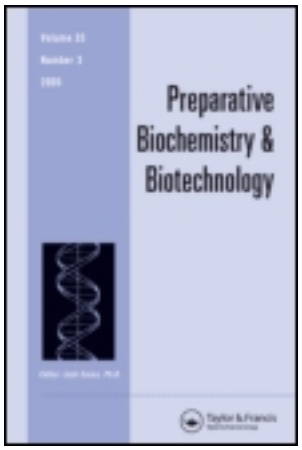

Preparative Biochemistry and Biotechnology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lpbb20

ENANTIOSELECTIVE ACYLATION OF β-PHENYLALANINE ACID AND ITS DERIVATIVES CATALYZED BY PENICILLIN G ACYLASE FROM Alcaligenes faecalis

Dengchao Li [a] , Lilian Ji [a] , Xinfeng Wang [a] & Dongzhi Wei [b]
[a] Jiangsu Key Laboratory for Biomass-Based Energy and Enzyme Technology, Huaiyin Normal University, Huai'an, Jiangsu Province, P.R. China
[b] State Key Laboratory of Bioreactor Engineering, New World Institute of Biotechnology, East China University of Science and Technology, Shanghai, P.R. China
Accepted author version posted online: 22 Aug 2012.Published online: 10 Jan 2013.

To cite this article: Dengchao Li , Lilian Ji , Xinfeng Wang & Dongzhi Wei (2013) ENANTIOSELECTIVE ACYLATION OF β-PHENYLALANINE ACID AND ITS DERIVATIVES CATALYZED BY PENICILLIN G ACYLASE FROM Alcaligenes faecalis , Preparative Biochemistry and Biotechnology, 43:2, 207-216, DOI: 10.1080/10826068.2012.719847

To link to this article: http://dx.doi.org/10.1080/10826068.2012.719847

Preparative Biochemistry & Biotechnology, 43:207–216, 2013

ISSN: 1082-6068 print/1532-2297 online
DOI: 10.1080/10826068.2012.719847

Taylor & Francis
Taylor & Francis Group

ENANTIOSELECTIVE ACYLATION OF β-PHENYLALANINE ACID AND ITS DERIVATIVES CATALYZED BY PENICILLIN G ACYLASE FROM *Alcaligenes faecalis*

Dengchao Li,[1] Lilian Ji,[1] Xinfeng Wang,[1] and Dongzhi Wei[2]

[1]*Jiangsu Key Laboratory for Biomass-Based Energy and Enzyme Technology, Huaiyin Normal University, Huai'an, Jiangsu Province, P.R. China*
[2]*State Key Laboratory of Bioreactor Engineering, New World Institute of Biotechnology, East China University of Science and Technology, Shanghai, P.R. China*

□ *This study developed a simple, efficient method for producing racemic β-phenylalanine acid (BPA) and its derivatives via the enantioselective acylation catalyzed by the penicillin G acylase from* Alcaligenes faecalis *(Af-PGA). When the reaction was run at 25°C and pH 10 in an aqueous medium containing phenylacetamide and BPA in a molar ratio of 2:1, 8 U/mL enzyme and 0.1* M *BPA, the maximum BPA conversion efficiency at 40 min only reached 36.1%, which, however, increased to 42.9% as the pH value and the molar ratio of phenylacetamide to BPA were elevated to 11 and 3:1, respectively. Under the relatively optimum reaction conditions, the maximum conversion efficiencies of BPA derivatives all reached about 50% in a relatively short reaction time (45–90 min). The enantiomeric excess value of product* (ee_p) *and enantiomeric excess value of substrate* (ee_s) *were all above 98% and 95%, respectively. These results suggest that the method established in this study is practical, effective, and environmentally benign and may be applied to industrial production of enantiomerically pure BPA and its derivatives.*

Keywords β-phenylalanine acid, *Alcaligenes faecalis*, derivatives, enantioselective acylation, penicillin G acylase

