Title : Process and Enzyme Engineering for Lauryl Glucoside Biosynthesis in *Escherichia coli*

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ABSTRACT

Lauryl glucoside is a mild, non-ionic surfactant used in many personal care products. It is biodegradable and low in toxicity. However, traditional chemical synthesis is unsustainable as it relies on petroleum-based substrates. Despite the development of *de novo* lauryl glucoside biosynthesis, this sustainable route currently faces challenges regarding low titers and yields. The current study focuses on the heterologous expression and catalytic efficiency of MtH2, a UDP-glycosyltransferase responsible for *O*-glycosylation, the final step in the lauryl glucoside biosynthetic pathway. Different cultivation media (Luria-Bertani (LB) and M9 minimal medium) and *Escherichia coli* host strains (BL21 (DE3) and JM109) were evaluated to optimize MtH2 expression and lauryl glucoside production. Under optimized conditions, both strains showed similar growth over 48 h. The highest lauryl glucoside titer was obtained in *E. coli* BL21 (DE3) cultivated in M9 minimal medium, reaching 6.30 ± 0.45 mg/L for intracellular fraction and 1.74 ± 1.02 mg/L for extracellular fraction, as determined by HPLC. Moreover, *in silico* alanine scanning and molecular docking analyses revealed that alanine substitutions at histidine 21 (H21A) and aspartic acid 125 (D125A) decreased binding affinity toward 1-dodecanol relative to the native structure, whereas alanine substitutions at histidine 378 (H378A) reduced binding affinity toward UDP-glucose indicating that these residues play critical roles in substrate binding and may consequently influence the catalytic performance of the enzyme.