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High yield production of D-xylonic acid from D-xylose using engineered *Escherichia coli*

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ABSTRACT

An engineered *Escherichia coli* was constructed to produce D-xylonic acid, one of the top 30 high-value chemicals identified by US Department of Energy. The native pathway for D-xylose catabolism in *E. coli* W3110 was blocked by disrupting xylose isomerase (XI) and xylulose kinase (XK) genes. The native pathway for xylonic acid catabolism was also blocked by disrupting two genes both encoding xylonic acid dehydratase (*yagE* and *yjhG*). Through the introduction of a D-xylose dehydrogenase from *Caulobacter crescentus*, a D-xylonic acid producing *E. coli* was constructed. The recombinant *E. coli* produced up to 39.2 g L^{-1} D-xylonic acid from 40 g L^{-1} D-xylose in M9 minimal medium. The average productivity was as high as 1.09 g L^{-1} h^{-1} and no gluconic acid byproduct was produced. These results suggest that the engineered *E. coli* has a promising application for the industrial-scale production of D-xylonic acid.

1. Introduction

It has become a common view now that shifting the world's dependence from fossil-based to biomass-based resources is an important approach to the effective management of global warming (Ragauskas et al., 2006). Innumerous scientists have devoted themselves to the biofuels research since the latter part of last century. Compared with biofuels, research on bio-sourced chemicals and materials traditionally derived from fossils has not attracted much interest until the last decade. However, in recent years, biomass processing for the production of value-added chemicals has come to the forefront of both biological and chemical engineering research (FitzPatrick et al., 2010). To support the campaign on integrated conversion of biomass, the US Department of Energy (DOE) identified 30 chemicals which could be used for the production of high-value chemicals and fuels in 2004. These compounds were selected from a list of more than 300 candidates that can be produced via biomass conversion. A list of top 12 chemicals, the so-called building block chemicals, was further identified from the top 30 list (PNNL and NREL, 2004).

As one of the top 30 high-value chemicals, D-xylonic acid has found its applications in many fields. It has been utilized as the substrate for biosynthesis of 1,2,4-butanetriol; a valuable precursor for the energetic material 1,2,4-butanetriol trinitrate (Niu et al., 2003). D-xylonic acid can also be used as a concrete additive which improves concrete dispersion (Chun et al., 2006). In addition, other applications of D-xylonic acid have been reported as well in the fields of food, pharmaceutical, and agriculture (Tomoda et al., 2004; Pujos, 2006; Richard, 1990).

D-xylonic acid can be produced from D-xylose by microbial conversion. Enzymatic hydrolysis of D-xylose to D-xylonolactone then subsequently to D-xylonic acid has been observed in several bacterial species such as *Pseudomonas*, *Gluconobacter* and *Caulobacter* (Buchert et al., 1988; Buchert and ViiKari, 1988; Stephens et al., 2007). Another enzyme, D-xylose dehydrogenase, is also found in the fungus *Hypocrea jecorina*, but its function remains unclear (Berghäll et al., 2007). Recently, two recombinant yeast strains have been constructed aiming to produce D-xylonic acid from lignocellulosic hydrolysates (Nygård et al., 2011; Toivari et al., 2010). But so far, no commercial production of D-xylonic acid has been established. Reasons are either because bacteria strains produce many other oxidizing enzymes resulting in the conversion of other sugars present in lignocellulosic hydrolysates or because the engineered yeast strains have low D-xylonic acid accumulation rate and yield. In addition, the high cost of peptone and/or yeast extract media as nitrogen sources is generally uneconomical for industrial scale production of D-xylonic acid.

In this paper, the construction of a D-xylonic acid producing *Escherichia coli* strain is presented. The *Caulobacter crescentus xylB* gene encoding NAD^+-dependent D-xylose dehydrogenase was expressed in *E. coli* W3110. To avoid the consumption of D-xylose and D-xylonic acid for cellular growth, both the native D-xylose and D-xylonic acid catabolic pathways were successfully blocked by disrupting four genes in *E. coli* W3110. The engineered strain

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