INTRODUCTION

β-Amino acids are increasingly attracting interest due to their biological activities and importance as key compounds in the synthesis of pharmaceuticals.[1] Optically pure β-phenylalanine (3-amino-3-phenylpropionic acid, BPA) and its derivatives have become an important group of β-amino acids

Address correspondence to Dengchao Li, Department of Bioengineering, School of Life Sciences, Huaiyin Normal University, 111 Western Changjiang Road, Huai'an, Jiangsu Province, 223300, P.R. China. E-mail: dcli@hytc.edu.cn

in producing antibiotics and chiral building blocks. For instance, (*R*)-BPA is a component of astins A–C, the antitumor cyclopentapeptides isolated from the roots of the medicinal plant *Aster tataricus*,[2] while (*S*)-BPA can be used to synthesize pyloricidins A–D, novel anti-*Helicobacter pylori* antibiotics.[3] Compared to the chemical catalyst, the biocatalyst (enzyme) usually has higher catalytic efficiency and enantioselectivity, and its optimum reaction conditions are also milder. Therefore, the enzyme-catalyzed synthetic method has been viewed as a potential approach to the production of optically pure BPA and derivatives, and a number of enzymes have been used in this field, including lipase,[4] α-chymotrypsin,[5] penicillin G acylase,[6] amino-acylase,[7] and *β*-phenylalanine amino transferases.[8] However, the application of this method to industrial production of optically pure BPA and its derivatives is still at a preliminary stage and needs to be developed.

Penicillin G acylase (PGA) has traditionally served as an industrial catalyst for production of 6-aminopenicillanic acid[9] and the synthesis of *β*-lactam antibiotics[10] and peptides,[11] and for the resolution of racemates.[12,13] The PGA-catalyzed enantioselective resolution of racemic substances could be an alternative to the existing biocatalytic approaches because of its fast reaction rate, high enantioselectivity, and simple isolation procedures. For example, the acylation of amines catalyzed by PGA from *Alcaligenes faecalis* (Af-PGA) in an aqueous solution was shown to be surprisingly efficient and highly enantioselective for (*R*)-amines.[14] The direct condensation of phenylglycinonitrile with phenylacetic acid catalyzed by PGA from *Escherichia coli* (Ec-PGA) was effective and exclusively enantioselective for (*S*)-phenylglycinonitrile, which led to nearly stoichiometric acylation.[13]

In our earlier study on Ec-PGA-catalyzed production of enantiomerically pure *β*-phenylalanine in an aqueous medium, an alkaline medium (pH 10) was found to favor Ec-PGA-catalyzed acylation reaction.[15] However, Af-PGA, which was less studied than Ec-PGA, has been demonstrated to bear a broader optimum pH range (8–10) and to maintain nearly 80% of its maximum catalytic activity at pH 11,[14] with a higher thermostability (due to the presence of a disulfide), catalytic activity, and ratio of synthesis to hydrolysis.[16] Recently, some researchers indicated that an Af-PGA-catalyzed enantioselective acylation in an aqueous medium resulted in the production of pharmacologically interesting α -D-phenylalanine and its *p*-substituted derivatives.[17] However, to our knowledge, Af-PGA-catalyzed enantioselective acylation of *β*-amino acids has not been reported. Here, we present the results of the Af-PGA-catalyzed enantioselective acylation of BPA and its derivatives (Figure 1), as well as optimized reaction conditions.

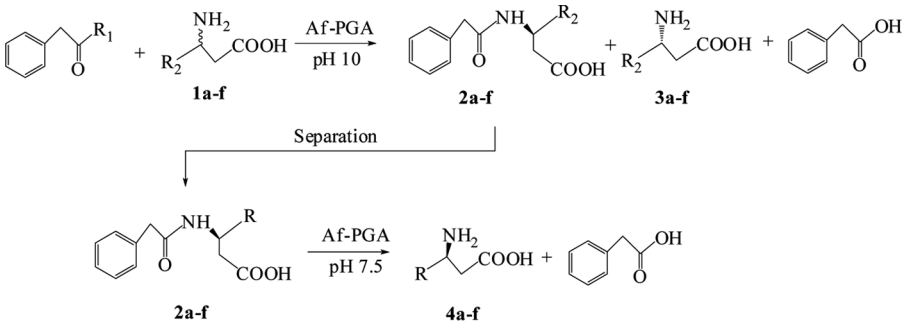

FIGURE 1 The scheme of Af-PGA-catalyzed enantioselective acylation of BPA and its derivatives.

