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Synthetic Bacterial Cell Factory for Highly Efficient Protein Secretion and Consolidated Lignin Bioconversion to Lipid

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Extended Abstract

Lignin is the second most abundant biopolymer on earth, yet the utilization of lignin for fungible fuels and chemicals has become a bottleneck for biorefining. Some bacteria show high capacity in of aromatic compounds catabolism, like *Rhodococcus opacus* PD630. However, their lignin bioconversion efficiency was significantly hindered by their low lignin depolymerization capacity, where the bacteria lack efficient extracellular secreted lignin-degrading enzymes. Despite extensive research, secretory production of heterologous protein in bacteria remains highly challenging. The challenge is particularly true for the lignin degradation enzymes with high-redox potential like laccase. We hereby demonstrated that proteomics-guided engineering could enable efficient heterologous secretion with a total protein yield at 13.7g/L by balancing the processes among transcription, translation, secretion, and protein folding of ligninolytic laccase. The engineered secretory laccase in *R. opacus* PD630 well complemented its biochemical limits on lignin depolymerization. Further proteomics analysis revealed the key factor of efficient lipid biosynthesis for the *R. opacus* PD630, where a distinct multi-unit fatty acid synthase I drove the carbon partition to storage lipid. The discovery guided the design of efficient lipid conversion from lignin and carbohydrate. The integration of laccase-secretion based lignin depolymerization module and enhanced FASI lipid biosynthese module enabled a high titer (2.54 g/L) in converting lignin-enriched biorefinery waste to lipid. The fundamental mechanisms, engineering components, and design principle could empower transformative platforms for biomanufacturing and biorefining.