Enhancement of enzymatic transformation of mulberry flavonoid glycosides by surface display of covalent isopeptide bond

Cheng-Hai Yan[1], Yu-Lu Yang[1], Fang-Hui Chen[1], Lu-Chan Gong[1,2], Jun Wang[1,2,*]
[1] School of biotechnology, Jiangsu University of Science and Technology, 212018 Zhenjiang, China;
[2] Sericultural Research Institute, Chinese Academy of Agricultural Sciences, 212018 Zhenjiang, China.
Key words: SpyTag/SpyCatcher, Whole cell catalysis, Surface display; Biocatalytic, Isoquercitrin.
Presenting author email: wangjun@just.edu.cn; biojustych@163.com

Biocatalysis continues to be favored by green processes due to its high stereoselectivity, green process, and mild reaction conditions (Alcantara et al., 2022). As a green and efficient catalyst, whole-cell catalysis has the advantages of good enzyme stability, a long half-life, and strong adaptability (Pinto et al., 2020). However, due to the mass transfer obstruction of the plasma membrane, the transmembrane transport of substances is greatly limited, which often makes the whole-cell catalytic efficiency lower than that of free enzyme catalysis (Domach, 2015). In order to reduce the adverse effect of intracellular enzyme mass transfer obstruction on the catalytic rate, many attempts have been made, such as the development of enzyme surface display technology. Enzymes and substrates do not need to pass through the cell membrane barrier, and the substrate directly contacts the enzyme, which greatly improves the reaction efficiency (Ye et al., 2021). However, the traditional gene fusion surface display system has the disadvantages of low surface display efficiency and the limited display range of target proteins, which limits the application of cell surface display technology in biocatalysis to a certain extent.

In recent years, isopeptide bond-mediated self-assembly techniques have been used to successfully construct several surface display systems (Hatlem et al., 2019). Gallus et al. successfully constructed a surface display system for cytochrome P450 (119 kDa) using the SpyTag/SpyCatcher system derived from the CnaB2 structural domain (Gallus et al., 2020). In addition, the SnoopCatcher/SnoopTag-mediated surface display system for lactic acid bacteria showed a 21% increase in display efficiency compared to the conventional method (Plavec and Berlec, 2019). Thus, heteropeptide bond-mediated self-assembly technology provides the feasibility for efficient display of cell surfaces of macromolecules and complex proteins.

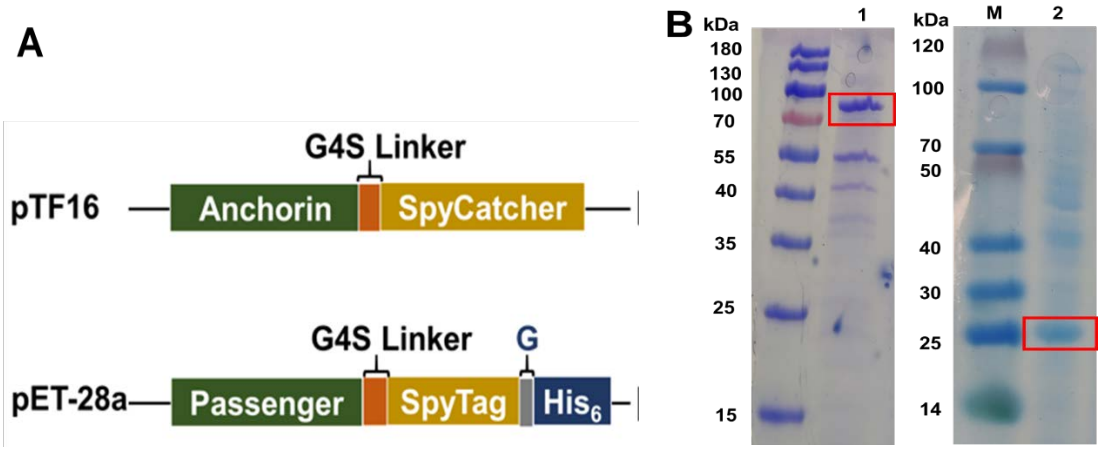

Figure. 1 Construction of RhaB1-ΔN1 recombinant protein mediated by isopeptide bond (A) and SDS-PAGE analysis (B)M, protein molecular mass marker; lane 1, Rhab1-ΔN1-SpyTag; lane 2, Lpp-OmpA-SpyCatcher

In this study, the *E. coli* surface display system was successfully constructed through the self-assembly technology mediated by the isopeptide bond SpyTag / SpyCatcher. As shown in Figure 1, two conformational proteins (Lpp-OmpA-SpyCatcher and Rhab1-ΔN1-SpyTag) were designed. Figure 1B shows the SDS-PAGE results of the two recombinant proteins, and Rhab1-ΔN1-SpyTag (79.7 kDa) and Lpp-OmpA-SpyCatcher (28.26 kDa) were successfully expressed. The surface display system of *α*-L-rhamnosidase was successfully constructed by spontaneous coupling of covalent isopeptide bonds between lysine and aspartate residues.