EXPERIMENTAL

Materials

Potassium penicillin G was purchased from Shijiazhuang Pharmaceutical (Shijiazhuang, Hebei Province, China). *p*-Dimethylaminobenzaldehyde (PDAB) was supplied by SSS Reagent Company (Shanghai, China). Amberzyme oxirane resin, a polymeric support for enzyme immobilization, was a gift kindly provided by Rohm and Haas Company (Philadelphia, PA). All other reagents and chemicals used were of analytical grade.

The recombinant Af-PGA was produced by the *E. coli* strain DH5α that was transformed with the plasmid *pSMLFPGA* carrying the *A. faecalis pga* gene, whose expression was controlled by the *trc* promoter.[18] The cell-free extract of the cultured bacterial cells was subjected to a series of purification processes: fractionating the precipitate with a 40–80% saturation ammonium sulfate solution and then dialyzing in potassium phosphate buffer (pH 8.0). The enzyme preparation was loaded on a DEAE Sepharose column chromatograph (2.5 cm diameter × 35 cm length, Amersham Pharmacia Biotech Co.) and then concentrated with PEG 20,000. The purity of the isolated free enzyme was above 80% and its activity was about 332 U/mL. To immobilize the free enzyme on Amberzyme matrix, 4000 U free enzyme was mixed with 10 g (wet weight) matrix in 1 *M* phosphate buffer (pH 8.0) and incubated at room temperature with agitation at 300 rpm for 24 hr. The resultant immobilized enzyme activity was 260 U/g (wet weight).

Synthesis of Racemic β-Amino Acids 1a–1f and *N*-Phenylacetyl-β-amino Acids 5a–5f

Racemic β-amino acids 1a–1f were synthesized as described previously[19] with some modifications. Benzaldehyde or its derivatives (0.2 mol), maionic acid (0.2 mol), and ammonium acetate (0.6 mol) were refluxed in EtOH (500 mL) for 6 hr. The reaction mixture was allowed to cool to room temperature before the white precipitate was collected by filtration. The precipitated crude amino acids la–1f were washed with methanol, dried in vacuum, and then used for phenylacetylation.

N-Phenylacetyl-β-amino acids 5a–5f were synthesized according to the methods previously described.[6] Phenylacetyl chloride (0.13 mol) in 15 mL acetone was added to the homogeneous solution of racemic β-amino acid (0.10 mol) and triethylamine (0.24 mol) in 60 mL aqueous acetone (the ratio of H_2O to MeCOMe was 3:1) at −5°C with stirring for 0.5 hr. The mixture was stirred for 2 hr at −5°C and then 3 hr at room temperature. After the reaction mixture was filtered and acetone was evaporated, the residue was washed with diethyl ether (3×25 mL) to remove unreacted phenylacetyl chloride. The aqueous phase was acidified to pH 2.0 with 2 *N* HC1 and then extracted with ethyl acetate (3×50 mL). The combined organic layers were dried with anhydrous sodium sulfate and then the solution was concentrated under reduced pressure. The residue was recrystallized from ethyl acetate/hexane to give a crystalline product. The yields and melting points (mp) of 1a–1f and 5a–5f are presented in Tables 1 and 2, respectively.

