ARTICLE IN PRESS

Metabolic Engineering ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Metabolic Engineering

journal homepage: www.elsevier.com/locate/ymben

Aromatic catabolic pathway selection for optimal production of pyruvate and lactate from lignin

Q1 Christopher W. Johnson, Gregg T. Beckham*

National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO 80401, United States

ARTICLE INFO

Article history:
Received 29 September 2014
Received in revised form
8 January 2015
Accepted 13 January 2015

Keywords:
Lignin valorization
β-ketoadipate pathway
Aromatic degradation
Pseudomonas putida KT2440
Sphingobium sp. strain SYK-6

ABSTRACT

Lignin represents an untapped feedstock for the production of fuels and chemicals, but its intrinsic heterogeneity makes lignin valorization a significant challenge. In nature, many aerobic organisms degrade lignin-derived aromatic molecules through conserved central intermediates including catechol and protocatechuate. Harnessing this microbial approach offers potential for lignin upgrading in modern biorefineries, but significant technical development is needed to achieve this end. Catechol and protocatechuate are subjected to aromatic ring cleavage by dioxygenase enzymes that, depending on the position, *ortho* or *meta* relative to adjacent hydroxyl groups, result in different products that are metabolized through parallel pathways for entry into the TCA cycle. These degradation pathways differ in the combination of succinate, acetyl-CoA, and pyruvate produced, the reducing equivalents regenerated, and the amount of carbon emitted as CO_2—factors that will ultimately impact the yield of the targeted product. As shown here, the ring-cleavage pathways can be interchanged with one another, and such substitutions have a predictable and substantial impact on product yield. We demonstrate that replacement of the *ortho* catechol degradation pathway endogenous to *Pseudomonas putida* KT2440 with an exogenous *meta*-cleavage pathway from *P. putida* mt-2 increases yields of pyruvate produced from aromatic molecules in engineered strains. Even more dramatically, replacing the endogenous protocatechuate *ortho* pathway with a *meta*-cleavage pathway from *Sphingobium* sp. SYK-6 results in a nearly five-fold increase in pyruvate production. We further demonstrate the aerobic conversion of pyruvate to L-lactate with a yield of $41.1 \pm 2.6\%$ (wt/wt). Overall, this study illustrates how aromatic degradation pathways can be tuned to optimize the yield of a desired product in biological lignin upgrading. Q2

© 2015 International Metabolic Engineering Society. Published by Elsevier Inc.

1. Introduction

Lignin is a branched, aromatic polymer found in the cell walls of plants that accounts for 15–30% of the dry weight of terrestrial biomass. While it contains more energy and a lower oxygen-to-carbon ratio than either cellulose or hemicellulose (Ragauskas et al., 2014), the structural heterogeneity and recalcitrance have limited its utility in lignocellulosic upgrading strategies, where it is routinely slated for combustion to generate process heat (Chundawat et al., 2011; Ragauskas et al., 2014; Zakzeski et al., 2010). Recent reports have suggested, however, that incorporation of lignin in upgrading strategies is critical for the economic viability of bio-based fuels and chemicals (Davis et al., 2013; Ragauskas et al., 2014).

When depolymerized in nature by oxidative enzymes secreted by ligninolytic fungi and bacteria, lignin yields a heterogeneous mixture of aromatic monomers that many bacteria use as sources of carbon and energy (Bugg et al., 2011a; 2011b; Martínez et al., 2005). In these organisms, "upper pathways" degrade such mixtures to form conserved metabolic intermediates, acting as a "biological funnel" to reduce the heterogeneity of carbon for catabolism. In aerobic organisms, these central intermediates, which include catechol (1,2dihydroxybenzene) and protocatechuate (3,4dihydroxybenzoate), are then subjected to O_2 dependent ring opening by dioxygenase enzymes that exhibit either *ortho* (intradiol) or *meta* (extradiol) cleavage, between or adjacent to the hydroxyl groups, respectively (Fuchs et al., 2011). The cleavage products are degraded through distinct pathways that yield different molecules for entry into central metabolism (Fig. 1). The *ortho*-cleavage pathways of both catechol and protocatechuate produce succinate and acetyl-CoA (Jiménez et al., 2002). The *meta*-cleavage of catechol, as well as the protocatechuate 2,3 *meta*-cleavage pathway yield pyruvate and acetyl-CoA, while the 4,5 *meta*-cleavage pathway of protocatechuate ultimately yields two pyruvate molecules (Greated et al., 2002; Harayama and Rekik, 1990; Kamimura et al., 2010; Kamimura and Masai, 2014; Kasai et al., 2009).

