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Characteristic of L-threonine transaldolase for asymmetric synthesis of β-hydroxy-α-amino acids

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Abstract

L-threonine transaldoase (LTTA) was a putative serine hydroxymethyltransferase (SHMT) that could catalyze the trans-aldehyde reaction of L-threonine and aldehyde to produce L-threo-β-hydroxy-α-amino acids with excellent stereoselectivity. In present study, a L-threonine transaldolase from *Pseudomonas* sp. (PsLTTA) was mined and expressed in *Escherichia coli* BL21 (DE3). Substrate spectrum assay indicated that PsLTTA only consumed L-threonine as donor substrate and could accept a wide range of aromatic aldehydes as acceptor substrates. Among those substrates, PsLTTA could catalyze *p*-methylsulfonyl benzaldehyde and L-threonine to produce L-threo-*p*-methylsulfonylphenylserine with high conversion (74.4%) and high *de* value (79.9%). It was found that the conversion and stereoselectivity of PsLTTA were dramatically influenced by concentration of whole-cell, co-solvent and reaction temperature. By conditional optimization, L-threo-*p*-methylsulfonylphenylserine was obtained with 67.1% conversion and near perfect *de* value (94.5%), the highest stereoselectivity of L-threo-β-hydroxy-α-amino acids reported by enzymatic synthesis so far. Finally, synthesis of L-threo-*p*-methylsulfonylphenylserine in a 100 mL scare by a whole-cell biocatalyst was conducted. This was for the first time systemically reported that L-threonine transaldolase as a robust biocatalyst for preparation of β-hydroxy-α-amino acids which could provide new insights for β-hydroxy-α-amino acids synthesis.

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1. Introduction

β-hydroxy-α-amino acids composed of numerous important building blocks which were essential for the synthesis of agriculturally or medicinally bioactive products.[1-3] For example, some β-hydroxy-α-amino acids such as L-threo-*p*-methylsulfonylphenylserine and L-threo-*p*-nitrophenylserine were known as key intermediates of thiamphenicol and chloramphenicol, respectively.[4,5] L-threo-phenylserine could be found in cyclomarin A, a marine cyclopeptide with anti-tuberculosis and anti-malaria activities.[6] In addition, L-threo-3,4-dihydroxyphenylserine (Droxidopa) itself served as a crucial drug for the treatment of Parkinson's disease. [7] As a result, β-hydroxy-α-amino acids had attracted extensive attentions because of their important role in chemical synthesis and pharmaceutical manufacturing.

β-hydroxy-α-amino contained two-chiral centers and four isomers (L-threo, D-threo, L-erythro and D-erythro) were produced simultaneously during the chemical processes. [8,9] This made optically pure of β-hydroxy-α-amino acid difficult to synthesize. Many approaches including Sharpless asymmetric dihydroxylation, epoxidation or enzymatic aldol addition had been developed for the synthesis of β-hydroxy-α-amino acids.[10-12] Among them, enzymatic approach was most promising as it could establish two new chiral-centers in a single reaction with inexpensive substrates as well as under mild and unprotected conditions. [12] Threonine aldolases (TAs) were such a kind of enzymes that catalyzed the aldol addition of glycine and various aldehyde to produce β-hydroxy-α-amino acids. TA was a pyridoxal

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5'-phosphate (PLP)-dependent enzyme and could be classified into LTA and DTA based on the stereospecificity at the C_α of its products.[13-15] In most cases, TA was highly stereoselective (>99% enantiomeric excess, *ee*) at the C_α while showed moderate stereoselectivity at the C_β (about 10-45% diastereomeric excess, *de*).[16] As a result, both threo and erythro isomers were produced in a TA-catalyzed reaction. This "C_β problem" had greatly hampered and limited the synthetic application of TA. One possible reason for the poor stereoselectivity at C_β was that two active histidine residues in TA could both interact with the hydroxyl group on C_β from the opposite direction.[16] It was noteworthy that up to now, attempts to improve the C_β stereoselectivity of TA by protein engineering had ended in failure mainly due to the complicated interactions in the enzyme active center participating in the aldol addition reactions.[14,17,18] DTA was reported could obtain a high stereoselectivity by keeping the reactions under kinetically control (usually in conditions of low temperature and low amount of enzyme).[19,20] Unfortunately, almost all of active intermediates were L-threo isomers and only produced by LTA[4-7]. Recently, Chen et al. had developed a high-throughput screening method and improved the stereoselectivity of LTA. However, the *de* value of mutant enzyme was up to about 74% which was still too low for the industrial application.[18] As a result, the mining and characterization of novel L-threonine aldolase-like enzyme with high stereospecificity was requisite to meet the needs of industrial production as well as to expand toolkit for asymmetric synthesis of β-hydroxy-α-amino acids.

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In recent years, L-threonine transaldoase (LTTA) was reported as a member of antibiotic gene cluster and participated in the biosynthesis of antibiotics. [21-23] The characteristic of LTTA was that it could catalyze the formation of β-hydroxy-α-amino acids with high stereoselectivity at C_β using L-threonine and aldehyde as substrates. [22] Phylogenetic analysis indicated that LTTA, LTA and serine hydroxymethyl transferase (SHMT) shared a common evolutionary origin and thus might have a similar catalytic mechanism.[22] In present study, we characterized a LTTA form *Pseudomonas* sp. with excellent stereoselectivity in β-hydroxy-α-amino acids synthesis. It showed that PsLTTA only consumed L-threonine as donor substrate and could accept a wide range of aromatic aldehydes as acceptor substrates. The conversion and *de* value of L-threo-*p*-methylsulfonylphenylserine by PsLTTA were up to 67.1% and 94.5% respectively after reaction conditions optimization. Finally, synthesis of L-threo-*p*-methylsulfonylphenylserine in a 100 mL scare by a whole-cell biocatalyst was conducted. Taken together, our results systematic evaluated the catalytic property of PsLTTA and suggested that PsLTTA could serve as a promising biocatalyst for preparation of L-threo-β-hydroxy-α-amino acids.

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2. Results and discussion

2.1 Sequence analysis and expressing of PsLTTA

PsLTTA was identified as a SHMT-like enzyme as it showed about 26-33% identity in amino acid sequence with SHMTs (Table S1). Multiple sequences alignment demonstrated that the PLP-binding residues were quite conservative among PsLTTA and SHMTs, suggesting that they might have a similar catalytic mechanism (Fig S1). This was consistent with the previous report that LTTA and SHMT shared a common evolutionary origin.[22]

PsLTTA was inducing expressed under the optimum conditions (0.2 mM IPTG, 28℃, 16h) and further purified with His-tag affinity chromatography. The purified PsLTTA revealed a pink color in solution (Fig S2A). SDS-PAGE indicated that most of PsLTTA was soluble protein and its monomer molecular weight was about 48 kDa, which was in accordance with the predicted molecular size (Fig S2B). PsLTTA could catalyzed the trans-aldehyde reaction of L-threonine and aldehyde to produce β-hydroxy-α-amino acids and acetaldehyde (Fig S3). Transaldolase activity assay using *p*-methylsulfonyl benzaldehyde and L-threonine as substrates demonstrated that the cell-free extracts and purified enzyme had specific activities of 0.87 U mg^{-1} and 1.32 U mg^{-1}, respectively.