Enzymatic Acylation Reactions

The enzymatic acylation reactions were run in a pH-stat at a constant temperature with continuous stirring. First, 0.1 *M* 1a–1f was dissolved in 30 mL of an aqueous solution (20 m*M* dipotassium hydrogen phosphate); next, 0.2 or 0.3 *M* acyl donor (phenylacetamide or methyl phenylacetate)

TABLE 1 Yields and Melting Points (mp) of Synthesized β-Amino Acids 1a–1f

		mp (°C)	
Product	Molar Yield (%)	Found	Reported
1a	74	218–221	218–219[6]
1b	68	214–219	219[19]
1c	60	213–215	no report
1d	76	233–235	237[6]
1e	58	215–217	216–217[19]
1f	61	230–232	228–229[6]

TABLE 2 Yields and Melting Points (mp) of Synthesized *N*-Phenylacetic-*β*-amino Acids 5a–5f

Product	Molar Yield (%)	mp (°C)	
		Found	Report
5a	70	136–138	134–140[6]
5b	62	169–171	No report
5c	88	170–174	No report
5d	70	191–192	189–193[6]
5e	61	157–160	159–165[6]
5f	74	153–156	155–158[6]

was added into the solution and incubated for 10 min at a constant pH and 25°C. The reaction was started by adding 0.93 g Af-PGA to the reaction mixture and the pH was kept constant by automatic titration of 2 *M* KOH solution. To monitor the reaction, aliquots of 100 μL were taken from the reaction mixture at intervals of 10 or 15 min, diluted about 10 times with the mobile phase of high-performance liquid chromatography (HPLC), and assayed using HPLC.

Enzyme Activity Assay

PGA activity was determined by a spectrophotometric assay with PDAB as a colorimetric substrate.[20] First, a certain amount of Af-PGA (0.10 g immobilized enzyme or 0.10 mL of diluted free enzyme) was mixed with 5 mL of a penicillin potassium salt solution (prepared by adding 0.4 g penicillin to 10 mL of 0.1 *M* phosphate buffer, pH 7.8) and incubated at 37°C for 5 min, and then the reaction was terminated by adding 5 mL of 95% ethanol to the reaction mixture. Second, the resultant solution was centrifuged at 12,000 rpm for 5 min, and then 1 mL of the resultant supernatant was diluted with distilled water about 10-fold. Third, after 0.5 mL of the diluted supernatant was mixed with 3.5 mL PDAB, the mixture was settled at room temperature for 5 min, and its absorbance was then measured at 415 nm. One unit of PGA was defined as the amount of enzyme required to produce 1 μmol 6-APA per min from 4% (w/v) penicillin G in 0.1 *M* phosphate buffer (pH 7.8) at 37°C.

Assay Methods

Samples were analyzed using HPLC with a Zorbax XDB C-18 column (250 × 4.6 mm, 5 μm); the mobile phase was 7 m*M* phosphate (pH 3.0), containing acetonitrile 35% (v/v) and 2.36 m*M* sodium dodecyl sulfate (SDS); the flow rate was set at 1 mL/min and absorbance was measured

at 210 nm. The enantiomeric excess value (*ee*) of the 2a–2f was determined using HPLC with a Chirobiotic T column (250 × 4.6 mm, 5 μm), the mobile phase was the mixture of methanol and 20 m*M* acetic acid buffer (pH 5.5) in a ratio of 1:4 (v:v), the flow rate was set at 0.5 mL/min, and absorbance was measured at 210 nm. The *ee* of 3a–3f was determined using HPLC with a Chirobiotic R column (150 × 4.6 mm, 5 μm); the mobile phase was the mixture of methanol, acetic acid, and triethylamine in a ratio of 100:0.4:0.1 (v:v:v); the flow rate was set at 0.4 mL/min; and absorbance was measured at 254 nm.

RESULTS AND DISCUSSION

We previously had demonstrated the Ec-PGA-catalyzed production of enantiomerically pure (*S*)-BPA and (*R*)-BPA in an aqueous medium under optimum reaction conditions: at 25°C and pH 10 with 0.15 *M* BPA, 0.3 *M* phenylacetamide, and 8 U/mL enzyme.[15] Under these conditions, Af-PGA-catalyzed acylation of BPA at 40 min only achieved the maximum BPA conversion efficiency of 36.1% (50% conversion efficiency is the maximum in the stereoisomer reaction and the following reactions) (Figure 2A). Therefore, we investigated the effects of pH, acyl donor, and molar ratio of phenylacetamide to BPA on this acylation reaction.

