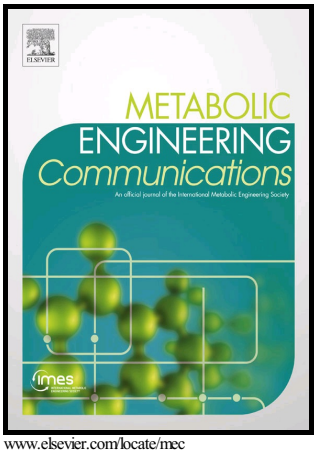

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Eliminating a global regulator of carbon catabolite repression enhances the conversion of aromatic lignin monomers to muconate in *Pseudomonas putida* KT2440

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ABSTRACT

Carbon catabolite repression refers to the preference of microbes to metabolize certain growth substrates over others in response to a variety of regulatory mechanisms. Such preferences are important for the fitness of organisms in their natural environments, but may hinder their performance as domesticated microbial cell factories. In a *Pseudomonas putida* KT2440 strain engineered to convert lignin-derived aromatic monomers such as *p*-coumarate and ferulate to muconate, a precursor to bio-based nylon and other chemicals, metabolic intermediates including 4-hydroxybenzoate and vanillate accumulate and subsequently reduce the muconate yield and productivity. We hypothesized that these metabolic bottlenecks may be, at least in part, the effect of carbon catabolite repression caused by glucose or acetate, more preferred substrates that must be provided to the strain for supplementary energy and cell growth. Using mass spectrometry-based proteomics, we have identified the 4-hydroxybenzoate hydroxylase, PobA, and the vanillate demethylase, VanAB, as targets of the Catabolite Repression Control (Crc) protein, a global regulator of carbon catabolite repression. By deleting the gene encoding Crc from this strain, the accumulation of 4-hydroxybenzoate and vanillate are reduced and, as a result, muconate production is enhanced. In cultures grown on glucose, the yield of muconate produced from *p*-coumarate after 36 hours was increased nearly 70% with deletion of the gene encoding Crc (94.6 ± 0.6% vs. 56.0 ± 3.0% (mol/mol)) while the yield from ferulate after 72 hours was more than doubled (28.3 ± 3.3% vs. 12.0 ± 2.3% (mol/mol)). The effect of eliminating Crc was similar in cultures grown on acetate, with the yield from *p*-coumarate just slightly higher in the Crc deletion strain after 24 hours (47.7 ± 0.6% vs. 40.7 ± 3.6% (mol/mol)) and the yield from ferulate increased more than 60% after 72 hours (16.9 ± 1.4% vs. 10.3 ± 0.1% (mol/mol)). These results are an example of the benefit that reducing carbon catabolite repression can have on conversion of complex feedstocks by microbial cell factories, a concept we posit could be broadly considered as a strategy in metabolic engineering of pseudomonads for conversion of renewable feedstocks to value-added chemicals.

Keywords

Carbon catabolite repression, Catabolite repression control, Crc, *Pseudomonas putida* KT2440, *cis*,*cis*-Muconate, Muconic acid, Lignin valorization

1. Introduction

To successfully compete in the environmental niches they occupy, most microorganisms have developed innate preferences for certain growth substrates over others. This phenomenon has been singularly termed carbon catabolite repression (CCR), but the mechanisms that govern these preferences are as diverse as the organisms in which they have evolved (Reviewed in Görke and Stülke, 2008).

In pseudomonads, a preference for organic acids and amino acids over glucose, which is generally preferred over hydrocarbons and aromatic compounds, is imparted by a complex combination of global and operon-specific mechanisms (Reviewed in Rojo, 2010). Perhaps the most important of these mechanisms is the action of the Crc (catabolite repression control) protein, a global regulator that inhibits translation of targeted mRNAs by binding near ribosome binding sites. This binding occurs in association with another protein, Hfq, at catabolite activity (CA) sequence motifs that contain a AANAANAA core and the presence of Crc, Hfq, and the CA motif is essential for Crc regulation (Moreno et al., 2014; 2009b; Sonnleitner et al., 2009; 2012). Crc has been shown to target catabolic pathways directly, by inhibiting translation of the enzymes themselves, and indirectly, by inhibiting translation of transcriptional regulators that drive expression of genes encoding catabolic enzymes as well as transporters required for substrates to enter the cell (Hernández-Arranz et al., 2013).

CCR is undoubtedly important for the fitness of saprophytic soil bacteria like *Pseudomonas putida* KT2440, which is well-suited for its native environment because of its ability to judiciously degrade a wide range of natural and xenobiotic substrates, including those derived from the three major fractions of plant biomass, namely cellulose, hemicellulose, and lignin. Lignin is a heterogeneous polymer of aromatic monomers that is an important component of the plant cell wall and can account for up to 40% of the carbon in terrestrial biomass (Ragauskas et al., 2014; Zakzeski et al., 2010). We have recently reported the development of *P. putida* KT2440-based biocatalysts for production of muconate (Fig. 1), which can be converted to adipic acid (Vardon et al., 2015; 2016) and diethyl terephthalate (Lu et al., 2015), precursors to the commodity plastics nylon and polyethylene terephthalate, respectively. Muconate can also be utilized directly or partially hydrogenated to produce novel materials (Rorrer et al., 2016; 2017). Biologically, *cis*,*cis*-muconate can readily be produced from aromatic