

# 不对称还原制备光学纯 (R)-2-羟基-4-苯基丁酸乙酯的双酶共表达重组菌的构建

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**摘要** 克隆了来自于枯草芽孢杆菌的羧基还原酶基因 *IoIS* 和葡萄糖脱氢酶基因 *GDH*, 采用 Ni-NTA 镍亲和和层析柱对重组蛋白 *IoIS* 进行纯化, 并对纯酶进行了酶学性质研究. 结果表明, 该羧基还原酶的最适温度和 pH 值分别为 30 °C 和 6.0; 在 40 °C 以下具有较好的热稳定性; 在 pH 5.5-7.0 的偏酸性范围内能保持 75% 以上的酶活. 采用三种策略构建了 *IoIS* 和 *GDH* 的共表达重组质粒, 结果发现, 采用双启动子的重组质粒能够实现羧基还原酶 *IoIS* 的高效表达, 粗酶液中的 *IoIS* 和 *GDH* 的比酶活均达到 1.5 U/mg. 运用该重组菌对 10 g/L 的 OPBE 进行不对称还原, 反应 15 h 后, 底物转化率大于 99%, 产物 (R)-2-羟基-4-苯基丁酸乙酯的 ee 值达到 99.5%.

**关键词:** 羧基还原酶 葡萄糖脱氢酶 共表达 (R)-2-羟基-4-苯基丁酸乙酯 不对称还原

**Abstract:** (R)-2-Hydroxy-4-phenylbutyrate (HPBE) is an important chiral intermediate for the synthesis of angiotensin-converting enzyme (ACE) inhibitors. Asymmetric reduction of ethyl 2-oxo-4-phenylbutyrate (OPBE) to (R)-HPBE using a recombinant strain can provide high enantioselectivity. Cofactor regeneration is a critical issue in the application of a recombinant strain. A carbonyl reductase gene (*IoIS*) and a glucose dehydrogenase (*GDH*) gene from *Bacillus subtilis* were cloned. Recombinant *IoIS* was purified using a Ni-NTA column and its enzyme activity properties were investigated. The purified *IoIS* exhibited maximum activity at pH 6.0 and 30 °C, and the enzyme showed good thermostability below 40 °C. It retained over 75% of its activity in the acidic pH range of 5.5-7.0. Three coexpression strategies were used for the recombinant vectors. The recombinant *E. coli* strain containing polycistronic plasmid pET-G-T7-1 showed excellent carbonyl reductase activity, and the specific activity of both *IoIS* and *GDH* in the crude cell extract reached 1.5 U/mg. In the asymmetric reduction of OPBE by recombinant *E. coli* cells in aqueous system, the yield of (R)-HPBE reached over 99% with an enantiomeric excess of 99.5% at 10 g/L of OPBE within 15 h.

**Keywords:** carbonyl reductase, glucose dehydrogenase, coexpression, (R)-2-hydroxy-4-phenylbutyrate, asymmetric reduction

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[1] Herold P, Indolese A F, Studer M, Jalett H P, Siegrist U, Blaser H U. *Tetrahedron*, 2000, 56: 6497 


[2] 张文. [硕士学位论文]. 无锡: 江南大学 (Zhang W. [MS Dissertation]. Wuxi: Jiangnan Univ), 2009


[3] 张宪锋, 郑裕国. 生物加工过程 (Zhang X F, Zheng Y G. *Chin J Bioprocess Eng*), 2003, 11(2): 33

[4] Fadnavis N W, Radhika K R, Madhuri K V. *Tetrahedron: Asymmetry*, 2004, 15: 549 

[5] Huang S H, Tsai S W. *J Mol Catal B*, 2004, 28: 65 

[6] Liese A, Kragl U, Kierkels H, Schulze B. *Enzyme Microb Technol*, 2002, 30: 673 

[7] Shi Y G, Fang Y, Ren Y P, Guan H L, Zhang J Y. *J Chem Technol Biotechnol*, 2009, 84: 681 

[8] Fadnavis N W, Radhika K R. *Tetrahedron: Asymmetry*, 2004, 15: 3443 

[9] Bergo de Lacerda P S, Ribeiro J B, Leite S G F, Ferrara M A, Coelho R B, Bon E P S, Lima E L S, Antunes O A C. *Tetrahedron: Asymmetry*,