As shown in Figure 2, the maximum BPA conversion efficiency at 40 min increased to 38.5% when methyl phenylacetate was used as an acyl donor (Figure 2B), which indicated that methyl phenylacetate was a better acyl donor than phenylacteamide for this type of reaction. As the molar ratio of phenylacetamide to BPA was elevated from 2:1 to 3:1, the conversion efficiency increased to 47.8%, but the reaction time at the maximum conversion was prolonged to 130 min because of the low solubility of phenylacetamide in the aqueous medium (Figure 2A). Compared to Ec-PGA, Af-PGA is more stable and active in alkaline conditions, so the acylation reaction was then run under the condition of pH 11; consequently, the conversion efficiency increased to 42.9% and 46.5% at 60 min and 90 min, respectively (Figures 2C and 2D). The result also showed that the maximum BPA conversion efficiency rose as the pH value was elevated from 10 to 11, which indicated that the alkaline condition favored this acylation reaction. However, it needed a longer time to accomplish the maximum conversion efficiency, which might be due to the fact that the enzyme activity and stability at pH 11 were lower than that at pH 10.

In the kinetic acylation reaction, an acyl donor binds to the immobilized enzyme to form an acyl–enzyme complex, which can either be hydrolyzed to produce the enzyme and phenylacetic acid, or react with a nucleophilic amino acid to form 3a–3f. Furthermore, the hydrolysis of the resultant 2a–2f can also occur simultaneously. Therefore, it is necessary to reduce