2.2 Enzymatic properties

Thermostability profile indicated that PsLTTA was quite stable in the range of 4-30℃. The enzymatic activity of PsLTTA was rapidly decreased when the temperature was above 37℃ and was complete inactivation at 60℃ (Fig 1A). The

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optimum reaction temperature of PsLTTA was determined at 30℃ (Fig 1B). PsLTTA was active at pH 6.0-8.0 as more than 80% activity could be detected (Fig 1C). The maximum activity of PsLTTA was observed at 100 mM potassium phosphate buffer with a pH value of 7.0 (Fig 1D). Those results suggested that PsLTTA was a pH and temperature sensitive enzyme. The effects of metal ions on the activity of PsLTTA were further investigated at concentrations of 1 mM and 10 mM. As shown in Fig 1E, the transaldolase activity of PsLTTA was slightly promoted by Mg^{2+} and Li^{+} when compared to the control group. Ni^{2+}, Cu^{2+}, Fe^{2+} and Zn^{2+} could inhibit PsLTTA both in 1 mM and 10 mM. Other metal ions such as Cs^{2+}, Ba^{2+}, Ca^{2+} and Mn^{2+}, showed little effect on PsLTTA in 1 mM but a moderate inhibition in 10 mM. PLP concentration assay suggested that PLP was required for the catalysis of PsLTTA since a PLP-free group showed 65.8% activity compared to the control group (250 μM PLP) (Fig 1F). This result was similar to the previous report that LTTA was a PLP-dependent enzyme.[24] The partial activity in PLP-free group was likely on account that the enzyme itself combined with a portion of PLP during the process of folding intracellularly.

2.3 Substrate specificity of PsLTTA

The donor substrate specificity of PsLTTA was evaluated with *p*-methylsulfonyl benzaldehyde as the acceptor. The K_m and k_{cat} of PsLTTA using L-threonine as donor substrate was 9.85 mM and 63.4 min^{-1} while PsLTTA was not active toward L-allo-threonine, D-threonine, D-allo-threonine, L-serine and glycine (Table S2 and Fig S4). Similar to the enzyme assay results, HPLC analysis indicated that PsLTTA

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could catalyze *p*-methylsulfonyl benzaldehyde and L-threonine to form L-threo-*p*-methylsulfonylphenylserine with high stereoselectivity (80.1% *de*) (Fig 2A). However, no products were detected when using L-allo-threonine, D-threonine, D-allo-threonine, and glycine as donor substrates (Fig 2C-2F). PsLTTA could also catalyzed the trans-aldehyde action of *p*-methylsulfonyl benzaldehyde and L-serine, however, the transaldolase activity sharply declined and the major product was L-erythro-*p*-methylsulfonylphenylserine (-43.8% *de*) (Fig 2B). Those data suggested that PsLTTA was high selective in donor substrate. Coincidentally, the configuration of L-threonine was L-threo form which was the same as the major product, this finding let us speculated that the strict donor specificity might contribute to the high stereoselectivity of PsLTTA.

The acceptor substrate specificity was further assessed using L-threonine as the donor. We evaluated the catalytic activity of PsLTTA on aliphatic aldehydes such as formaldehyde or acetaldehyde. The results showed that PsLTTA could not consume those two aldehydes mainly due to their oxidability. It demonstrated that PsLTTA could utilize a wide range of aromatic aldehydes as acceptor substrate to produce β-hydroxy-α-amino acids. As expected, all the major products were L-threo forms (Table 1). This was quite important since the active intermediates of thiamphenicol, chloramphenicol and Droxidopa were L-threo isomers. [4-7] We found the substituent type on the phenyl ring of aromatic aldehydes could affect the activity of PsLTTA. For example, PsLTTA could catalyze aromatic aldehydes with electron-withdrawing substituents such as $-CH_3SO_2$, $-NO_2$, -F, -Cl and -Br, while electron-donating

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substituents (-CH_3, -OCH_3 and -OH) could not serve as the substrates of PsLTTA. For -Cl and -Br substituted benzaldehydes, it showed that PsLTTA only catalyzed *o*-substituted benzaldehydes, suggesting that the position of substituent also had an effect on PsLTTA. Those results were quite similar when using DTA to catalyze aromatic aldehydes and glycine. Chen et al. reported that the type of substituting as well as the position on the phenyl ring played an essential role in the catalytic performance of DTA by affecting the interaction between the β-OH-group of the substrate and the manganese ion during the process of substrate recognition.[20] This conclusion seemed to be useful for PsLTTA and could be the basis for protein engineering of PsLTTA.

It was worth mentioning that PsLTTA could catalyze *p*-methylsulfonyl benzaldehyde with a high conversion (74.4%) and high stereoselectivity (79.9% *de*). The product L-threo-*p*-methylsulfonylphenylserine was the main intermediate of thiamphenicol and florfenicol. L-threonine aldolase (LTA) also reported that could catalyze the formation of *p*-methylsulfonylphenylserine using *p*-methylsulfonyl benzaldehyde and glycine as substrates. [25] We assessed the catalytic activity and stereoselectivity of PsLTTA and a LTA from *Clavibacter michiganensis* (ClLTA). The conversion of *p*-methylsulfonyl benzaldehyde by PsLTTA and ClLTA were 75.2% and 85.6%, respectively. However, the *de* value of L-threo-*p*-methylsulfonylphenylserine by PsLTTA was 79.6%, much higher than that of ClLTA with a *de* value of 28.6% (Fig S5). This result suggested that PsLTTA was more stereoselective compared to ClLTA. Furthermore, the reverse activities of

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PsLTTA and ClLTA were evaluated using L-threo-*p*-methylsulfonylphenylserine as substrate. As shown in Fig S6, ClLTA could total change L-threo-*p*-methylsulfonylphenylserine into *p*-methylsulfonyl benzaldehyde and glycine within 3 hours. This result was consistence with the conclusion that LTA-catalyzed reaction was reversible. [26] To our surprise, the reverse transaldol activity of PsLTTA was almost undetectable when using L-threo-*p*-methylsulfonylphenylserine and acetaldehyde as substrates, suggesting the LTA-catalyzed reaction might not a reversible reaction. In LTA catalyzed-reaction, the excess substrate (usually glycine was 10 folds to aldehyde) was required to make sure that the reaction equilibrium shifted in the direction of producing β-hydroxy-α-amino acids, which resulted in a waste of substrates. [18] In PsLTTA, the use of superfluous substrates could be avoidable as its poor reverse transaldol activity. Considering the stereoselectivity and substrate availability, it revealed that PsLTTA was more suitable for industrialized application of producing β-hydroxy-α-amino acids.

2.4 Effects of reaction conditions on conversion and stereoselectivity of PsLTTA

Although the enzyme activity assay indicated that PsLTTA achieved the maximum activity at phosphate buffer (Fig 1D), it revealed that the transformation of β-hydroxy-α-amino acid by whole-cell biocatalyst could be improved more than 30% when using Tris-HCl buffer (100 mM Tris-HCl, pH 7.0) as reaction buffer. This result suggested Tris-HCl buffer was more suitable for whole-cell biocatalyst and was used in the following reactions. 10% acetonitrile (v/v) was selected for co-solvent since

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p-methylsulfonyl benzaldehyde dissolved well in acetonitrile and the optimal reaction temperature (30 ℃) was used as initial reaction temperature.

