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Selecting better biocatalysts by complementing recoded bacteria

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PURPOSE OF THE ABSTRACT

Assaying enzymatic activities often proves a critical bottleneck for the directed evolution of useful biocatalysts. Commonly-employed, multi-well screens that evaluate enzyme variants one-by-one are laborious and slow when compared to nature's approach to select improved variants from large populations through adaptation to a selection pressure. To apply analogous selections for the directed evolution of industrially-useful biocatalysts, I will present an in vivo directed evolution platform that leverages recoded organisms addicted to non-canonical amino acids (ncAAs) to evolve biocatalysts that can provide these building blocks from synthetic precursors.[1] Repurposing recoded organisms to link enzymatic activities to bacterial proliferation requires the introduction of three readily-available genetic components into Escherichia coli (see Figure 1A): (1) an enzyme able to convert an appropriate precursor to a ncAA (=input); (2) an orthogonal translation system that enables the site-selective incorporation of this ncAA (=sensor); and (3) a β-lactamase featuring an in-frame stop-codon, whose activity to degrade the hydrolytically-stable penicillin derivative, carbenicillin, is strictly dependent on the incorporation of the same ncAA. Critically, in our selection platform, the growth rates of bacteria in presence of carbenicillin and the synthetic precursor correlate with the activities of the enzyme they produce. As such, improved biocatalysts are readily identified by subjecting bacteria harboring vast enzyme libraries to continuous growth-dilution cycles in presence of increasing carbenicillin concentrations (=selection pressure, Figure 1B). In my talk, I will showcase how our selection platform can be employe for the directed evolution of hydrolases as well as biocatalysts that catalyze C-H activation or C-C-bond-forming reactions. Lastly, I will discuss how our platform that requires minimal human intervention and no specialized equipment will enable the autonomous exploration of many evolutionary trajectories in a continuous fashion.

FIGURES

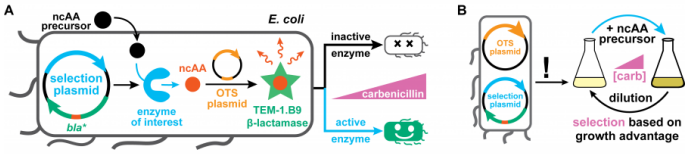

FIGURE 1

Principles of selecting better biocatalysts by complementing recoded bacteria

A: Blueprint of our directed evolution platform that functions by complementation of recoded bacteria. B: Selection of improved biocatalysts from vast libraries by serial passaging.

FIGURE 2

KEYWORDS

directed evolution | in vivo selection | genetic-code expansion | enzyme engineering

BIBLIOGRAPHY

[1] Rubini, R., Jansen, S. C., Beekhuis, H., Rozeboom, H. J., Mayer, C., Angew. Chem. Int. Ed. 2023, 62, e202213942; Angew. Chem. 2023, 135, e202213942.