0.50	0.7	1.6	2.0	3.0
Specific activity (kU/g DCW[d])	0.89	1.00	0.97	0.87	0.83	1.11	1.42	0.93	0.72	0.81	1.49	1.03	0.90

All tests were carried out in 250-ml shake flasks. Firstly, the *E. coli* JM 109 (DE3) was cultured at 37 °C until OD_{600} reached appropriate value and then IPTG was added and the temperature was shifted immediately to 15 °C. The post-induction expression time was 16 h

DCW dry cell weight

[a] IPTG was added at OD_{600} about 1.6 with final concentration of 0.17 mM

[b] Medium load was 30 ml; IPTG was added at OD_{600} about 1.6

[c] Medium load was 30 ml; IPTG was added at different OD_{600}s with final concentration of 0.17 mM

[d] Enzyme activity was assayed according to "Materials and Methods." 1 kU=1,000 IU

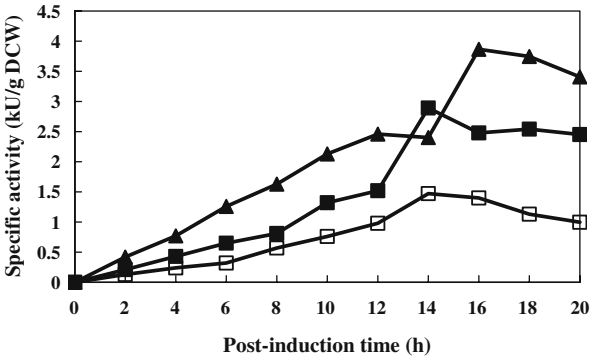


Fig. 2 Effects of different kinds of media on the production of *levo*-lactonase in *E. coli* JM109 (DE3). *Filled triangles* MLB + 1.5 g/L glucose; *filled squares* MLB; *empty squares* LB; the experiments were carried out in 250-ml flasks, and culture volume was 30 ml. Firstly, the *E. coli* JM109 (DE3) was cultured at 37 °C until OD_{600} reaching 1.6, then IPTG was added to a final concentration of 0.17 mM when the temperature was immediately shifted to 15 °C

The results were summarized in Table 1. The specific activity of reFPL was improved to 1.49 kU/g DCW (3.5 times).

Subsequently, the effects of medium composition on enzyme production were also studied. Among three different media, i.e., LB, MLB, and MLB media supplemented with 1.5 g/L glucose, the glucose-supplemented MLB medium was found to be the most effective for enzyme production, as shown in Fig. 2. Glycerol and glucose were first examined and were capable of increasing both cell growth and reFPL production equally (data not shown). Considering that glycerol was relatively expensive, glucose was selected for the following experiments. Glucose concentration was quite crucial to enzyme fermentation in *E. coli* JM109 (DE3) for acetate accumulation in high-cell-density *E. coli* JM109 (DE3) cultures [16, 17]. Three levels of glucose concentration were examined (Fig. 3), indicating that 1.5 g/L glucose might be more suitable for enzyme production than the others. It was consumed completely when IPTG was added. Finally, the ratio of

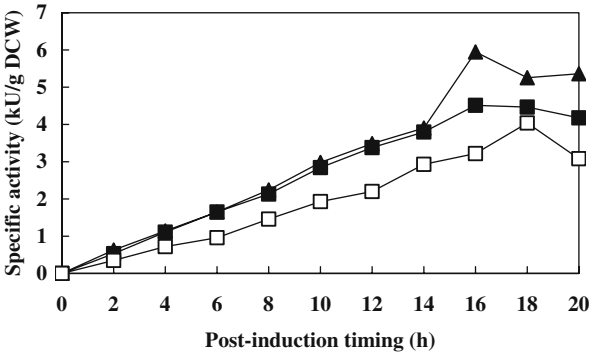


Fig. 3 Effects of glucose concentration on the production of *levo*-lactonase in *E. coli* JM109 (DE3). *Filled triangles* MLB + 1.5 g/L glucose; *filled squares* MLB + 2.5 g/L glucose; *empty squares* MLB + 3.5 g/L glucose; the experiments were carried out in 250-ml flask, and the culture volume was 30 ml. Firstly, the *E. coli* JM109 (DE3) was cultured at 37 °C until OD_{600} reaching 4.0, then IPTG was added to a final concentration of 0.17 mM when the temperature was immediately shifted to 15 °C