Descriptions of aromatic catabolism date back to the 1960s (Crawford, 1975; Dagley et al., 1960; Dagley and Gibson, 1965; Feist and Hegeman, 1969a; 1969b; Hegeman, 1966; Ornston, 1966;

* Corresponding author.
E-mail address: gregg.beckham@nrel.gov (G.T. Beckham).

http://dx.doi.org/10.1016/j.ymben.2015.01.005
1096-7176/© 2015 International Metabolic Engineering Society. Published by Elsevier Inc.

Please cite this article as: Johnson, C.W., Beckham, G.T., Aromatic catabolic pathway selection for optimal production of pyruvate and lactate from lignin. Metab. Eng. (2015), http://dx.doi.org/10.1016/j.ymben.2015.01.005

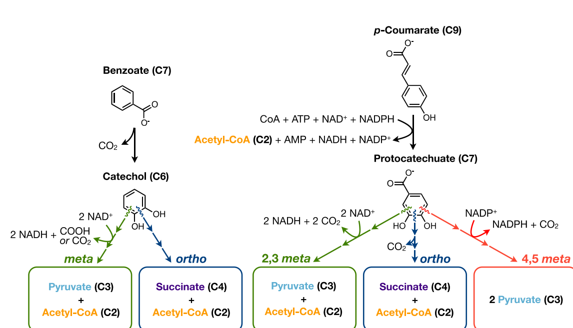

Fig. 1. The catechol and protocatechuate *ortho* and *meta* degradation pathways. Aromatic molecules such as benzoate and *p*-coumarate are degraded aerobically through intermediates catechol and protocatechuate. O_2-dependent dioxygenase enzymes cleave the aromatic rings of catechol or protocatechuate *ortho* or *meta* to the hydroxyl groups. The different products of ring cleavage are degraded through separate pathways (each represented as three colored arrows) and enter the TCA cycle as different combinations of acetyl-CoA, succinate and pyruvate. Deletion of *aceEF*, which encodes components of the pyruvate dehydrogenase complex, prevents the conversion of pyruvate to acetyl-CoA. Reducing equivalents and ATP generated in the TCA cycle have been omitted for simplicity. See the main text for further details.

Ornston and Stanier, 1966). Research during the decades since has led to a wealth of knowledge regarding the enzymes that comprise these pathways, the genes that encode them, and the regulatory mechanisms that govern their activity (Fuchs et al., 2011; Harwood and Parales, 1996; Kamimura and Masai, 2014; Pérez-Pantoja et al., 2010). Until recently, the application of these pathways in the synthesis of bio-based products has been limited to just a few examples, such as the production of muconic acid, an intermediate in the catechol *ortho* degradation pathway, for conversion to adipic acid (Curran et al., 2013; Draths and Frost, 1994; Lin et al., 2014; van Duuren et al., 2012; Weber et al., 2012).

