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Chemical intervention in bacterial lignin degradation pathways: Development of selective inhibitors for intradiol and extradiol catechol dioxygenases

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ABSTRACT

Bacterial lignin degradation could be used to generate aromatic chemicals from the renewable resource lignin, provided that the breakdown pathways can be manipulated. In this study, selective inhibitors of enzymatic steps in bacterial degradation pathways were developed and tested for their effects upon lignin degradation. Screening of a collection of hydroxamic acid metallo-oxygenase inhibitors against two catechol dioxygenase enzymes, protocatechuate 3,4-dioxygenase (3,4-PCD) and 2,3-dihydroxyphenylpropionate 1,2-dioxygenase (MhpB), resulted in the identification of selective inhibitors D13 for 3,4-PCD (IC_{50} 15 μM) and D3 for MhpB (IC_{50} 110 μM). Application of D13 to *Rhodococcus jostii* RHA1 in minimal media containing ferulic acid led to the appearance of metabolic precursor protocatechuic acid at low concentration. Application of 1 mM disulfiram, an inhibitor of mammalian aldehyde dehydrogenase, to *R. jostii* RHA1, gave rise to 4-carboxymuconolactone on the β-ketoadipate pathway, whereas in *Pseudomonas fluorescens* Pf-5 disulfiram treatment gave rise to a metabolite found to be glycine betaine aldehyde.

1. Introduction

Lignin is an aromatic heteropolymer found as 15–25% of lignocellulose in plant cell walls, hence is a major component of plant biomass. The aromatic content of lignin represents an attractive raw material for conversion to renewable aromatic chemicals, provided that biocatalytic or chemocatalytic conversion routes can be found for lignin valorization [1]. Despite research into fungal lignin degradation since the early 1980s [2], there is currently no commercial process for lignin valorization. We have recently identified a number of soil bacteria with activity for lignin oxidation [3,4], and we have identified Dyp-type peroxidase enzymes with activity for lignin oxidation in *Rhodococcus jostii* RHA1 [5] and *Pseudomonas fluorescens* Pf-5 [6].

Following the initial oxidation of the lignin polymer, the pathways for microbial degradation of lignin fragments are only partly understood [7]. Identification of metabolites from lignin breakdown in *R. jostii* RHA1 and *Pseudomonas putida* mt-2 has led to hypotheses for lignin degradation pathways in these bacteria [3,7]. Several pieces of evidence indicate that the vanillic acid degradation pathway, shown in Fig. 1, is involved in bacterial lignin degradation: vanillic acid (**2**) has been observed as a lignin breakdown product in *R. jostii* and *P. putida* [8]; lignin-degrading bacteria isolated from environmental samples can utilise vanillic acid from growth on minimal media [4]; gene deletion of vanillin dehydrogenase in *R. jostii* RHA1 leads to accumulation of vanillin (**1**) up to 96 mg/L when grown on minimal media containing wheat straw lignocellulose [8].

The accumulation of vanillin (**1**) via gene deletion demonstrates that intervention in bacterial lignin degradation pathways is a possible strategy for accumulation of aromatic bioproducts from lignin [8]. However, genetic modification via gene deletion is only possible in well characterised genetically tractable bacteria, therefore we wished to also investigate whether lignin degradation pathways could be intercepted via chemical intervention using selective chemical inhibitors of enzymes on the breakdown pathways.

Oxidative demethylation of vanillic acid (**2**) generates protocatechuic acid (**3**), which can be metabolized as shown in Fig. 1 either via intradiol oxidative cleavage by protocatechuate 3,4-dioxygenase via the β-ketoadipate pathway [7], or via extradiol oxidative cleavage by protocatechuate 4,5-dioxygenase, a well-characterised enzyme in *Sphingobium* SYK6 [9]. Oxidative cleavage of protocatechuic acid (**3**) is therefore a key branch-point in aromatic degradation pathways [7]. The ability to selectively block either ortho- or meta-cleavage would be potentially valuable for pathway