Figure. 2 shows the results of immunofluorescence analysis to verify the surface display of recombinant Rhab1-ΔN1 in *E. coli*. The results show that the surface displayed Rhab1-ΔN1 enzyme has obvious green fluorescence under fluorescence microscope, while the control has no obvious fluorescence (Figure 2A). This indicates that Rhab1-ΔN1 was successfully displayed to the surface of *E. coli* cells with better display efficiency. The novel whole-cell catalyst was used for the transformation of mulberry flavonoid glycosides, and the results showed that the yield of isoquercitrin was 79.8 ± 3.1% at the reaction conditions of pH 5.0 and 60 °C, which was

2.9-fold higher catalytic efficiency compared to the conventional whole-cell catalyst. Thus, the whole-cell surface display biocatalyst achieved efficient biotransformation of natural flavonoid glycosides, which is beneficial to improve the biotransformation and exploitation of natural flavonoid glycosides.

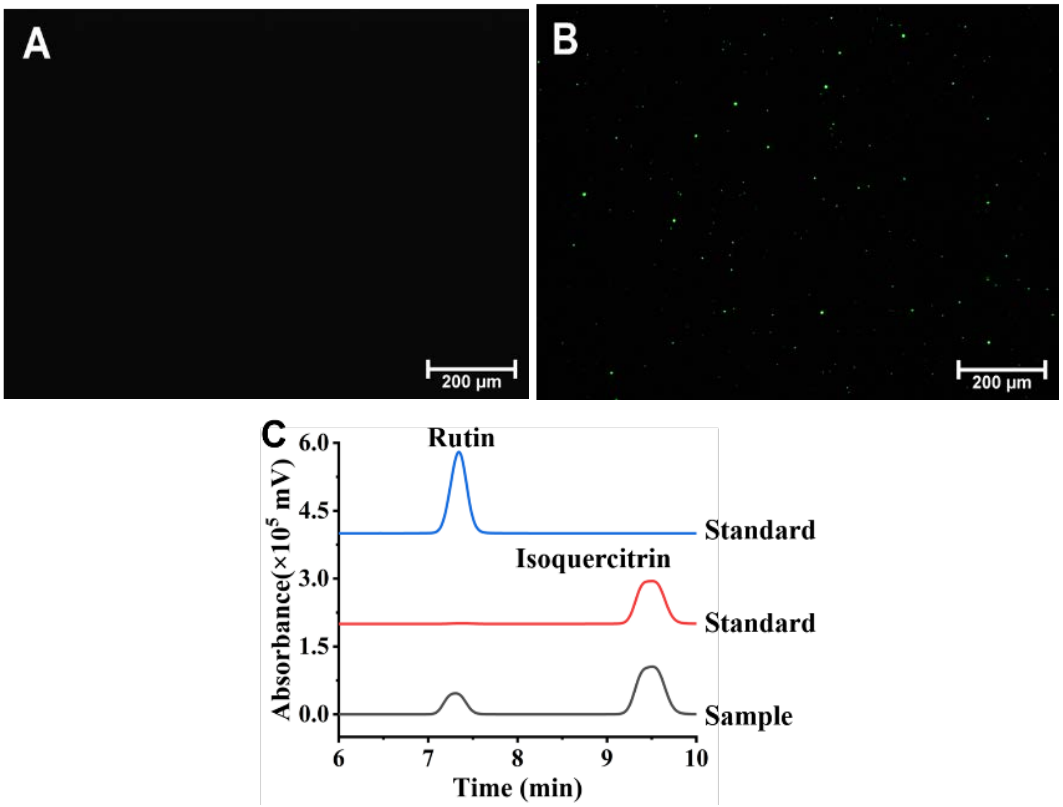

Figure. 2 Detection of whole-cell surface display catalyst by immunofluorescence (A and B) and HPLC chromatogram for the transformation of mulberry flavonoid glycosides (C)

Acknowledgements: This research was supported by the National Natural Science Foundation of China (22278196)

References:

[1] Alcantara, A. R., de Maria, P. D., Littlechild, J. A., Schurmann, M., Sheldon, R. A., Wohlgemuth, R., 2022. 'Biocatalysis as key to sustainable industrial chemistry'. *CHEMSUSCHEM*, 15 (9): e202200709.

[2] Domach, M. M., 2015. 'Perspectives and prospects for whole cell catalysis'. *CATALYSIS LETTERS*, 145: 346-359.

[3] Gallus, S., Peschke, T., Paulsen, M., Burgahn, T., Niemeyer, C. M., Rabe, K S. 2020. 'Surface display of complex enzymes by in situ SpyCatcher-SpyTag interaction'. *CHEMBIOCHEM*, 21: 2126-2131.

[4] Hatlem, D., Trunk, T., Link, D., Leo, J. C., 2019. 'Catching a SPY: Using the SpyCatcher-SpyTag and related systems for labeling and localizing bacterial proteins'. *INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES*, 20 (9): 2129.

[5] Pinto, A., Contente, M. L., 'Tamborini, L., 2020. Advances on whole-cell biocatalysis in flow'. *CURRENT OPINION IN GREEN AND SUSTAINABLE CHEMISTRY*, 25:100343.

[6] Plavec, T. V., Berlec, A., 2019. 'Surface anchoring on lactococcus lactis by covalent isopeptide bond'. *ACTA CHIMICA SLOVENICA*, 66: 18-27.

[7] Ye, M., Ye, Y., Du, Z., Chen, G., 2021. 'Cell-surface engineering of yeasts for whole-cell biocatalysts'. *BIOPROCESS AND BIOSYSTEMS ENGINEERING*, 44: 1003-1019.