We first evaluated the concentration of wet cells (3 to 50 mg/ml) and substrate (10 to 40 mM) on the conversion and stereoselectivity of PsLTTA using *p*-methylsulfonyl benzaldehyde and L-threonine as substrates. As shown in Fig 3, similar trends were constantly observed that the increase of wet cells led to a decrease of stereoselectivity in PsLTTA under different substrate concentration. The *de* values of products were about 86.1% when the wet cells concentrations were 3.0 and 6.25 mg/ml. Then the *de* values declined from 86.1% to 72.0% with the increase of wet cells concentrations from 6.25 up to 50 mg/ml. Those results strongly indicated that low dose of wet cells contributed to the formation of L-threo isomer. The 3.0 and 6.25 mg/ml of wet cells concentrations had little difference in the stereoselectivity of PsLTTA, but greatly affected its conversion (about 5-22% improvement in conversions), suggesting that 6.25 mg/ml of wet cells was suitable for achieving high stereoselectivity performance. The concentration of substrates affected the conversion of PsLTTA but not its stereoselectivity. Under 6.25 mg/ml wet cells concentration, the conversions fall from 90.1% to 50.5% when the substrate concentrations ranged from 10 mM to 40 mM. We found that the products could reach the maximum yield at 30 mM substrate with a conversion of 69.5%. Based on those results, 6.25 mg/ml and 30 mM were selected as the final concentration of wet cells and substrate, respectively.

The effects of co-solvents were further investigated. As shown in Table 2, the none organic solvents group gave a comparatively low conversion (41.6%) and *de* value

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(71.3%), suggesting that organic solvents were required for the catalysis of PsLTTA. It demonstrated that low percentage of organic solvents (10%) greatly promoted the conversion of PsLTTA while high percentage of organic solvents (30%) leaded to a decline of conversion. Among the tested organic solvents, high conversion (80.4%) and *de* value (90.2%) were obtained when using 10% ethyl acetate as co-solvents. 10% MeOH and 10% DMSO exerted promotion effects on the conversion but showed little enhancements on the stereoselectivity of PsLTTA when compared to 10% CH_3CN group. The addition of 10% acetone and 10% EtOH groups both lowered the conversion and the stereoselectivity of PsLTTA. Thus, 10% ethyl acetate was selected as an optimal co-solvent for enzymatic reaction.

The trans-aldehyde reaction of PsLTTA was evaluated under different temperature for 6 hours. As shown in Fig 4, the highest conversion of products (79.8%) were observed at 30℃, which was consistence with the conclusion that the optimum reaction temperature of PsLTTA was 30℃. For stereoselectivity, we surprising found that increasing temperature from 10℃ to 35℃ led to a reduction of stereoselectivity in PsLTTA. The *de* values had little change (about 94.5%) but the conversion rates were rapidly increased from 44.7% to 67.8% at the temperature of 10, 15 and 20 ℃, respectively. The *de* values sharply declined (from 94.5% to 87.2%) when the temperature was 25, 30 and 37℃. Those results indicated that lower temperature contributed to the formation of L-threo isomer and suggested 20 ℃ as the best temperature for catalysis reaction of PsLTTA.

Taken together, our results strongly suggested that low concentration of wet cells

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(6.25 mg/ml), co-solvent (10% ethyl acetate) and low temperature (20 °C) could dramatically improve the stereoselectivity of PsLTTA with a *de* value from 71.3% up to 94.5%. Our conclusion that low concentration of wet cells and low temperature contributed to the stereoselectivity of PsLTTA was exactly similar to DTA.[20] Under such conditions, the catalysis reaction was under kinetic control but not thermodynamic control which was benefit for producing L-threo isomer. [27]

In recent years, lots of attentions were put on TAs as they were the only reported aldolases for production β-hydroxy-α-amino acids using cheap substrates (glycine and aldehyde) and under mild conditions. However, the poor stereoselectivity at C_β (about 10-45% *de*) severely blocked its industrialized application.[16] In present study, the characterized PsLTTA not only displayed TAs-like merits such as inexpensive substrates and mild conditions, but also excellent stereoselectivity at C_β (94.5% *de*) and poor reverse transaldol activity. Under the optimized conditions, we further assessed the stereoselectivity of PsLTTA and ClLTA on other aromatic aldehydes (Table 3 and Fig S7-14). Similar to *p*-methylsulfonyl benzaldehyde, PsLTTA could consume multiple aromatic aldehydes to produce β-hydroxy-α-amino acids with excellent stereoselectivity. Among those products, L-threo-*p*-nitrophenylserine (92.8% *de*), L-threo-phenylserine (89.1% *de*), L-threo-*o*-chlorophenylserine (87.1% *de*) and L-threo-*o*-bromophenylserine (86.9% *de*) were obtained with excellent stereoselectivity. The de values of those products were much higher than that catalyzed by PsLTTA before reaction optimization (Table 2) or by ClLTA (Fig S7-14). Those results demonstrated that the optimized reaction conditions were

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universally applicable for aromatic aldehydes with different substituents. Considering that L-threo-*p*-nitrophenylserine was widely reported as an intermediate of chloramphenicol and L-threo-phenylserine could be found in serval bioactive substances, [4-6] it strongly suggested that PsLTTA might take TA' s place as a promising and robust biocatalyst for production of useful β-hydroxy-α-amino acids.

2.5 Synthesis of L-threo-*p*-methylsulfonylphenylserine by whole-cell biocatalyst

A 100-mL scale reaction was then carried out under the optimal reaction conditions, and time courses of transformation of L-threo-*p*-methylsulfonylphenylserine were monitored from 0 to 24 h. As expected, L-threo-*p*-methylsulfonylphenylserine were produced with excellent stereoselectivity (90.4% *de*). The conversion rate was 67.1% and the concentration of *p*-methylsulfonyl benzaldehyde decreased from 31.0 mM to 10.2 mM within 24 hours. The synthesis of L-threo-*p*-methylsulfonylphenylserine reached 13 mM (95.1% *de*) during the first 2 hours indicated that the beginning reaction was efficient. The products added up to 21 mM at 12 h and was not increased in yield from 12 to 24 h. However, the *de* value at 12 h was 93.1% and decreased to 90.4% at 24 h. In our opinion, L-threo-*p*-methylsulfonylphenylserine was synthesized preferentially in the initial reaction stage. As time went by, the reaction tended to produce more L-erythro-*p*-methylsulfonylphenylserine which led to a decline of *de* value. Those results suggested that short time reaction contributed to the formation of L-threo-*p*-methylsulfonylphenylserine. Considering that L-threo-*p*-methylsulfonylphenylserine was instable, we changed it into L-threo-*p*-methylsulfonylphenylserine ethyl ester by esterification. The final purified

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product was 189 mg in white solid, yield 33.0% and further confirmed by HRMS (Fig S15), ^{1}H NMR (Fig S16) and ^{13}C NMR (Fig S17) analysis. As far as we knew, these results represent the highest *de* value reported far for asymmetric catalysis of L-threo-*p*-methylsulfonylphenylserine.

