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

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Chapter 1

General introduction

Lignin is the second most abundant natural terrestrial polymer after cellulose (Zevallos Torres et al., 2020). Together with cellulose and hemicellulose, it constitutes lignocellulose, which represents a major fraction of plant biomass (Brenelli et al., 2016). Each year, approximately 100 million tons of lignin are produced, of which an estimated 50-70 million tons are produced through the Kraft process by the paper and pulp industry (Muthamil Selvam et al., 2021). Lignin remains largely underutilized in biorefineries due to its structural complexity, rigidity, and inherent chemical recalcitrance, which limit efficient depolymerization and conversion into value-added products (Shrestha et al., 2024). Nevertheless, lignin is potentially a renewable resource of aromatic carbon in nature and is rich in functional groups (e.g., phenolic and aliphatic hydroxyls), making it a highly promising feedstock for producing renewable aromatics, polymer precursors, and functional materials (Sheldon & Woodley, 2018). Developing more effective strategies to utilize lignin has become a major research focus in recent years.

In nature, the ability to decompose lignified plant biomass is widespread among microorganisms - most in lignin-degrading fungi (Kijpornyongpan et al., 2025), but also in bacteria that contribute to lignin transformation in decaying wood (Janusz et al., 2017a). The biodegradation of lignin became a focus of some research in the 1970s, and remains a major research focus today (Atiwesh et al., 2022). Studies have shown that soil bacteria, including *Pseudomonas* and *Bacillus*, can contribute to lignin depolymerization and degradation (Huang et al., 2013b; Lee et al., 2019; Liang et al., 2025). Furthermore, some enzymes involved in lignin depolymerization have been explored, including laccases and peroxidases (de Gonzalo et al., 2016).

Among the *Pseudomonas* family, *Pseudomonas putida* strains are considered promising candidates for lignin depolymerization and related biotechnological applications, owing to their metabolic versatility and robustness under industrially relevant conditions (Nikel & de Lorenzo, 2018; Poblete-Castro et al., 2012). *P. putida* KT2440 is one of the best-characterized representatives (Regenhardt et al., 2002). This strain is highly tolerant to oxidative stress, which is consistent with its capacity to degrade or utilize compounds that can themselves impose oxidative and other physiological stresses (Kim & Park, 2014; Nikel & de Lorenzo, 2014).

Bacillus are widespread soil bacteria with notable robustness and biotechnological potential. Beyond this, many *Bacillus* strains produce large amounts of extracellular

polymeric substances (EPS) (Arnaouteli et al., 2021; Yadav et al., 2023). These substances are widely recognized to be related to the formation of biofilms, which can enhance the biodegradation of polymers by facilitating close cell–substrate contact and strengthening microbial and enzymatic interaction. This has been confirmed in plastic degradation systems (Chattopadhyay, 2022).

Lignin degradation is typically driven by microbial communities rather than by individual strains in nature, with different members contributing complementary functions (Chen et al., 2025; Ding et al., 2025). Thus, artificial microbial consortia with functional partitions provide a novel approach for lignin degradation. In recent years, synthetic microbial communities (SynComs) have ben increasingly applied in diverse fields, including plant growth promotion (Xu et al., 2025), environmental remediation, and microbiota engineering (van Leeuwen et al., 2023).

The aim of this thesis is to understand bacterial lignin conversion by identifying and characterizing key enzymes in *P. putida* and by developing controllable microbial platforms - both single-chassis and synthetic community - for lignin degradation and future valorization.

Scope and outline of this thesis

This PhD project focuses mainly on lignin conversion and degradation by bacteria and the mechanism behind it. This **Chapter 1** provides a general introduction on lignin as a recalcitrant component of plant biomass, outlines the scientific and technological motivation for lignin valorization, and defines the aim and scope of this thesis.