tryptone to yeast extract was changed while the total weight was controlled at the same level (25 g/L). When yeast extract was used as sole nitrogen source without tryptone, the highest volumetric and specific activity were observed (Fig. 4) with 20.1 kU/L and 6.4 kU/g DCW (605 U/g wet cells), respectively. This was consistent with many reports showing that yeast extract was often used as additional nitrogen source to improve recombinant protein expression [18, 19], although Viitanen et al. observed the opposite phenomenon [20].

One-Step Purification of Recombinant *F. proliferatum lactonase*

Trivial processes have been reported for purifying *Fusarium* lactonase to near homogeneity [5, 9, 11, 21]. While in this study, reFPL was purified in one step by Ni^{2+}-chelating chromatography, giving a purification factor of 14.4-fold and an overall yield of 5.8%,

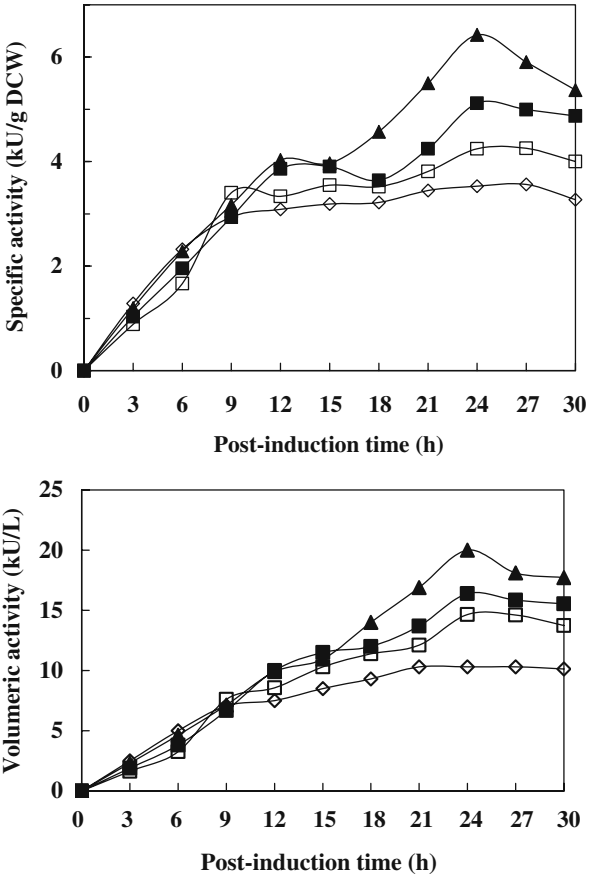


Fig. 4 Effects of different kinds of nitrogen sources on the production of *levo*-lactonase in *E. coli* JM109 (DE3). *Filled triangles* tryptone/yeast extract=0; *filled squares* tryptone/yeast extract=9; *empty squares* tryptone/yeast extract=6; *empty diamonds* tryptone/yeast extract=1.5. The experiments were carried out in 250-ml flask, and the culture volume was 30 ml. Firstly, the *E. coli* JM109 (DE3) was cultured at 37 °C until OD_{600} reaching 4.5, then IPTG was added to a final concentration of 0.17 mM when the temperature was immediately shifted to 15 °C

respectively. On the SDS-PAGE (Fig. 5), this recombinant enzyme corresponded to the molecular mass of about 50 kDa. These were consistent with a previous report showing that recombinant *Fusarium* lactonohydrolase corresponded to 49–51 kDa on SDS-PAGE, which was produced by *E. coli* JM109 (DE3) containing plasmid pFLC40E [10]. The specific activity of reFPL was 341.2 U/mg proteins under the standard assay conditions with respect to pantolactone.