3. Conclusions

A L-threonine transaldolase from *Pseudomonas* sp. (PsLTTA) was mined and expressed in *Escherichia coli* BL21 (DE3). Substrate spectrum assay indicated that PsLTTA only consumed L-threonine as donor substrate and could accept a wide range of aromatic aldehydes as acceptor substrates. Through reaction condition optimization, the reaction was under kinetic control and L-threo-*p*-methylsulfonylphenylserine was obtained with more than 60% conversion and near perfect stereoselectivity (94.5% *de*), the highest stereoselectivity of L-threo-β-hydroxy-α-amino acids reported by enzymatic synthesis so far. Finally, synthesis of L-threo-*p*-methylsulfonylphenylserine in a 100 mL scare by a whole-cell biocatalyst was conducted. It was for the first time systematically demonstrated that L-threonine transaldolase as a powerful biocatalyst for preparation of L-threo-β-hydroxy-α-amino acids. However, it should be admitted that the production of L-threo-*p*-methylsulfonylphenylserine by PsLTTA was comparatively low since the maximum concentration was only about 20 mM under the optimized conditions. In our opinion, the moderate production by PsLTTA mainly due to its less-efficient catalytic ability. Furthermore, the produced acetaldehyde during the

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reaction could also inhibit the synthesis of L-threo-*p*-methylsulfonylphenylserine (data were not shown). So further studies such as engineering PsLTTA or establishing an acetaldehyde removal system was under way to improve L-threo-β-hydroxy-α-amino acids production.

4. Experimental

4.1 Materials

Escherichia coli BL21 (DE3) and plasmid pET28a were kept in our laboratory and served as host strain and expression vector, respectively. Yeast alcohol dehydrogenase (ADH), Pyridoxal 5'-phosphate (PLP) and NADH were purchased from Aladdin (China). O-phthaldialdehyde (OPA) and N-acetyl-cysteine (NAC) were obtained from Sigma-Aldrich (America). L-threo-*p*-methylsulfonylphenylserine standard was purchased from Shanghai yuanye Bio-Technology Co., Ltd (China). All other chemicals and reagents used in this work were obtained commercially with the highest purity unless otherwise stated.

4.2 Gene cloning, expression and purification

The DNA sequences of *Pseudomonas* sp. L-threonine transaldolase (PsLTTA) and *Clavibacter michiganensis* low specificity L-threonine aldolase (ClLTA) based on their protein sequences (GenBank No. WP_065936857 and WP_011931605) were optimized and synthesized by Wuhan GeneCreate Biological Engineering Co., Ltd. (China) and cloned into plasmid pET28a. The recombinant plasmids were further

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transformed into *E. coli* BL21 (DE3).

500 mL *E. coli* BL21 (DE3) cells harbouring pET28a-*PsLTTA* or pET28a-*ClLTA* were grown in LB medium containing 50 μg/ mL kanamycin. The expression of PsLTTA and ClLTA were induced overnight at 28℃ with 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG) after $OD_{600\ nm}$ reached 0.5. Cells were pelleted at 6000 g under 4℃ and were resuspended with Tris-HCl buffer (100 mM Tris-HCl, 200 mM NaCl, 25 mM imidazole, pH 7.0). Cells were lysed by ultrasonication and the soluble PsLTTA protein was purified by a Ni-IDA Prepacked Column (Sangon, China) according to the instruction manual. The purified enzyme was exchanged into Tris-HCl buffer (100 mM Tris-HCl, 20% glycerol, pH 7.0) by a PD-10 desalination column (GE Healthcare, America) and further assessed by SDS-PAGE. Protein concentration was determined using a BCA Protein Assay Kit (Beyotime, China). For whole cell biocatalyst, wet cells were collected, weighted and resuspended in Tris-HCl buffer (100 mM Tris-HCl, pH 7.0) with a concentration of 250 mg/ml. The purified enzymes and wet cells were stored at -80℃ until used.

4.3 Enzyme activity assay

Enzyme activities of PsLTTA was determined by an NADH coupled with ADH assay as previous described.[28] The reduction of NADH was detected by monitoring the decrease in absorbance at 340 nm (ε=6220 $M^{-1}cm^{-1}$) using Multiskan Go microplate reader (Thermo Scientific, America) at 25 °C. Reaction mixtures (180 μL) consisting of 10 mM *p*-methylsulfonyl benzaldehyde, 30 mM L-threonine, 0.2 mM PLP, 0.2 mM NADH and 10 U ADH in Tris-HCl buffer (100 mM Tris-HCl, pH 7.0,

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10% acetonitrile) were incubated at 25 ℃ for 1 min. Reactions were initiated by introduction of 10 mg PsLTTA enzyme (in 20 μL Tris-HCl buffer) and monitored at 340 nm for 2 min. A boiled enzyme sample was used as a negative control. One unit (U) of transaldolase activity was defined as the amount of enzyme catalyzing the conversion of 1 μmol of *p*-methylsulfonylphenylserine per minute.[29] All experiments were conducted in triplicate.

4.4 Determination of kinetic parameters

The kinetic parameters were determined by measuring the initial rate of enzymatic reaction at 10 mM *p*-methylsulfonyl benzaldehyde and varying concentrations of L-threonine (0.1-200 mM).[29] Five independent replicates were performed for each concentration of L-threonine assay and the data were fitted to the Michaelis-Menten equation using Origin 8.0 software.

4.5 Effect of temperature and pH on the stability and activity of PsLTTA

The pH stability of PsLTTA was evaluated by investigating the transaldolase ability after incubation in 4℃ for 12h at various pHs using 100 mM citric acid-citrate (pH 3.0-6.0), phosphate (pH 6.0-8.0), Tris-HCl (pH 8.0-9.0) and carbonate-bicarbonate (pH 9.0-11.0) as buffer. The thermostability of enzyme was assessed after 1h incubation at varied temperatures from 4 to 80℃.

The pH dependence of enzyme activity was studied at various pHs (pH 6.0-8.0) in 100 mM phosphate buffer. The effect of temperature on enzyme activity was assessed at varied temperatures from 25 to 45 ℃. The enzyme activity was measured as described above. All experiments were conducted in triplicate.

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4.6 Effect of metal ions on transaldolase activity of PsLTTA

The influences of different metal ions on transaldolase activity of PsLTTA were studied in the presence of specific metal ions at final concentrations of 1 mM and 10 mM. The activity measured in the absence of metal ion was taken as a negative control and all experiments were performed in triplicate.

4.7 Chiral derivatization analysis of stereospecificity of PsLTTA

The standard catalysis reaction was performed in 1 ml volume comprising 100 mM L-threonine, 40 mM *p*-methylsulfonyl benzaldehyde, 0.2 mM PLP, 1 mM $MgCl_2$ and 2 μM PsLTTA (or 25mg/ml wet cells) in Tris-HCl buffer (100 mM Tris-HCl, pH 7.0). Reaction was incubated at 30℃ for 4h with constantly shaking and terminated by addition of 2 ml MeOH. The reaction mixtures were incubated at 4℃ for 12 h and the supernatant was used for further study.

