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Surface Display of Lipase on Yarrowia lipolytica Cells

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Abstract : Cell-surface display of lipase is of great interest as it has many applications in the field of biotechnology owing to its unique advantages: simplified product purification, and cost-effective downstream processing. One promising area of application for whole-cell biocatalysts with surface displayed lipase is biodiesel synthesis. Biodiesel is biodegradable, renewable, and nontoxic alternative fuel for diesel engines. Although the alkaline catalysis method has been widely used for biodiesel production, it has a number of limitations, such as rigorous feedstock specifications, complicated downstream processes, including removal of inorganic salts from the product, recovery of the salt-containing by-product glycerol, and treatment of alkaline wastewater. Enzymatic synthesis of biodiesel can overcome these drawbacks. In this study, Lip2p lipase was displayed on Yarrowia lipolytica cells via C- and N-terminal fusion variant. The active site of lipase is located near the C-terminus, therefore to prevent the activity loosing the insertion of glycine-serine linker between Lip2p and C-domains was performed. The hydrolytic activity of the displayed lipase reached 12,000–18,000 U/g of dry weight. However, leakage of enzyme from the cell wall was observed. In case of C-terminal fusion variant, the leakage was occurred due to the proteolytic cleavage within the linker peptide. In case of N-terminal fusion variant, the leaking enzyme was presented as three proteins, one of which corresponded to the whole hybrid protein. The calculated number of recombinant enzyme displayed on the cell surface is approximately 6–9 × 105 molecules per cell, which is close to the theoretical maximum (2 × 106 molecules/cell). Thus, we attribute the enzyme leakage to the limited space available on the cell surface. Nevertheless, cell-bound lipase exhibited greater stability to short-term and long-term temperature treatment than the native enzyme. It retained 74% of original activity at 60°C for 5 min of incubation, and 83% of original activity after incubation at 50°C during 5 h. Cell-bound lipase had also higher stability in organic solvents and detergents. The developed whole-cell biocatalyst was used for recycling biodiesel synthesis. Two repeated cycles of methanolysis yielded 84.1–% and 71.0–% methyl esters after 33–h and 45–h reactions, respectively.

Keywords : biodiesel, cell-surface display, lipase, whole-cell biocatalyst
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