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Sensing of plant hydrolysate-related phenolics with an *aaeXAB::luxCDABE* bioreporter strain of *Escherichia coli*

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HIGHLIGHTS

- Successful use of previous biomarker results to develop a bioreporter strain.
- Low detection of hydrolysate-related phenolics in artificial and real samples.
- Stable response and broad specificity to phenolic acids and aldehydes.
- Rapid, dose-dependent and sensitive responses, down to 4.8 mg/L.

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ABSTRACT

A bioluminescent *Escherichia coli* bioreporter strain to detect hydrolysate related phenolics was developed by cloning the *aaeXAB* promoter from *E. coli* upstream of the *luxCDABE* genes. *E. coli* str. DH5α carrying this plasmid (pDMA3) was responsive to sub-inhibitory concentrations of plant hydrolysate-related phenolics, such as ferulic and vanillic acids, responding to these compounds at concentrations as low as 9.8 and 4.9 mg/L, respectively. Experiments with a mixture of the compounds showed similar responses as with single compound tests, with a minimum detectable concentration of 19.5 mg/L. Finally, tests using rice straw hydrolysates were conducted, with *E. coli* str. DH5α/pDMA3 showing a maximum induction of 33-fold and a minimum detectable phenolic concentration of 9.3 mg/L, based upon Folin–Ciocalteu's reagent. These results demonstrate that this bioreporter maintains its sensitivity even with hydrolysate samples and that it can be potentially applied within biofuel industries to detect phenolics present within plant hydrolysates.

1. Introduction

Plant biomass hydrolysis is an attractive process for renewable energy, particularly the generation of biofuels, such as ethanol and biohydrogen. The main reason for this is that woody plants, by dry weight, can consist of 60% or more of hemicellulose and cellulose. Hydrolysis of the biomass exposes the cellulose within the plant cell wall, thereby making it available for cellulase enzymes to cleave it to simple sugars. However, it also releases the lignin fraction which, when hydrolyzed, forms many different aromatics, such as 4-hydroxybenzoic acid and vanillin. Several of these have been shown to be inhibitory towards fermentative microbes when present at concentrations as low as 0.1–1 g/L leading to significant loss in the activity and growth of the microbes (Clark and Mackie, 1984; Ezeji et al., 2007; Lee et al., 2012; Mills et al., 2009).

In response, several groups have sought out a variety of means to remove these compounds or to mitigate their activity through detoxification, such as through vacuum evaporation (Parajo et al., 1998; Rodrigues et al., 2001). Other groups have used activated carbon (Canilha et al., 2004; Lee et al., 1999; Mussatto and Roberto, 2001), resins (de Carvalho et al., 2004), other lignin residues as an absorbent (Bjorklund et al., 2002) and even enzymatic or biological processes to reduce the inhibitory activities of these compounds(Cho et al., 2009; Okuda et al., 2008; Sakurabayashi et al., 1993). To date, however, the most common method has been to over-lime the solution to precipitate the compounds (Martinez et al., 2000; Mills et al., 2009; Persson et al., 2002).

Since the inherent activity of the compounds shows that they can have an adverse effect even at low concentrations (Ezeji et al., 2007; Mills et al., 2009), this necessitates a sensitive method for their detection. Whereas conventional physico-chemical analyses, such as HPLC and GC, offer many benefits, one main limitation

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