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Enhanced D-HPG Synthesis Using Surface-Displayed Enzymes in E. coli

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Abstract

D-p-Hydroxyphenylglycine (D-HPG) is an essential building block for the synthesis of semisynthetic antibiotics, such as amoxicillin and cefadroxil. Traditionally, D-HPG is produced through chemical synthesis, which involves harsh reaction conditions, high energy consumption, and generates significant environmental waste [1,2]. While enzymatic production offers a greener alternative, conventional approaches require the purification of the two key enzymes involved, D-hydantoinase (D-Hase) and D-carbamoylase (D-Case), before use. This purification step is labor-intensive, costly, and time-consuming, limiting the practicality of enzymatic methods. The surface display system, which anchors enzymes directly to the surface of the cell, addresses this limitation by bypassing the need for enzyme purification. [3-5]. In this study, we employed a surface display system on *E. coli* BL21(DE3) to produce D-HPG efficiently. D-Hase hydrolyzes hydantoin derivatives to N-carbamoyl-D-amino acids, which are further converted to D-amino acids by D-Case. We constructed recombinant *E. coli* BL21(DE3) strains with surface-displayed D-Hase and D-

Case by designing fusion constructs for efficient enzyme localization. Expression conditions were optimized to enhance enzyme activity. Semi-quantitative enzymatic activity analysis using Ehrlich's reagent confirmed the functionality of the surface-displayed enzymes. D-HPG production was analyzed using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Quantitative HPLC measurements demonstrated a high production yield of approximately 95%. This method offers significant advantages over traditional approaches, including mild reaction conditions, reduced environmental impact, and cost-effectiveness. In conclusion, this study highlights the successful application of a surface display system on *E. coli* for whole-cell biocatalysis of D-HPG. This innovative method provides a sustainable and efficient alternative to traditional chemical synthesis, offering great potential for industrial-scale production of D-HPG.

Key words: Bacterial surface display, D-p-hydroxyphenylglycine, D-hydantoinase; D decarbamoylase

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