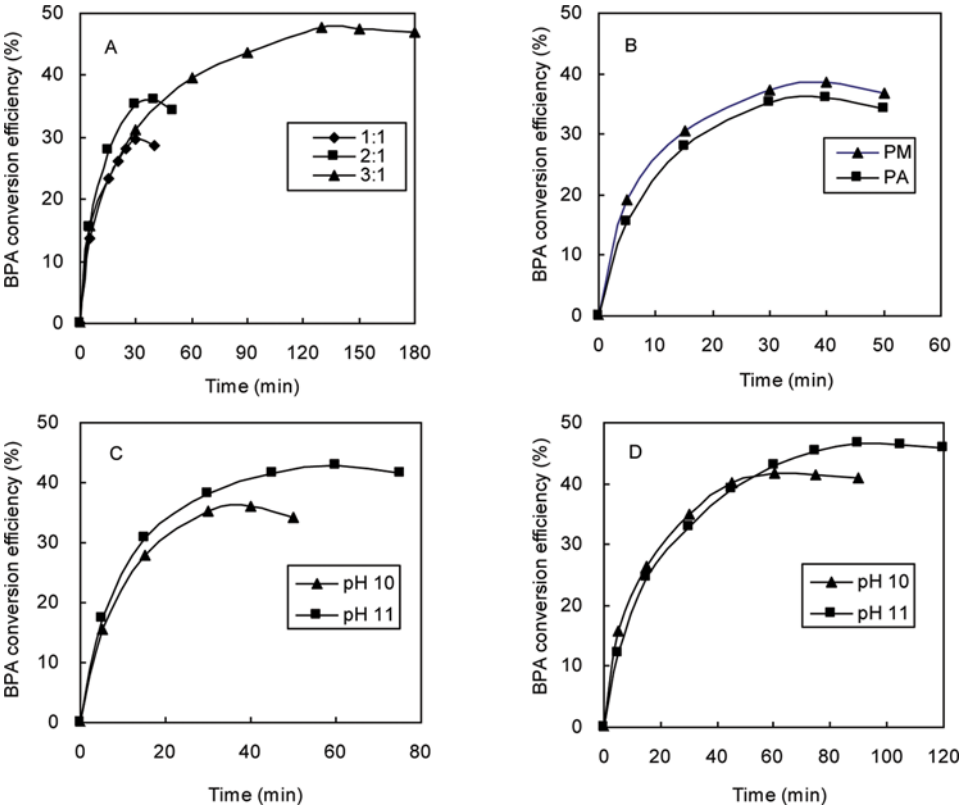

FIGURE 2 Effects of molar ratio of acyl donor to BPA, acyl donor, and pH on BPA conversion efficiency. (A) The effect of the molar ratio of phenylacetamide to BPA on BPA conversion. (B) The effect of acyl donor on BPA conversion. (C) The effect of pH on BPA conversion using phenylacetamide as acyl donor. (D) The effect of pH on BPA conversion using methyl phenylacetate as acyl donor. Reaction conditions: (A) 0.1 *M* BPA, 0.1–0.3 M phenylacetamide and 8 U/mL enzyme at 25°C and pH 10. (B) 0.1 *M* BPA, 0.2 M acyl donor and 8 U/mL enzyme at 25°C and pH 10. (C) 0.1 *M* BPA, 0.2 M phenylacetamide and 8 U/mL enzyme at 25°C, and pH 10 and 11. (D) 0.1 *M* BPA, 0.3 M methyl phenylacetate and 8 U/mL enzyme at 25°C, and pH 10 and 11 (color figure available online).

TABLE 3 Af-PGA-Catalyzed Acylation of BPA and Its Derivatives

Entry	[1a–1f] Conversion[a] (%)	[1a–1f] Time[b] (min)	$(s/h)_0$[c]	[2a–2f] ee_p (%)	[3a–3f] ee_s[d] (%)
a	46.5	90	0.7	>99	96.5
b	48.6	60	0.99	>99	98.3
c	49.8	90	1.71	98.4	97.6
d	49.8	45	1.36	98.0	95.2
e	49.9	75	1.04	>99	98.5
f	49.4	75	2.88	>99	98.6

[a]Conversion—the maximum conversion efficiency of 1a–1f.
[b]Time—the reaction time for achieving the maximum conversion efficiencies of 1a–1f.
[c]Determined as a ratio of initial rates for accumulation of both reaction products.
[d]Determined by chiral HPLC.

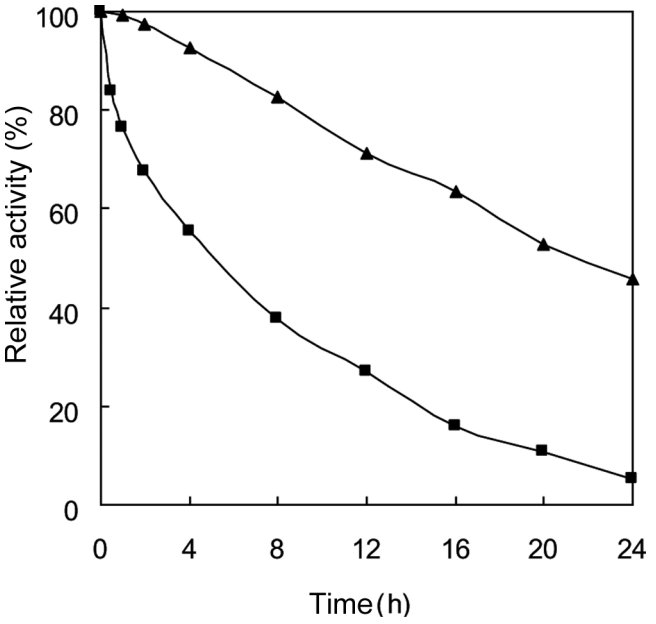

FIGURE 3 The stability of Af-PGA at 25°C, and pH 10 and 11. (▲) pH 10; (■) pH 11.

the occurrence of hydrolytic side reaction or to improve the ratio of synthesis to hydrolysis (s/h). The preceding scheme was also used to examine the acylation of several BPA derivatives, and the results are shown in Table 3. Af-PGA exhibited an excellent enantioselectivity for *R*-isomer from the corresponding racemates ($ee_p > 98\%$), leaving the remaining *S*-BPA derivatives in moderate to high ee_s (>95%). The maximum conversion efficiencies of 1a–1f all reached about 50% (it means 100% conversion efficiency of the desired stereoisomer) in a relatively short time (within 45–90 min). Both the conversion efficiency and the ee_s of 1b–1f slightly increased in comparison with those of 1a. It might be mainly due to that the benzene ring of BPA was substituted by electron-withdrawing groups substituted, which caused the hydrolytic rate of 2b–2f to be slower than that of 2a, leading to Af-PGA-catalyzed BPA derivatives with a higher s/h than BPA.

