Engineering Compositionally Uniform Whole-Cell Biocatalyst for Biofuel

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As the most abundant biopolymer on Earth, cellulose represents a promising and sustainable feedstock for the production of biofuels and value-added chemicals. Due to its recalcitrant nature, researchers have engineered whole-cell biocatalysts to display designer multi-enzyme cellulosomes for efficient cellulose hydrolysis. Previous research demonstrated that enzyme density rather than enzyme proximity is the most important parameter for catalytic performance in converting biomass to ethanol. However, due to the heterogeneous nature of the most commonly used yeast surface display platform, Aga1-Aga2, which is known to yield a mixture of non-displaying and displaying cells (i.e., inactive and active biocatalysts), the achieved anchor scaffold (aScaf) display level and enzyme density in their study were limited to relative lower level. The present study replaced the plasmid-based Aga1-Aga2 expression system with genomic integration (CRISPR-Cas9), yielding uniformly active whole-cell biocatalysts. The highest aScaf display level and the enzyme density achieved using CRISPR-Cas9 is about three folds of that with a plasmid-based expression system. By adjusting the gene copy number, different aScaf display levels were attained. More importantly, creating these compositionally uniform yeast biocatalysts with high enzyme display levels improved the accuracy of the previously developed mathematical model, enabling the rational design of the optimal yeast biocatalyst to achieve the highest cellulose to ethanol conversion yield reported to date.

Awards Won:

Third Award of $1,000