Kinetic Parameters of Recombinant *F. proliferatum lactonase*

Based on Lineweaver–Burk plots (Fig. 6a and b), the K_M and V_{max} were calculated to be 121 mM and 4.2 μM/min, respectively. These were somewhat different from those of the native FPL probably due to the fusion with N-terminal 6× His tags (2.7 mM and 11.5 μM/min, respectively) [5].

Effect of Additives on Enzyme Activity

Organic solvents are frequently used in enzymatic reactions to improve substrate solubility. Five common solvents were examined in this work to study their effects on the lactonase activity (Table 2). Methanol inhibited the enzyme activity less than any other solvents. When the concentration of methanol was increased up to 20% (*v/v*), 60.5% of the initial activity was remained. While at the same concentration, acetone and acetonitrile caused loss of the enzyme activity up to 70.0%. This phenomenon indicated that methanol would be more suitable for the reFPL catalyzed reactions than the others.

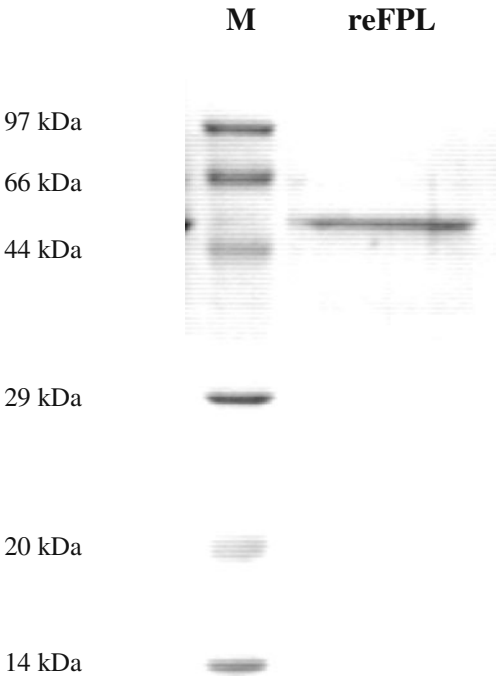


Fig. 5 SDS-PAGE of reFPL. *Left* a low molecular mass SDS calibration standard: 97, 66, 44, 29, 20, 14 kDa. *Right* reFPL