The optimal pH for the acylation of BPA and its derivatives was 11, whereas the Af-PGA has an optimum pH range between 8 and 10. The stability of Af-PGA at pH 10 and pH 11 is indicated in Figure 3. The half lives of Af-PGA at pH 10 and 11 were about 24 hr and 5 hr, respectively.

CONCLUSIONS

In this study, we developed a method of producing enantiomerically pure BPA and its derivatives by Af-PGA-catalyzed acylation of racemates.

Under relatively optimum reaction conditions, the maximum conversion efficiencies of racemates reached about 50% in a relatively short time (45–90 min), and the products also had high enantiomeric purity. Considering the short half-life (5 hr) of Af-PGA at pH 11 and its relatively high thermostability, in the future we may attempt to improve its efficiency by appropriately increasing reaction temperature, and to enhance its stability using gene engineering techniques and enzyme immobilization methods. All in all, due to its effectiveness and environmentally friendly nature, this method appears to be a promising approach to the green synthesis of BPA and its derivatives.

ACKNOWLEDGMENTS

This study was supported by the Huai'an City Industrial Fund for Science and Technology Support Program (HAG2010020) and the Foundation of Key Disciplines of Shanghai.

REFERENCES

1. Spiteller, P.; von Nussbaum, F. *β*-Amino Acids in Natural Products. In *Enantioselective Synthesis of β-Amino Acids*, 2nd ed.; Juaristi, E., Soloshonok, V.A., Eds.; Wiley-VCH, New York, NY, 2005; pp. 19–93.
2. Morita, H.; Nagashima, S.; Takeya, K.; Itokawa, H.; Staka, Y. Structures and Conformation of Antitumor Cyclic *β*-Peptides, Astin A, B and C, From *Aster tataricus*. *Tetrahedron* **1995**, *51*, 1121–1132.
3. Nagano, Y.; Ikedo, K.; Fujishima, A.; Izawa, M.; Tsubotani, S.; Nishimura, O.; Fujino, M. Pyloricidins, Novel Anti-*Helicobacter pylori* Antibiotics Produced by *Bacillus sp. J. Antibiot.* **2001**, *54*, 934–947.
4. Faulconbridge, S.J.; Holt, K.E.; Sevillano, L.G.; Lock, C.J.; Tiffin, P.D.; Tremayne, N.; Winter, S. Preparation of Enantiomerically Enriched Aromatic *β*-Amino Acids via Enzymatic Resolution. *Tetrahedron Lett.* **2000**, *41*, 2679–2681.
5. Cohen, S.G.; Weinstein, S.Y. Hydrolysis of D(-)-Ethyl-*β*-phenyl-*β*-hydroxypropionate and D(-)-Ethyl-*β*-phenyl-*β*-acetamidopropionate by α-Chymotrypsin. *J. Am. Chem. Soc.* **1964**, *86*, 725–728.
6. Soloshonok, V.A.; Fokina, N.A.; Rybakova, A.V.; Shishkina, I.P.; Galushko, S.V.; Sorochinsky, A.E.; Kukhar, V.P.; Savchenko, M.V.; Švedas, V.K. Biocatalytic Approach to Enantiomerically Pure *β*-Amino Acids. *Tetrahedron: Asymmetry* **1995**, *6*, 1601–1610.
7. Groeger, H.; Trauthwein, H.; Buchholz, S.; Drauz, K.; Sacherer, C.; Godfrin, S.; Werner, H. The First Aminoacylase-Catalyzed Enantioselective Synthesis of Aromatic *β*-Amino Acids. *Org. Biomol. Chem.* **2004**, *2*, 1977–1978.
8. Makoto, H.; Junichi, M.; Tairo, H.; Jun, S.; Sakayu, S.; Jun, O. *β*-Aryl-*β*-Amino Acid Amino Transferase From *Variovorax sp.* JH2 Is Useful for Enantioselective *β*-Phenylalanine Production. *Biocatal. Agric. Biotechnol.* **2012**, *1*, 253–258.
9. Shewale, J.G.; Deshpande, B.S.; Sudhakaran, V.K.; Ambedkar, S.S. Penicillin Acylase: Applications and Potentials. *Process Biochem.* **1992**, *27*, 131–143.
10. Bruggin, K.; Roos, E.C.; Devroom, E. Penicillin Acylase in the Industrial Production of *β*-Lactam Antibiotics. *Org. Process Res. Dev.* **1998**, *2*, 128–133.
11. Khimiuk, A.Y.; Korennykh, A.V.; van Langen, L.M.; van Rantwijk, F. Penicillin Acylase-Catalyzed Peptide Synthesis in Aqueous Medium: A Chemoenzymatic Route to Stereoisomerically Pure Diketopiperazines. *Tetrahedron: Asymmetry* **2003**, *14*, 3123–3128.