The determination of conversion and stereospecificity of PsLTTA was performed by HPLC after derivatization with O-phthaldialdehyde/N-acetyl-cysteine (OPA/NAC).[30] Briefly, the OPA/NAC reagent was obtained by dissolving of 100 mg of NAC in 20 mL derivatization buffer (0.2 M Boric acid, 0.2 M KCl) and then 25.6 mg of OPA in 5 mL MeOH was added. OPA/NAC reagent was mixed with diluted reaction solution at a ratio of 4:1 and kept for 10 min in room temperature. Chromatographic analysis was carried out using Shimadzu LC-20AT HPLC system (Japan) with an UV detector at 236 and 340 nm. Column: Agilent ZORBAX reversed-phase columns (250×4.6 mm, 5 μm), mobile phase: 50 mM KH_2PO_4, pH 8.0/acetonitrile (81/19 or 79/21), flow rate: 1 ml min^{-1}, temperature: 30 ℃.

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4.8 Donor and acceptor substrate specificity

Donor substrate specificity of PsLTTA was evaluated using L-threonine, L-allo-threonine, D-threonine, D-allo-threonine, L-serine and glycine as donors while *p*-methylsulfonyl benzaldehyde as acceptor. For acceptor substrate specificity, a series of aromatic aldehydes and L-threonine were selected as acceptor and donor, respectively. Reaction mixtures were detected by enzyme activity assay or by analytical HPLC after OPA/NAC derivatization.

4.9 Effect of reaction condition on activity and stereospecificity of PsLTTA

Effect of reaction conditions on whole-cell biocatalyst were further determined. The initial catalysis condition was performed at 30℃ for 3h, in a 1 ml volume comprising 100 mM L-threonine, 40 mM *p*-methylsulfonyl benzaldehyde, 0.2 mM PLP, 1 mM $MgCl_2$ and 25 mg wet cells in Tris-HCl buffer (100 mM Tris-HCl, 10% CH_3CN, pH 7.0). The reaction conditions including amount of enzyme or substrate, co-solvents and reaction temperature were further evaluated. The conversion and stereospecificity catalyzed by whole-cell biocatalyst were detected by analytical HPLC after OPA/NAC derivatization.

4.10 Reverse activity of PsLTTA

The reverse activity of PsLTTA was assessed using L-threo-*p*-methylsulfonylphenylserine and acetaldehyde as substrates. The reaction was performed in 1 ml volume comprising 100 mM L-threo-*p*-methylsulfonylphenylserine, 50 mM acetaldehyde, 0.2 mM PLP, 1 mM $MgCl_2$ and 25mg/ml wet cells in Tris-HCl buffer (100 mM Tris-HCl, pH 7.0).

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Reaction was incubated at 30℃ for different hours with constantly shaking and terminated by addition of 2 ml MeOH. The conversions were detected by analytical HPLC at 236 nm. The reaction catalyzed by ClLTA was performed as a positive control.

4.11 Synthesis of L-threo-*p*-methylsulfonylphenylserine in a large scare of 100 mL system by whole-cell biocatalyst

Transformation of L-threo-*p*-methylsulfonylphenylserine at a 100 mL system by whole-cell biocatalyst were performed at the optimized reaction conditions. Briefly, L-threonine (1.2 g, 10 mmol), *p*-methylsulfonyl benzaldehyde (0.55 g, 3 mmol), PLP (5 mg, 0.02 mmol), $MgCl_2$ (10 mg, 0.1 mmol) and wet cells (0.625g) were added in 100 ml Tris-HCl buffer (100 mM Tris-HCl, 10% ethyl acetate, pH 7.0). The reaction mixture was constantly shaking at 20 °C for 24 h. Time course of catalysis processing was minored in triplicate. The conversion and stereoselectivity were detected by analytical HPLC after OPA/NAC derivatization.

After reaction, the crude reaction supernatant was added 20 mL ethanol and adjusted to pH 1.0 by concentrated sulfuric acid. For esterification, the mixture was incubated at 100℃ for 2 hours. The supernatant was extracted with chloroform twice. The chloroform layer was combined and evaporated to dryness. The residue was purified by a C18 SPE column ($CHCl_3$: MeOH = 2 :1) to give 189 mg white solid, yield 33.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.1 Hz, 2H), 7.62 (d, J = 8.1 Hz, 2H), 4.98 (d, J = 4.5 Hz, 1H), 4.19 (q, J = 7.0 Hz, 2H), 3.64 (d, J = 4.7 Hz, 1H), 3.08 (s, 3H), 1.23 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.79, 147.58,

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139.91, 127.48, 127.26, 73.41, 61.64, 60.22, 44.53, 14.08. HRMS (*m/z*) (M^+): calcd. for $C_{12}H_{18}NO_5S$, 288.0897, found 288.0900.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

We thank Dr. Bing-De Zheng (College of Chemistry, Fuzhou University) for the technical assistance of HRMS and NMR analysis. This study is financially supported by Industry University Research project of Fuzhou University (No. 2019090501).

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514 **Table**

515 **Table 1.** Acceptor substrate specificity of PsLTTA.

516 **Table 2.** Effects of co-solvents on the conversion and stereoselectivity of PsLTTA.

517 **Table 3** Stereoselectivity of PsLTTA and ClLTA.

518

519 **Figure captions**

520 **Fig. 1.** Enzymatic properties of PsLTTA. (A) Temperature stability profile of
521 PsLTTA after incubation of enzyme solution on various temperatures (4-80 °C) for 60
522 minutes. (B) Detection of the optimal reaction temperature of PsLTTA. (C) pH
523 stability profile of PsLTTA after incubation of enzyme solution on various pH (3-11)
524 for 12 hours at 4℃. (D) Detection of the optimal reaction pH of PsLTTA. (E) Effects
525 of metal ions (1 mM and 10 mM) on the trans-aldehyde activity of PsLTTA. (F)
526 Effects of PLP concentration on the trans-aldehyde activity of PsLTTA. All assays
527 were performed three times.

528 **Fig. 2.** Donor substrate specificity of PsLTTA detected by HPLC after OPA/NAC
529 derivatization. (A) L-threonine. (B) L-serine. (C) D-threonine. (D) L-allo-threonine.
530 (E) D-allo-threonine. (F) glycine. Product L-*p*-methylsulfonylphenylserine ($t_{L\text{-}threo}$=7.1
531 min, $t_{L\text{-}erythro}$=8.4 min)

532 **Fig. 3.** Concentration effect of wet cells and substrates on the conversion and
533 stereoselectivity of PsLTTA. The concentrations of *p*-methylsulfonyl benzaldehyde
534 were 10 mM (A), 20 mM (B), 30 mM (C) and 40 mM (D), respectively. The reactions
535 mixture contained 10% (v/v) CH_3CN as co-solvent and incubation at 30 ℃ for 4

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hours.

Fig. 4. Effect of temperature on the conversion and stereoselectivity of PsLTTA. (A) The conversion and *de* value of L-threo-*p*-methylsulfonylphenylserine under various temperatures. (B) Determination of stereospecificity of PsLTTA by HPLC analysis after OPA/NAC derivatization. Product L-*p*-methylsulfonylphenylserine ($t_{L\text{-}threo}$=7.1 min, $t_{L\text{-}erythro}$=8.4 min).

