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论文

多拷贝毕赤酵母重组菌表达GCL摇瓶优化和高密度发酵

智晓燕, 汪小锋, 孙永川, 柯锋, 代敏, 闫云君

分子生物物理教育部重点实验室 华中科技大学生命科学与技术学院 武汉 430074

摘要:

目的:构建高效表达白地霉脂肪酶的毕赤酵母重组菌株,并对筛选得到的菌株进行摇瓶发酵条件优化和分批补料高密度发酵工艺研究。方法:将诱导型表达载体pPIC9K-*gcl*电转化至毕赤酵母GS115。通过橄榄油-罗丹明B平板和摇瓶发酵筛选高脂肪酶活力的重组菌株,运用基于TaqMan探针的实时荧光定量PCR 法确定其拷贝数,并对菌株进行摇瓶发酵条件优化。在此基础上,研究重组菌在3L 发酵罐中的高密度发酵工艺。结果:筛选得到一株具有3 个白地霉脂肪酶基因拷贝的菌株GS115/pPIC9K-*gcl* 78#,初始酶活力为220 U/ml。当摇瓶发酵条件为甲醇诱导96 h,每24 h甲醇添加量1 %,接种量2 %,培养基初始pH 7.0,500 ml摇瓶装液量50 ml,甲醇诱导温度25℃ 时酶活力达735 U/ml。3L 发酵罐高密度发酵176.5 h,酶活力达到3360 U/ml,总蛋白含量达到4.30 g/L,且发酵过程中细胞活性一直保持在96 % 以上。结论:基因拷贝数与重组菌株的产酶水平呈正相关,摇瓶优化可显著提高重组菌株的产酶能力,为白地霉脂肪酶的工业化生产奠定了技术基础。

关键词: 巴斯德毕赤酵母 白地霉脂肪酶 基因拷贝数 摇瓶优化 高密度发酵

High Cell-density Fermentation and Shaking Flask Optimization of *Geotrichum candidum* Lipase Production in Multi-copy *Pichia pastoris*

ZHI Xiao-yan, WANG Xiao-feng, SUN Yong-chuan, KE Feng, DAI Min, YAN Yun-jun

Key Laboratory of Molecular Biophysics of the Ministry of Education, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China

Abstract:

Objective: To effectively express *Geotrichum candidum* lipase (GCL) in *Pichia pastoris*, optimize the culture conditions of the recombinant strain in shaking flask and investigate its high-level expression in a 3L bioreactor. Methods: The inducible secretion vector pPIC9K-*gcl* was transformed into *P. pastoris* GS115 by electroporation. Clones with high lipase activity were screened by olive oil-rhodamine B plates and shaking flask cultivation. Real-time PCR based TaqMan probe was applied to determine the copy number of *G. candidum* lipase gene in the target clones. Then six key factors of shaking flask cultivation were optimized, including induction time, methanol concentration per 24h, culture medium initial pH, inoculation concentration, culture medium volume in shake flask and induction temperature. Based on the results of shaking flask cultivation, fed-batch fermentation was implemented in a 3L bioreactor. Results: A clone GS115/pPIC9K-*gcl* 78# which possessed three copies of *G. candidum* lipase gene was obtained. Its initial lipase activity was up to 220 U/ml. The results also showed that when the cultivation conditions were 96 h after methanol induction, 1% of methanol addition per 24 h, 2% of inoculation concentration, initial pH 7.0 of culture medium, 50 ml of culture medium volume, and induction temperature at 25 ℃, the lipase activity reached 735 U/ml. In the 3L bioreactor, lipase activity achieved 3360 U/ml and the total protein concentration reached 4.30 g/L after 176.5 h cultivation. Moreover, the cell viability was above 96% during the high cell-density fermentation. Conclusion: *Pichia pastoris* GS115 is a suitable host for high-level expression of *G. candidum lipase*. Multiple gene copies had a positive effect on expression level of GCL. Single factor optimization can effectively enhance lipase expression. This high cell-density fermentation would offer a solid basis for large-scale production of GCL.

Keywords: *Pichia pastoris* *Geotrichum candidum* lipase Gene copy number Shaking flask cultivation optimization High cell-density fermentation

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通讯作者: 闫云君 **通讯作者**E_mail: yanyunjun@tom.com

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