12. Fadnavis, N.W.; Sharfuddin, M.; Vadivel, S.K. Resolution of Racemic 2-Amino-1-butanol With Immobilized Penicillin G Acylase. *Tetrahedron: Asymmetry* **1999**, *10*, 4495–4500.
13. Chilov, G.G.; Moody, H.M.; Boesten, W.H.J.; Švedas, V.K. Resolution of (*R*, *S*)- Phenylglycinonitrile by Penicillin Acylase-Catalyzed Acylation in Aqueous Medium. *Tetrahedron: Asymmetry* **2003**, *14*, 2613–2617.
14. Guranda, D.T.; van Langen, L.M.; van Rantwijk, F.; Sheldon, R.A.; Švedas, V.K. Highly Efficient and Enantioselective Enzymatic Acylation of Amines in Aqueous Medium. *Tetrahedron: Asymmetry* **2001**, *12*, 1645–1650.
15. Li, D.C.; Cheng, S.W.; Wei, D.Z.; Ren, Y.H.; Zhang, D.R. Production of Enantiomerically Pure (S)-*β*-Phenylalanine and (R)-*β*-Phenylalanine by Penicillin G Acylase From *Escherichia coli* in Aqueous Medium. *Biotechnol. Lett.* **2007**, *29*, 1825–1830.
16. Zhiryakova, D.; Guncheva, M.; Ivanov, I.; Stambolieva, N. Hydrolysis of Phenylacetanilides Catalyzed by Penicillin G Acylase From *Alcaligenes faecalis*: Sensitivity of the Reaction to Substitution in the Leaving Group. *Catal. Commun.* **2009**, *11*, 196–201.
17. Gong, X.Y.; Su, E.Z.; Wang, P.X.; Wei, D.Z. *Alcaligenes faecalis* Penicillin G Acylase-Catalyzed Enantioselective Acylation of D,L-Phenylalanine and Derivatives in Aqueous Medium. *Tetrahedron Lett.* **2011**, *52*, 5398–5402.
18. Cheng, S.W.; Song, Q.X.; Wei, D.Z.; Gao, B.X. High-Level Production Penicillin G Acylase From *Alcaligenes faecalis* in Recombinant *Escherichia coli* With Optimization of Carbon Sources. *Enzyme Microbial Technol.* **2007**, *41*, 326–330.
19. Tan, C.Y.K.; Weavera, D.F. A One-Pot Synthesis of 3-Amino-3-aryl-propionic Acids. *Tetrahedron* **2002**, *58*, 7449–7461.
20. Shewale, J.G.; Kumar, K.K.; Ambekar, G.R. Evaluation of Determination of 6-Aminopenicillanic Acid by *p*-Dimethylminobenzaldehyde. *Biotechnol. Technol.* **1987**, *1*, 69–72.