Fig. 5. Time course for synthesis of L-threo-*p*-methylsulfonylphenylserine in 100 mL scare.

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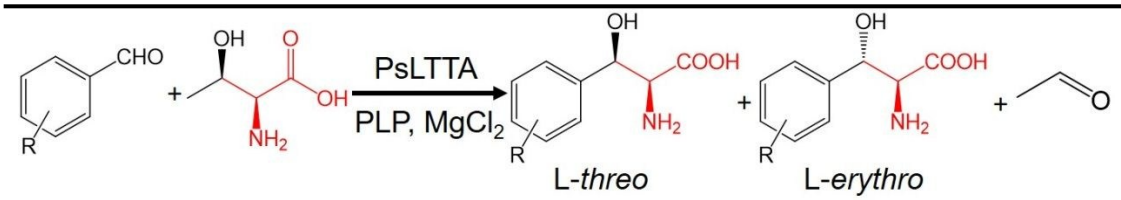
Table 1. Acceptor substrate specificity of PsLTTA.



R	Conversion (%)	*de* (%)	R	Conversion (%)	*de* (%)
H	51.0	77.2	*o*-Br	43.3	80.8
o-NO_2	40.5	50.2	*m*-Br	ND	ND
m-NO_2	25.2	29.9	*p*-Br	ND	ND
p-NO_2	59.4	76.7	*p*-$MeSO_2$	74.4	79.9
o-F	43.5	69.2	*p*-I	ND	ND
m-F	44.6	66.4	*o*-OH	ND	ND
p-F	ND	ND	*m*-OH	ND	ND
o-Cl	37.9	80.3	*p*-OH	ND	ND
m-Cl	ND	ND	*o*-CH_3	ND	ND
p-Cl	ND	ND	*p*-O(CH_3)	ND	ND

Reaction conditions: 1 mL reaction mixture containing 40 mM aromatic aldehydes, 100 mM L-threonine, 0.2 mM PLP, 1 mM $MgCl_2$ and 25 mg/mL wet cells in Tris-HCl buffer (100 mM Tris-HCl, 10% CH_3CN, pH 7.0) at 30 ℃ for 4 h.

ND, Not detected.

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552 **Table 2.** Effects of co-solvents on the conversion and stereoselectivity of PsLTTA.

co-solvents	Conversion (%)	*de* (%)	co-solvents	Conversion (%)	*de* (%)
none	41.6	71.3	10% CH_3CN	70.4	84.9
10% MeOH	81.1	85.3	20% CH_3CN	45.1	82.1
20% MeOH	76.9	86.9	30% CH_3CN	26.2	79.0
30% MeOH	44.6	82.6	10% DMSO	78.2	84.2
10% EtOH	45.1	80.8	20% DMSO	69.9	85.7
20% EtOH	55.9	81.0	30% DMSO	56.0	84.6
30% EtOH	17.7	80.2	10% Ethyl acetate	80.4	90.2
10% Acetone	64.5	80.0	20% Ethyl acetate	74.8	89.4
20% Acetone	46.2	85.1	30% Ethyl acetate	69.2	89.4
30% Acetone	39.7	84.5	40% Ethyl acetate	61.1	89.9

553 Reaction conditions: 1 mL reaction mixture containing 30 mM *p*-methylsulfonyl
554 benzaldehyde, 100 mM L-threonine, 0.2 mM PLP, 1 mM $MgCl_2$ and 6.25 mg/mL wet
555 cells in Tris-HCl buffer (100 mM Tris-HCl, pH 7.0) at 30 ℃ for 4 h.

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556 **Table 3** Stereoselectivity of PsLTTA and ClLTA.

	de (%)			*de* (%)	
R	PsLTTA	ClLTA	R	PsLTTA	ClLTA
H	89.1	17.0	*o*-F	78.1	38.5
o-NO_2	77.7	35.0	*m*-F	80.4	20.4
m-NO_2	67.9	25.6	*o*-Cl	87.1	53.9
p-NO_2	92.8	28.3	*o*-Br	86.9	53.8

557 Reaction conditions:

558 For PsLTTA, 1 mL reaction mixture containing 30 mM aromatic aldehydes, 100 mM
559 L-threonine, 0.2 mM PLP, 1 mM $MgCl_2$ and 6.25 mg/mL wet cells in Tris-HCl buffer
560 (100 mM Tris-HCl, 10% ethyl acetate, pH 7.0) at 20 ℃ for 6 h.

561 For ClLTA, 1mL reaction mixture containing 30 mM aromatic aldehydes, 300 mM
562 Glycine, 0.2 mM PLP and 6.25 mg/mL wet cells in Tris-HCl buffer (100 mM
563 Tris-HCl, 10% ethyl acetate, pH 7.0) at 20 ℃ for 6 h.

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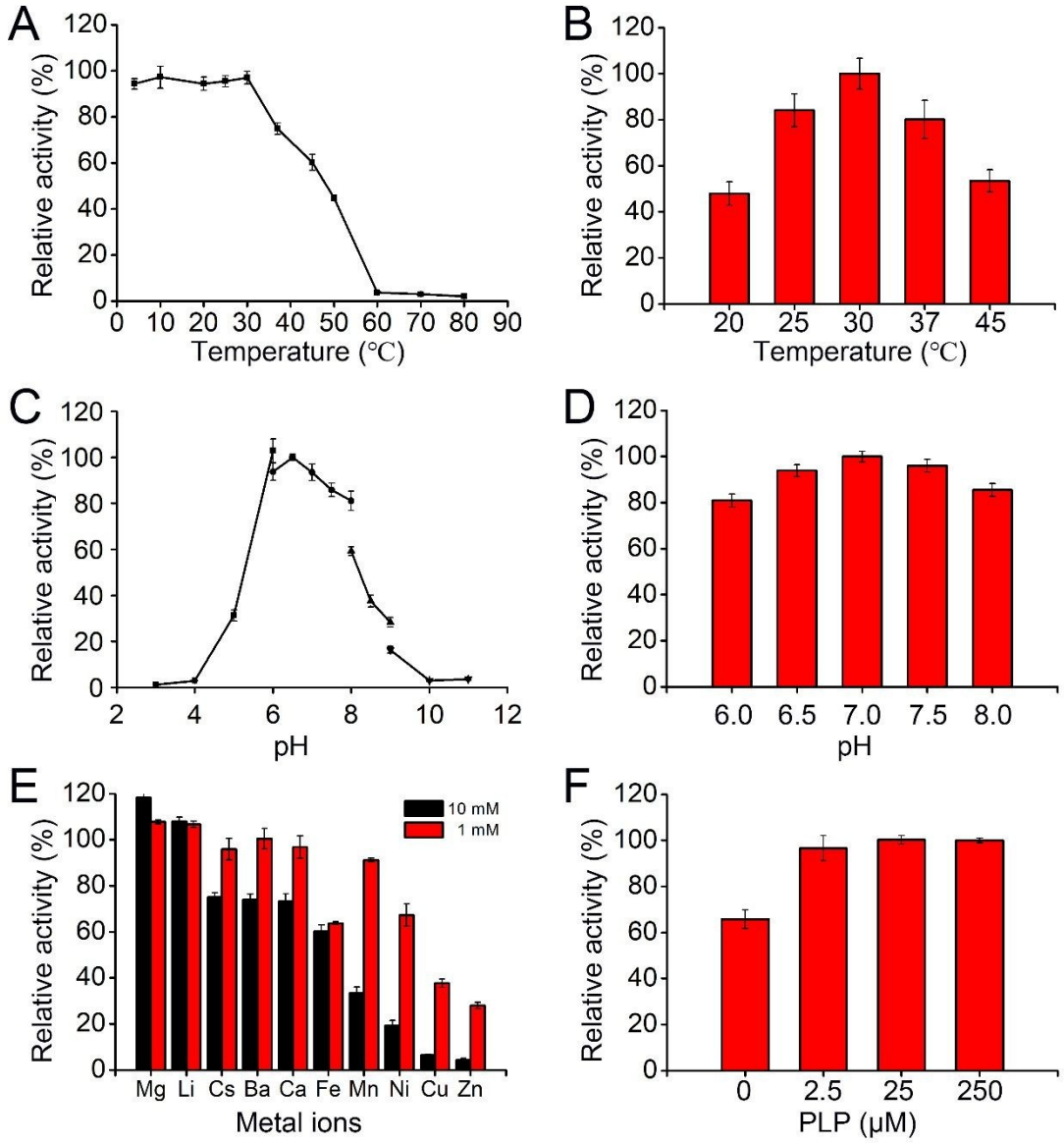
565 Fig 1.



