Postprint: System Construction and Application for Efficient Production of Tp-GUS in Bacillus subtilis

Authors: Zhang Zhenghui, Liu Hu, Li Chun

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Abstract

Glycyrrhizin (GL) is one of the most important active components in licorice, possessing anti-tumor, anti-viral, anti-allergic, anti-inflammatory, and adrenal cortex hormone-promoting activities. It also finds extensive applications in food additives and cosmetics, making it an important fine chemical product 1,2. Its molecule contains two glucuronic acid groups, which render it highly polar, leading to difficulty in effectively crossing cell membranes and exerting pharmacological effects within cells. Therefore, glycyrrhizin is not the optimal molecular form for achieving its best pharmacological activity 3,4. Derivatives of glycyrrhizin, such as glycyrrhetinic acid monoglucuronide (GAMG) and glycyrrhetinic acid (GA), are products obtained by hydrolyzing one or two glucuronic acid groups from the outer -1,2 glycosidic bonds of glycyrrhizin, respectively. GAMG lacks one glucuronic acid group compared to GA, resulting in moderate polarity that significantly improves its solubility and transmembrane transport capacity in vivo. Consequently, GAMG exhibits higher absorption rates and bioavailability than both GL and GA 5.

Our research group previously screened a novel -glucuronidase from Talaromyces pinophilum (TpGUS) that specifically converts glycyrrhizin to glycyrrhetinic acid monoglucuronide. Based on the extensive commercial and medical value of TpGUS, as well as our group' s previous research work, this project will employ Bacillus subtilis, which possesses excellent secretion capabilities, as the host organism. Through the establishment of promoter and signal peptide libraries, and via directed evolution of the enzyme, random mutagenesis of the enzyme gene will be implemented to improve and enhance the enzyme' s performance properties, providing a theoretical basis for its industrial application. Finally, through characterization of enzymatic properties, the enzymatic reaction activity of the enzyme molecule will be optimized.

Through a series of genetic and molecular manipulations, this study aims to achieve large-scale production of TpGUS, thereby circumventing the bottleneck issues of low yield, long fermentation cycles, and high production costs associated with TpGUS expression in Saccharomyces cerevisiae. This holds significant importance for its future industrial applications.

Full Text

System Construction and Application for Efficient Production of Tp-GUS in *Bacillus subtilis*

Zheng-Hui Zhang, Hu Liu, Chun Li*
Department of Bioengineering, School of Life Science, Beijing Institute of Technology, lichun@bit.edu.cn

Glycyrrhizin (GL) is one of the principal active components of licorice, exhibiting anti-tumor, anti-viral, anti-allergic, anti-inflammatory, and adrenocorticotropic properties. It finds extensive applications as a food additive and in cosmetics, making it an important specialty chemical product [1,2]. However, its molecular structure contains two glucuronic acid groups that impart strong polarity, impeding effective membrane permeability and subsequent intracellular pharmacological activity. Consequently, glycyrrhizin is not the optimal molecular form for exerting maximal pharmacological effects [3,4]. Derivatives such as glycyrrhetinic acid monoglucuronide (GAMG) and glycyrrhetinic acid (GA) are produced by hydrolyzing one or two -1,2 glycosidic bonds of the outer glucuronic acid groups from glycyrrhizin. GAMG lacks one glucuronic acid group compared to GA, giving it moderate polarity that significantly enhances its solubility and transmembrane transport capacity in vivo. As a result, GAMG demonstrates superior absorption and bioavailability compared to both GL and GA [5].

Our research group previously identified a novel -glucuronidase from *Talaromyces pinophilum* (TpGUS) that specifically converts glycyrrhizin to GAMG. Given the extensive commercial and medical value of TpGUS and our previous research foundation, this study employs *Bacillus subtilis*—a host with excellent secretory capabilities—as the production platform. Through construction of promoter and signal peptide libraries combined with directed evolution for random mutagenesis of the enzyme gene, we aim to improve and enhance the enzymatic properties, providing a theoretical foundation for industrial application. Finally, comprehensive enzymatic characterization will be performed to optimize the catalytic activity of the enzyme molecule.

Through systematic genetic and molecular engineering, we seek to achieve large-scale production of TpGUS, overcoming the critical bottlenecks of low yield, prolonged fermentation cycles, and high production costs associated with TpGUS expression in *Saccharomyces cerevisiae*. This advancement will be of significant importance for its future industrial applications.

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Author Information

Zheng-Hui Zhang (born 1993), Master' s student. Research interests: biocatalysis and enzyme engineering. E-mail: zzhui093@163.com.

***Corresponding author: Chun Li**, Professor. Research focus: biocatalysis and enzyme engineering. E-mail: lichun@bit.edu.cn.

Note: Figure translations are in progress. See original paper for figures.