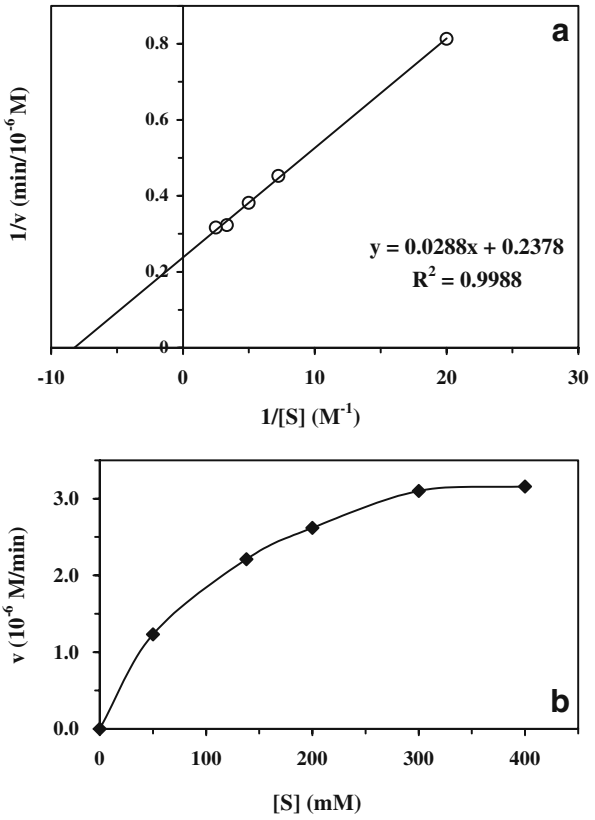


Fig. 6 **a** Lineweaver–Burk plot of $1/v$ vs. 1/[S], based on Michaelis–Menten equation. The intercept of X-axis is $-1/K_M$ and the intercept of Y-axis is $1/V_{max}$. **b** Plot of velocity (v) versus substrate concentration ([S]). All experiments were performed in triplicate

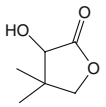
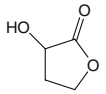
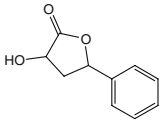
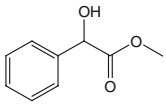
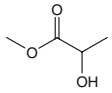
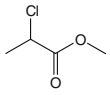
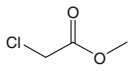
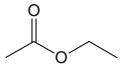
We are also interested in the effect of metal ions, especially Ca^{2+}, on the activity and stability of reFPL. Although reFPL was in a similarity of 98% with amino acid sequence of *F. oxysporum* lactonohydrolase which contained calcium and caused 15% activation, there was additional 34 amino acid residues at the N terminus of reFPL derived from pET-28a(+) vector. EDTA did not exhibit significant effect when incubated with reFPL at 30 °C for

Table 2 Effects of organic cosolvents on reFPL activity.

Additive	Relative activity (%)
None	100
Methanol	81
Ethanol	55
Acetone	49
Acetonitrile	55
DMF	51

Reactions were carried out at 30 °C in 10 ml system with substrate concentration of 200 mM and different organic cosolvents (10%, *v*/*v*).

Table 3 Hydrolytic activities of reFPL on substrates.

Entry	Substrate	Structure	Relative activity (%)
1	Pantolactone		100
2	2-Hydroxy butyrolactone		2450
3	4-Phenyl-2-hydroxyl -4-butyrolactone		22
4	Butyrolactone		<1
5	2-Bromide butyrolactone		<1
6	Methyl mandelate		<1
7	Methyl lactate		<1
8	Methyl 2-chloropropanoate		2.3
9	Methyl 2-chloroacetate		11.6
10	Ethyl acetate		<1

Reactions were carried out at 30°C in 10 ml system with substrate concentration of 30 mM and methanol (10%, v/v) as cosolvent.

30 min, only 3.3% of enzyme activity was lost at the concentration of 1.0 mM and 5.4% was lost at the concentration of 2.0 mM. After dialysis to remove the EDTA, Ca^{2+} was added and incubated with enzyme for 1 h at 30 °C. It caused 7.4% activation. This activation function was lost when EDTA was added again. We then investigated the effect of Ca^{2+} on the stability of reFPL at 4 °C (for storing enzyme) and 30 °C (for enzymatic reaction), respectively. However, no significant effect of Ca^{2+} on the stability of reFPL was observed, when incubated together for 60 h (data not show). These results were in accordance with Shimizu's reports about *F. oxysporum* lactonohydrolase [11, 21]. We then examined other metal ions on the effect of enzyme activity, which all showed negative effects on the activity (Table 4). Especially, Fe^{3+} and Hg^{2+} caused the loss of more than 95% of activity.