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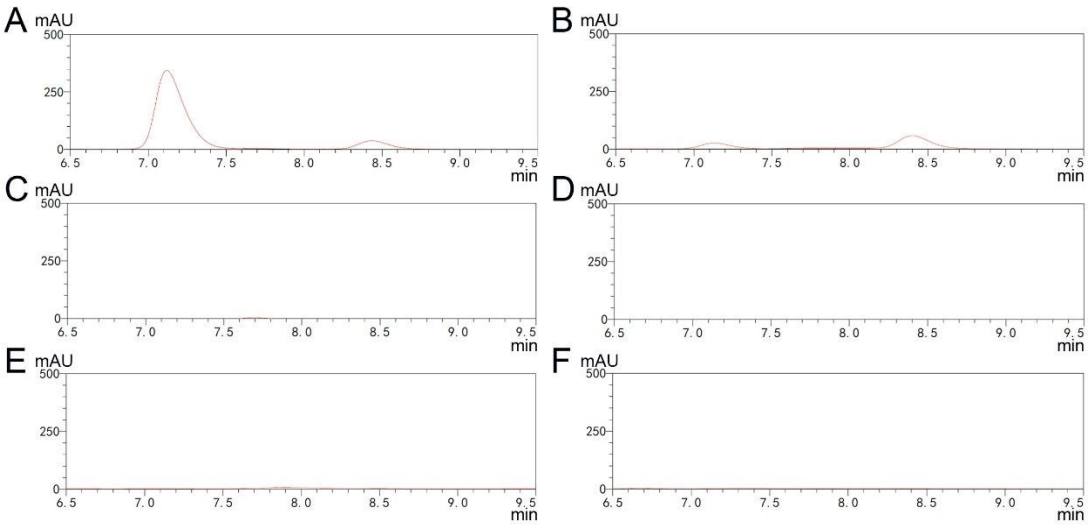
568 Fig 2.



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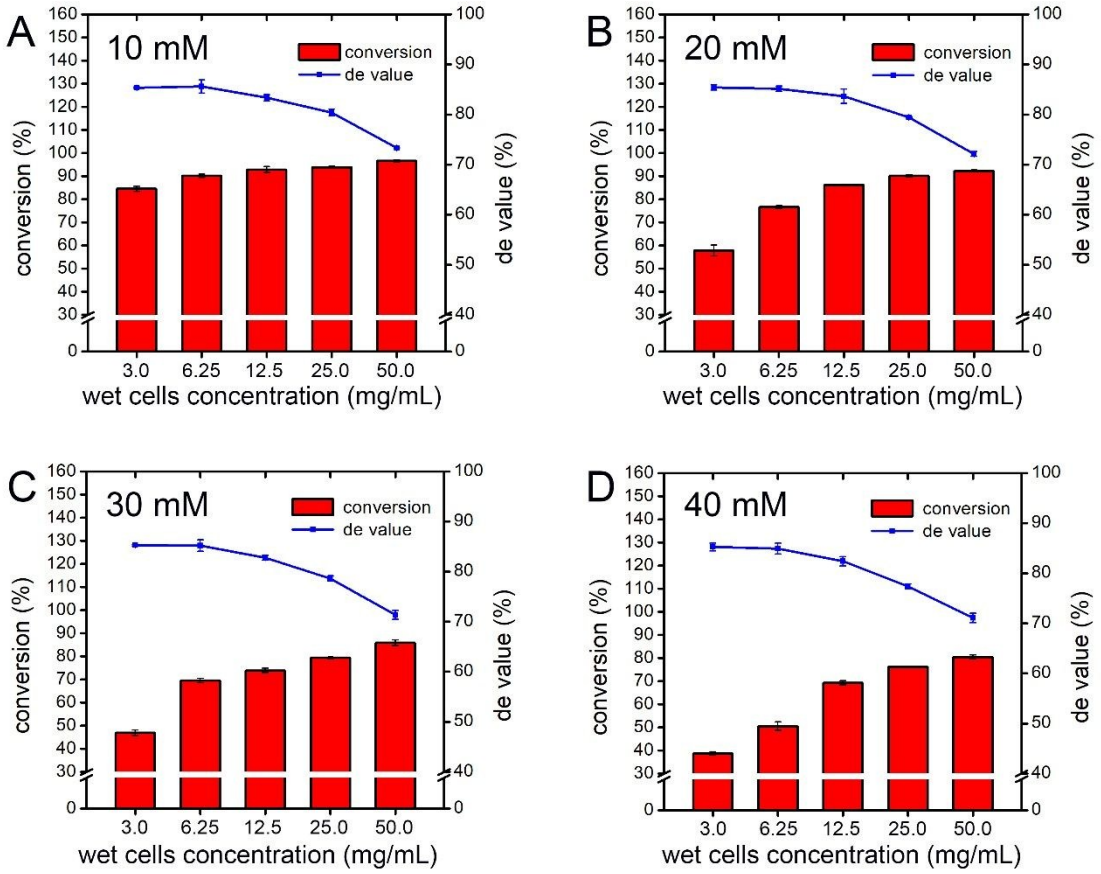