Nature has overcome the heterogeneity of molecules derived from lignin by evolving pathways that funnel carbon into central metabolism through a few common intermediates. Going beyond single intermediates, harnessing this evolutionary innovation to selectively convert a heterogeneous slate of lignin-derived molecules into fuels and chemicals represents a substantial opportunity, as demonstrated by our recent description of medium-chain-length polyhydroxyalkanoate (mcl-PHA) production from alkaline pretreated lignin (Linger et al., 2014). As we move toward utilizing lignin-derived substrates, it is likely that the appropriate deployment of the aromatic catabolic pathways described above will be central to achieve optimal yields of targeted molecules. In order to demonstrate this concept, we have constructed genetically integrated strains that degrade catechol or protocatechuate through *ortho* or *meta*-cleavage pathways using *Pseudomonas putida* KT2440, a fast growing, genetically amenable, and stress tolerant soil microbe, as the host (Nicolaou et al., 2010; Nikel and de Lorenzo, 2013; Nikel et al., 2014; Poblete-Castro et al., 2013). These strains were evaluated for production of pyruvate and subsequent conversion to L-lactate. Pyruvate is a key intermediate in central metabolism that is fundamental to metabolic engineering strategies for bio-based production of amino acids, alcohols including ethanol and isobutanol, terpenoids such as isoprene and farnesene, and lactate (Chandran et al., 2011; Lamsen and Atsumi, 2012; Lee et al., 2004; Park et al., 2007; Zhao et al., 2011; Zhu and Shimizu, 2004). Using our engineered strains, we show that higher yields of pyruvate from aromatic molecules degraded through catechol and protocatechuate can be achieved by utilizing exogenous *meta*-cleavage pathways rather than the endogenous *ortho*-cleavage pathways. We then demonstrate the aerobic conversion of pyruvate to L-lactate through the incorporation of a bovine lactate dehydrogenase, ultimately increasing product yield by providing greater competition for pyruvate that might otherwise enter the TCA cycle. Our findings here serve as an example of the benefit that appropriate selection of aromatic degradation pathways can have on biological lignin upgrading.

2. Materials and methods

2.1. Plasmid construction

Q5® Hot Start High-Fidelity 2X Master Mix (New England Biolabs) and primers synthesized by Integrated DNA Technologies (IDT) were used in all PCR amplification for plasmid construction. Plasmids were constructed using Gibson Assembly® Master Mix (New England Biolabs) according to the manufacturer's instructions. Plasmids were transformed into competent NEB 5-alpha F'I^q *Escherichia coli* (New England Biolabs) according to the manufacturer's instructions. Transformants were selected on LB (Lennox) plates containing 10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl, and 15 g/L agar, supplemented with either 10 μg/mL tetracycline or 50 μg/mL kanamycin grown at 37 °C. The sequences of all plasmid inserts were confirmed using Sanger sequencing performed by GENEWIZ, Inc. Specific plasmid construction details can be found in Supplementary materials and methods.

2.2. Strain construction

P. putida KT2440 (ATCC 47054) was used as the basis of strain engineering and gene replacements were made using the antibiotic/*sacB* system of selection and counter-selection (Marx, 2008). To prepare cells of *P. putida* KT2440 and strains derived from it for transformation by electroporation; LB broth was inoculated to an OD_{600} of about 0.02 and incubated shaking at 225 rpm, 30 °C, until an OD_{600} of 0.5–0.7 was reached. Cells were then centrifuged at 4 °C, washed twice in ice-cold water and once in ice-cold 10% glycerol before being resuspended in 1/100 of the culture's original volume of 10% glycerol. Cells were then stored at −80 °C or transformed by electroporation immediately. For transformation, 5 μL (200 ng–2 μg) of plasmid DNA was added to 50 μL of the electrocompetent cells, transferred to a chilled 0.1 cm electroporation cuvette, and electroporated at 1.6 kV, 25 uF, 200 Ω. 450 μL SOC outgrowth medium (NEB) was added to the cells immediately after electroporation and the resuspended cells were incubated shaking at 225 rpm, 30 °C, for one hour. The entire transformation was plated on an LB agar plate containing appropriate antibiotics (30 μg/mL tetracycline for pCM433-based plasmids, 50 μg/mL kanamycin for pK18mobsacB-based plasmids) and incubated at 30 °C overnight. Transformants were restreaked for single colonies on LB agar and incubated at 30 °C overnight to reduce the possibility of untransformed cells being transferred. For sucrose counter-selection, restreaked transformants were streaked for single colonies on YT+25% sucrose plates (10 g/L yeast extract, 20 g/L tryptone, 250 g/L sucrose, and 18 g/L agar), and incubated at

UNCORRECTED PROOF

Please cite this article as: Johnson, C.W., Beckham, G.T., Aromatic catabolic pathway selection for optimal production of pyruvate and lactate from lignin. Metab. Eng. (2015), http://dx.doi.org/10.1016/j.ymben.2015.01.005

Download English Version:

https://daneshyari.com/en/article/6494488

Download Persian Version:

https://daneshyari.com/article/6494488

Daneshyari.com