

Systems Metabolic Engineering of *Novosphingobium aromaticivorans* for Lignin Valorization

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Project Goals: To engineer a non-model bacterium, *Novosphingobium aromaticivorans*, for valorization of depolymerized lignin to value-added bioproducts. The project involves (1) discovery and optimization of pathways for assimilation of lignin-derived aromatic compounds, (2) engineering conversion pathways that match the stoichiometry of aromatic catabolism, and (3) development of genome-scale mapping techniques to identify new engineering targets in non-model bacteria.

In a typical biorefinery, biomass-derived sugars are fermented to fuels by microorganisms, while residual lignin is burned for process heat. Converting waste lignin into value-added bioproducts offers a potential source of additional revenue to improve the economics of biofuel production. While bacteria have been isolated and engineered to catabolize lignin-derived compounds, economically viable lignin valorization will require further work to discover and optimize these pathways in non-model bacteria. Extending beyond degradation pathway intermediates, new bioproducts must also be deployed that have sufficiently large markets and a competitive advantage for biosynthesis from lignin. Finally, engineered assimilatory and conversion pathways must be integrated with the host metabolic and regulatory networks to ensure efficient operation.

This project will optimize biochemical pathways for assimilation of lignin-derived compounds in *Novosphingobium aromaticivorans*. This strain is capable of efficiently catabolizing more lignin-derived compounds than were previously recognized, and we continue to identify and characterize catabolic pathways for relevant compounds including syringate, guaiacol, and β -1 aromatic dimers.

New pathways will then be engineered into *N. aromaticivorans* for conversion of lignin-derived aromatic compounds into valuable bioproducts. Most industrial biosynthetic pathways have been optimized for production from glucose or glycerol, and the alternate stoichiometry from aromatic catabolism offers potential advantages for certain classes of bioproducts.

To function efficiently, the assimilatory and conversion pathways described above must be carefully integrated into the host metabolism. To this end, we are applying novel techniques for bacterial quantitative trait-locus mapping to perform genome-wide screens for loci that affect these functions. This information will be used to build a predictive genetic model and identify additional targets for further metabolic optimization.

In combination, these efforts will result in new methods to predictively model and engineer a promising microbe for lignin valorization. The same methods can ultimately be applied to a wide range of emerging microorganisms relevant for BER's mission in sustainable bioenergy.

References

1. Cecil JH, Garcia DC, Giannone RJ, Michener JK. Rapid, parallel identification of catabolism pathways of lignin-derived aromatic compounds in *Novosphingobium aromaticivorans*. *Appl Environ Microbiol* 2018 84:e01185-18.

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