Chapter 2 outlines the theoretical background for this thesis. In this chapter, I introduce lignin as a highly recalcitrant and heterogeneous polymer and summarize its major structural units and key linages. I also review and summarize the current knowledge on various mechanisms of lignin breakdown by microorganisms. Both fungal and bacterial lignin degraders and the main ligninolytic enzymes in relation to which lignin linkages they can cleave are described. Furthermore, I discuss the potential of *P. putida* as a suitable chassis for lignin degradation and valorization from three perspectives (i) its natural capabilities and metabolic pathways for processing lignin-

derived aromatics, (ii) the available and emerging genetic engineering toolbox that enables targeted genome manipulation and pathway optimization, and (iii) biological conversion and reutilization strategies that couple aromatic assimilation to the production of value-added bioproducts.

P. putida is an attractive chassis because of its robust and flexible metabolism, as well as its high tolerance to many noxious and toxic compounds (Nikel & de Lorenzo, 2018). Building on the conclusion that *P. putida* is a promising host for lignin valorization, **Chapter 3** investigates how *P. putida* KT2440 metabolizes lignin-derived compounds, with a specific focus on the enzyme *PP_1686*, previously annotated as glutathione peroxidase, that was unexpectedly found to play a functional role in lignin-related growth and conversion.A mutant deleted for *PP_1686* shows impaired growth on lignin-derived aromatic compounds, indicating that *PP_1686* is important for efficient lignin-derived aromatics metabolism. Based on the results of transcriptome analysis, **Chapter 3** reveals the coordinated rewiring of lignin-derived aromatic catabolic and stress response pathways, including reduced expression of *pobA* (encode p-hydroxybenzoate hydroxylase) and DNA repair modules. Together, **Chapter 3** links lignin-derived aromatic metabolism to redox homeostasis in *P. putida* KT2440 and provides mechanistic targets for future lignin valorization engineering.

Inspired by nature, in **Chapter 4** we develop a defined *Bacillus* synthetic community for lignin degradation (LDSynCom). By combining top-down ecological filtering with bottom-up, trait-based selection, this chapter establishes a rational workflow to assemble functionally complementary community members. Our approach addresses the shortcomings of using either strategy alone—applying lessons learned from previous SynComs approaches. This dual strategy enables us to (i) retain taxa that may not directly degrade lignin but play essential ecological roles particularly biofilm formation while simultaneously (ii) enriching and preserving key ligninolytic strains that drive the core degradation process. The resulting LDSynCom combines strains with ligninolytic potential with strong biofilm formers that contribute indirectly to overall community performance, providing a controllable and mechanistically tractable model to study community-enabled lignin degradation.

Chapter 5 focuses on cell-factory development by heterologously expressing DyP-type peroxidases, reported to exhibit ligninolytic or lignin-oxidizing activity, in *P. putida*

KT2440. In this work, Dye-decolorizing peroxidase (DyP) genes from *Thermobifida fusca*, *Rhodococcus jostii* RHA1, and *Pseudomonas fluorescens* were heterologously expressed in *P. putida* KT2440 using 2 different expression systems. This chapter establishes an expression-and-validation workflow for deploying lignin-active enzymes in a genetically tractable bacterial chassis and evaluates recombinant DyP production and functionality through protein detection and peroxidase activity assays, providing a foundation for future engineering of *P. putida*–based lignin valorization platforms.

In **Chapter 6**, I discuss the current bottlenecks and challenges in microbial lignin degradation and valorization and provide suggestions for future research directions.

In summary, this thesis progresses from an overview of microbial lignin degradation to the new discovery that a glutathione peroxidase is involved in lignin-related growth and conversion in *P. putida* KT2440, then subsequently to the rational construction of microbial platforms for lignin degradation, including a defined LDSynCom, and towards an engineered *P. putida* cell factory expressing lignin-active enzymes. As such, this work may contribute to the development and implementation of more efficient, controllable, and scalable lignin valorization strategies with microorganisms in the near future.