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Towards microbial platforms for lignin valorization: Pseudomonas putida cell factories and Bacillus synthetic communities

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Summaries and Appendix

Summary

Lignin is the largest renewable source of aromatic carbon in plant biomass, yet it remains a major bottleneck for biorefineries, because its heterogeneous and complex structure and oxidative recalcitrance make controlled depolymerization difficult. In practice, much of the lignin generated in lignocellulose processing is heavily underutilized and often routed to low-value applications. This thesis addresses a central challenge in sustainable biotechnology: lignin degradation and valorization by bacteria.

Chapter 2 describes current knowledge on microbial lignin decomposition and the enzymatic strategies used by fungi and bacteria. This review highlights that fungal systems remain the most potent depolymerizers, yet bacterial platforms offer advantages for industrial deployment due to faster growth, easier cultivation, enhanced specificity, and superior genetic tractability. In particular, *Pseudomona putida* emerges as an attractive chassis because of its broad aromatic catabolism, robustness toward toxic compounds, and the availability of advanced genetic tools for pathway optimization. The chapter outlines how these features position *P. putida* as a potential "cell factory" that could integrate upstream oxidative attack on lignin with downstream "biofunneling" of released aromatics into defined intermediates and products.

In **chapter 3**, we experimentally interrogate secreted/oxidative enzymes that matter for growth and conversion on lignin-derived aromatics in *P. putida* KT2440. Candidate enzymes were first prioritized by comparison with a *P. putida* isolate previously shown to grow on lignin, with an initial focus on enzymes predicted to be secreted. This analysis unexpectedly pointed to *PP_1686*, annotated as a glutathione peroxidase (GPx) and homologous to "versatile peroxidase", as a key gene of interest, together with a DyP-type peroxidase homolog (*PP_3248*). Targeted deletions were constructed, and growth was measured on representative lignin-derived aromatics (*p*-coumaric acid, ferulic acid, and syringic acid) as well as on the lignin-mimicking dye methylene blue. Deletion of *PP_1686* caused a clear fitness defect on aromatic substrates and reduced dye decolorization, whereas deletion of *PP_3248* did not yield a pronounced phenotype under the tested conditions. Transcriptomics further showed that loss of *PP_1686* triggered coordinated reprogramming consistent with disrupted redox–metabolism coupling: repression of key aromatic funneling (involving pobA monooxygenase) alongside broader downregulation of translation and DNA repair modules. Together, these results indicate that *PP_1686*

has a broader function than a generic detoxification enzyme: it supports lignin-derived aromatic utilization by helping align oxidative balance with carbon flux, emphasizing redox regulation as a design constraint for robust lignin bioconversion in *P. putida*.

Chapter 4 shifts gears from single-strain engineering to a community perspective and develops a rational workflow to construct a lignin-degrading synthetic community (LDSynCom) from a diverse *Bacillales* collection by integrating bottom-up and top-down selection. The design principle is founded on the presumption that biofilm-associated lifestyles can promote lignin depolymerization by concentrating cells, extracellular matrices, and enzyme activities at the lignin interface while distributing functional roles across individual cells. The resulting 11-member LDSynCom formed biofilm structures on lignin particles, as visualized by scanning electron microscopy after growth on lignin-containing medium. Functional lignin modification was supported by shake-flask assays. Importantly, the LDSynCom increased phenolic product accumulation relative to abiotic controls. Overall, Chapter 4 provides an ecologically informed, tractable SynCom framework for lignin depolymerization, with a clear path toward mechanistic "drop-out/add-in" experiments to map variation and distribution of different roles and function among the members of the SynCom.

Chapter 5 returns to the "cell factory" concept by testing whether lignin-active oxidative enzymes can be produced functionally in *P. putida* KT2440. This chapter focuses on DyP-type peroxidases (Dyp) from *Thermobifida fusca*, *Rhodococcus jostii* RHA1, and *Pseudomonas fluorescens*, including constructs engineered with signal peptides to promote secretion or periplasmic access. Two genome-integrated expression platforms were evaluated: a constitutive mini-Tn7-based system (pBG) for stable single-copy insertion, and an inducible, orthogonal polymerase-driven system (pPUT) designed for stringent control and high production. Our results indicate that the DyP from *T. fusca* was expressed with clear peroxidase activity, validating *P. putida* as a workable host for active DyP enzymes under the tested conditions.

Chapter 6 critically discusses the bottlenecks and challenges encountered in the current process of microbial degradation and valorization of lignin and proposes suggestions for future research directions.

The work described in this thesis emphasizes that lignin bioconversion in *P. putida*

is not only an “enzyme availability” problem but also a redox and stress-management problem. In parallel, the SynCom results suggest that ecological traits such as biofilm formation can be treated as engineerable “process features” that enhance lignin depolymerization at interfaces. Together, these insights provide a roadmap for building more robust and predictable lignin biotechnologies—either as engineered single-organism platforms or as functionally partitioned microbial communities—capable of upgrading lignin-derived carbon into higher-value products and processes.