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- [10] Zhang W, Ni Y, Sun Z H, Zheng P, Lin W Q, Zhu P, Ju N F. Process Biochem, 2009, 44: 1270 
- [11] Li N, Ni Y, Sun Z H. J Mol Catal B, 2010, 66: 190 
- [12] Shen N D, Ni Y, Ma H M, Wang L J, Li C X, Zheng G W, Zhang J, Xu J H. Org Lett, 2012, 14: 1982 
- [13] Yun H, Choi H L, Fadnavis N W, Kim B G. Biotechnol Progr, 2005, 21: 366
- [14] Ni Y, Li C X, Zhang J, Shen N D, Bornscheuer U T, Xu J H. Adv Synth Catal, 2011, 353: 1213 
- [15] Ye Q, Cao H, Yan M, Cao F, Zhang Y Y, Li X M, Xu L, Chen Y, Xiong J A, Ouyang P K, Ying H J. Bioresour Technol, 2010, 101: 6761 
- [16] Bradford M M. Anal Biochem, 1976, 72: 248 
- [17] Kruger N J. The Protein Protocols Handbook. Totowa: Humana Press, 2002. 15
- [18] Ye Q, Cao H, Zang G L, Mi L, Yan M, Wang Y, Zhang Y Y, Li X M, Li J A, Xu L, Xiong J, Ouyang P K, Ying H J. Appl Microbiol Biotechnol, 2010, 88: 1277 
- [19] Ni Y, Li C X, Wang L J, Zhang J, Xu J H. Org Biomol Chem, 2011, 9: 5463 
- [20] Kataoka M, Yamamoto K, Kawabata H, Wada M, Kita K, Yanase, Shimizu S. Appl Microbiol Biotechnol, 1999, 51: 486 
- [21] 吴希, 张翀, 邢新会. 化工学报 (Wu X, Zhang Ch, Xing X H. CIESC J), 2009, 60: 2562

- [1] 倪晔, 张蓓花, 孙志浩. 采用通透性处理的安大略假丝酵母全细胞高效合成 (*R*)-2-氯-1-(3-氯苯基)乙醇[J]. 催化学报, 2012,33(4): 681-687
- [2] 宫敏敏, 聂尧, 徐岩, 肖荣, Gaetano T. MONTELLIONE. 羧基还原酶辅酶结合域位点突变对其不对称催化性能的影响[J]. 催化学报, 2012,33(2): 330-335
- [3] 王丹, 张强, 李旺, 戚南昌, 郭春晓, 杨志荣, 张杰. 酵母静息细胞催化丙酮酸乙酯不对称还原制 (*S*)-乳酸乙酯[J]. 催化学报, 2011,32(6): 1035-1039
- [4] 王梦亮; 闫甫昆. 类球红杆菌羧基还原酶不对称催化还原性能与底物结构的关系[J]. 催化学报, 2010,31(2): 195-199
- [5] 娄文勇; 郭强; 郁惠蕾; 宗敏华. 近平滑假丝酵母细胞催化乙酰基三甲基硅烷不对称还原反应[J]. 催化学报, 2009,30(12): 1276-1280
- [6] 倪宏亮; 姚善泾. 呼吸缺陷型面包酵母催化 $\beta$ -羧基酯的不对称还原[J]. 催化学报, 2008,29(6): 537-541
- [7] 张帆; 倪晔; 孙志浩; 郑璞; 林文清; 宋坡; 居年丰. 水/离子液体两相体系中出芽短梗霉催化4-氯-乙酰乙酸乙酯不对称还原合成(*S*)-4-氯-3-羟基-丁酸乙酯[J]. 催化学报, 2008,29(6): 577-582
- [8] 王梦亮; 胡锐; 郭学林; 闫甫昆; 刘滇生. 光控光合菌生物催化苯乙酮不对称还原的反应机理[J]. 催化学报, 2008,29(3): 233-237
- [9] 应小姣; 姚善泾; 关怡新. 高压CO<sub>2</sub>条件下粗状假丝酵母催化苯乙酮不对称还原合成 $\alpha$ -苯乙醇[J]. 催化学报, 2006,27(7): 631-635
- [10] 马小魁; 王喆之 陈五岭. 酵母发酵液直接催化4-氯-乙酰乙酸乙酯不对称还原生成4-氯-3-羟基-丁酸乙酯[J]. 催化学报, 2006,27(4): 314-318
- [11] 刘湘; 方志杰; 许建和. 酵母细胞催化芳香酮的不对称还原反应[J]. 催化学报, 2006,27(1): 20-24
- [12] 于明安; 朱晓冰; 祁巍; 赵领; 魏郁梦. CTAB透性化酵母细胞生物催化合成(*S*)-(+) -3-羟基丁酸乙酯[J]. 催化学报, 2005,26(7): 609-613
- [13] 敬科举; 徐志南; 林建平; 岑沛霖. 重组大肠杆菌细胞不对称还原4-氯乙酰乙酸乙酯合成(*R*)-(+) -4-氯-3-羟基丁酸乙酯[J]. 催化学报, 2005,26(11): 993-998
- [14] 杨忠华; 姚善泾; 赵珺. 活性酵母细胞不对称催化苯乙酮还原及树脂吸附对反应的促进作用[J]. 催化学报, 2005,26(10): 895-899
- [15] 杨忠华; 姚善泾. 水相中酵母细胞催化4-氯乙酰乙酸乙酯不对称还原反应[J]. 催化学报, 2004,25(6): 434-438