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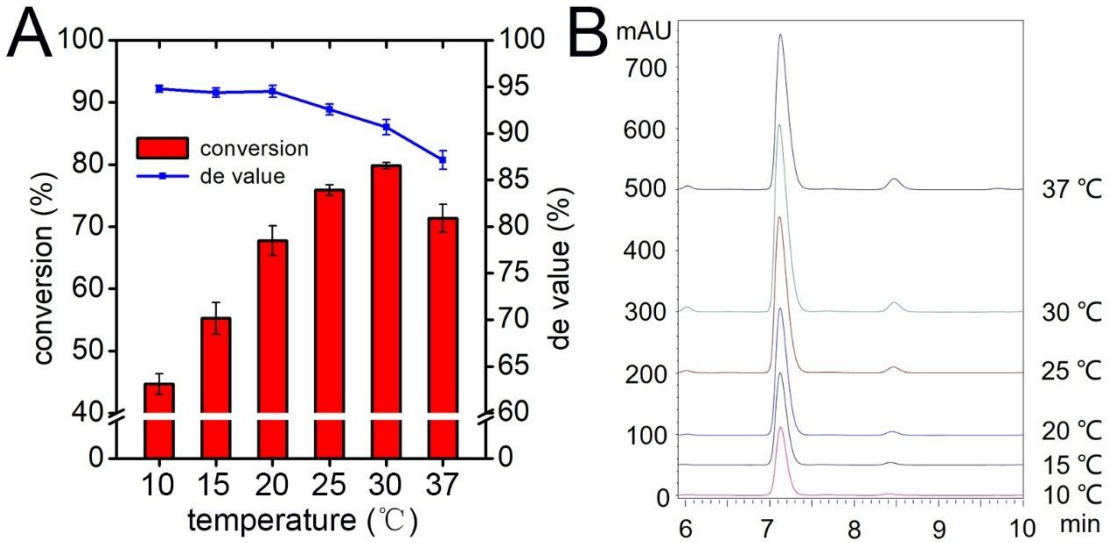
574 Fig 4.



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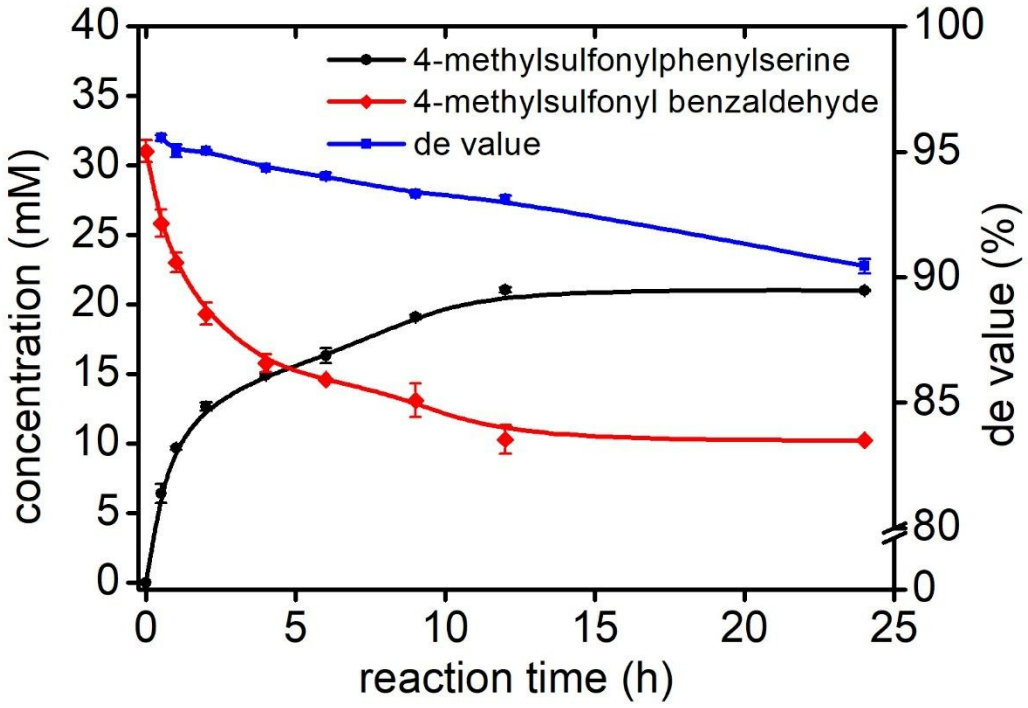
577 Fig 5.



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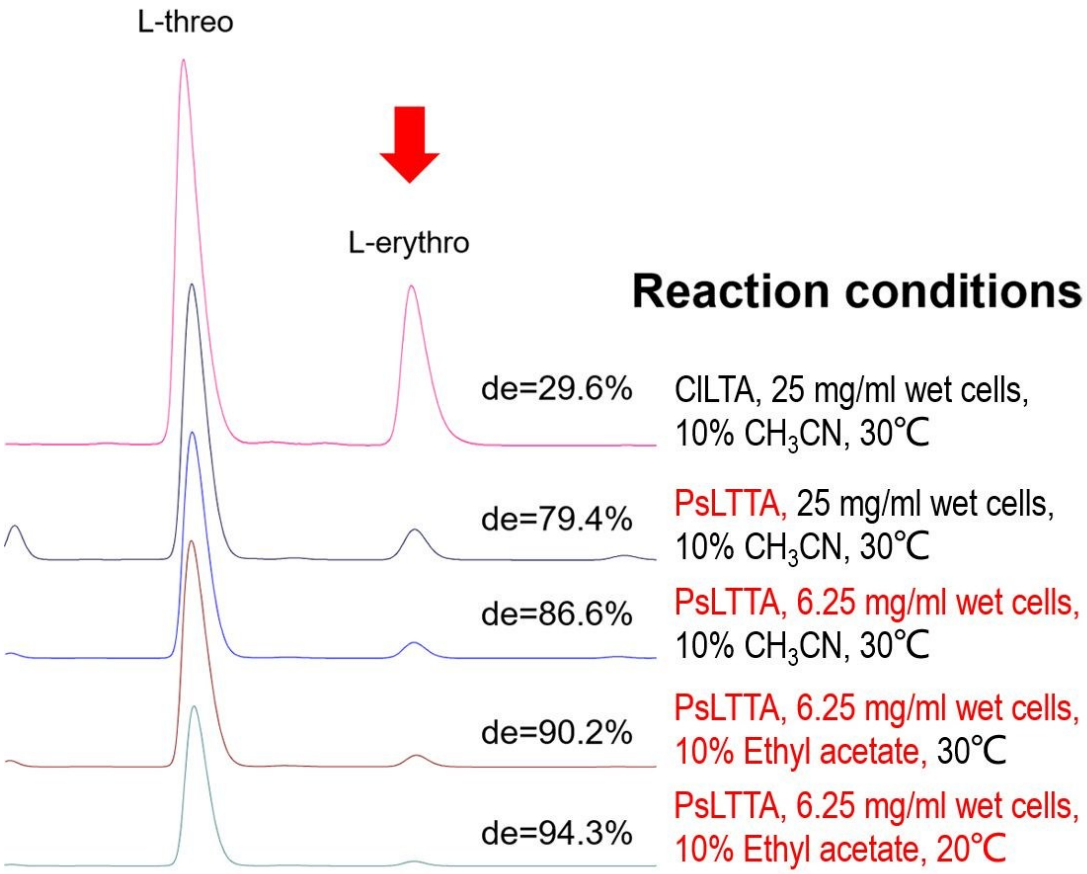
580 Figure abstract



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