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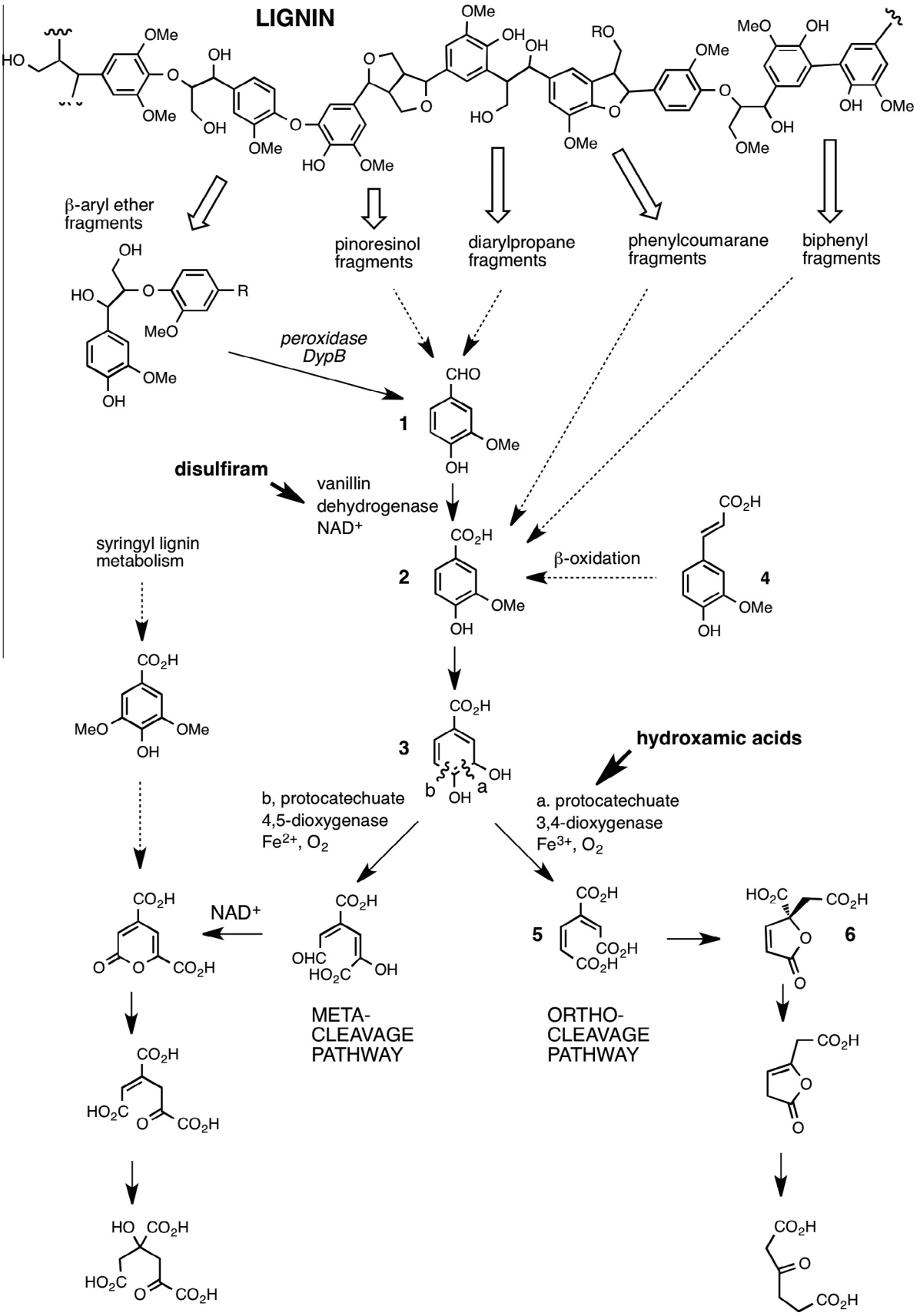

Fig. 1. Vanillic acid degradation pathway, showing enzymes for chemical intervention. Intermediates: **1**, vanillin; **2**, vanillic acid; **3**, protocatechuic acid; **4**, ferulic acid; **5**, 3-carboxymuconic acid; **6**, 4-carboxymuconolactone.