Resolution of Pantolactone by Recombinant *F. proliferatum lactonase*

The native FPL could catalyze the enantioselective hydrolysis of *dl*-pantolactone, giving ee_s of 51.9% and ee_p of 98.2% at a conversion of 38.2% [5]. In the case of *dl*-pantolactone resolution catalyzed by the purified reFPL, an ee_s of 89.7% and an ee_p of 94.9% were observed at a conversion of 48.6%, giving an enantiomeric ratio (*E* value) of 117.2 [22, 23]. After crystallization, optical purities of both the two enantiomers could be enhanced up to >99% *ee*, for *R*, $[\alpha]_D^{20} -50.2$ [5], for *S*, $[\alpha]_D^{20} +49.5 (c = 1.0, H_2O)$. This indicated that the reFPL was quite specific to the *l*-pantolactone and might become an efficient biocatalyst for preparing both the two isomers of 2-hydroxy-γ-butyrolactone derivatives at nearly 50% conversion.

Substrate Specificity of Purified Recombinant *F. proliferatum lactonase*

With this efficient biocatalyst in hand, we further investigated the substrate specificity with 10 probe compounds (Table 3). It was found that both the cyclized lactone structure and the hydroxyl group at the α-site to carbonyl group play important roles. Compounds 1–3 showed apparent activity while 4 and 5 showed none, suggesting the α-hydroxyl group was necessary for the hydrolysis by reFPL. However, in the presence of α-hydroxyl group,

Table 4 Effects of metal ions on the activity of reFPL.

Metal ion	Relative activity (%)	Metal ion	Relative activity (%)
None	100	Cu^{+}	13.6
Ca^{2+}	107.4	Al^{3+}	22.4
Na^{+}	100	Mg^{2+}	32.4
K^{+}	100	Zn^{2+}	14.3
Co^{2+}	10.7	Fe^{3+}	3.3
Li^{+}	24.7	Fe^{2+}	15.6
Ni^{2+}	55.4	Hg^{2+}	2.1
Mn^{2+}	22.7	Ag^{+}	33.2
Cu^{2+}	11.2	Pb^{2+}	13.9

Metal ions were removed from the purified enzymes by treatment with 1.0 mM EDTA at 30 °C for 30 min followed by overnight dialysis against 20 mM Tris–HCl buffer (pH 7.0) at 4 °C with several changes of buffer. After these, various metal ions at the concentration of 1.0 mM were added and incubated with enzyme for 1 h at 30 °C

compounds 6 and 7 still showed no activity, indicating the cyclized lactone structure was of the same importance with the hydroxyl group. Among compounds 1–3, 2 showed the strongest activity (K_m=290.2 mM and V_{max}=384.6 μM/min), probably due to the bigger steric hindrance in compounds 1 and 3. Interestingly, compounds 8 and 9 showed weaker activities than 1–3, while 10 was negligible. No apparent activity was detected when *p*-znitrophenyl acetate and *p*-nitrophenyl butyrate were used as substrates (Table 4).

Conclusions

Lactonases from *Fusarium* sp. have been studied by different groups for more than 10 years, but some problems remained unresolved. For example, the fermentation period of the wild-type *Fusarium* strains to produce native lactonase is time-consuming (as for *F. proliferatum* ECU2002, it is about 4 days). Sun et al. and Shimizu et al. tried to produce recombinant lactonase in *E. coli* JM109 (DE3), but no details of optimization for the lactonases production were disclosed. Shimizu et al. also produced recombinant lactonase in *A. oryzae* and obtained relatively high expression, but 4 days as a fermentation period was still necessary. In this work, a productivity of 20 kU $L^{-1}day^{-1}$ was achieved after careful optimization, which was about 80 times of that produced by the wild-type strain *F. proliferatum* ECU2002. Based on significantly enhanced production of lactonase, reFPL was purified by Ni^{2+}-chelating chromatography in one step and exhibited some new biocatalytic properties, among which the ability to prepare both isomers of pantolactone could probably make this enzyme a promising catalyst.

In summary, the whole cells of *E. coli* JM109 (DE3) expressing *F. proliferatum* lactonase gene have been made as an efficient and easy-to-make biocatalyst, with great potential to be applied not only in industrial production of both isomers of pantolactone and its derives [24–27] but also in biocatalytic synthesis of other chiral lactones or hydroxyl acids.

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