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Atypical ethanol production by carbon catabolite derepressed lactobacilli

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ABSTRACT

Cost effective use of lignocellulosic biomass for bio-based chemical production requires the discovery of novel strains and processes. *Lactobacillus pentosus* JH5XP5 is a carbon catabolite repression negative mutant which utilizes glucose and pentoses derived from lignocellulosic biomass in the media simultaneously. With a broad range of carbon substrates, *L. pentosus* JH5XP5 produced a significant amount of ethanol without acetate formation. The yields of ethanol were 2.0- to 2.5-fold higher than those of lactate when glucose, galactose or maltose was used either as a single carbon source or simultaneously with glucose. *L. pentosus* JH5XP5 was successfully used in an integrated process of simultaneous saccharification and mixed sugar fermentation of rice straw hydrolysate. During the fermentation, the enzyme activities for the saccharification of cellulose were not diminished. Moreover glucose, xylose, and arabinose sugars derived from rice straw hyrolysate were consumed concurrently as if a single carbon source existed and no sugars or cellulosic fiber remained after the fermentation.

1. Introduction

Currently, the production of many chemicals and energy are dependent on fossil fuels whose reserves are limited. In addition, fluctuations in oil prices necessitate changes in chemical and energy production from fossil fuel-based systems to bio-based systems (Hatti-Kaul et al., 2007). Environmental considerations also demand bio-based chemical production from sustainable feedstocks.

Bio-based production of platform chemical intermediates typically uses two different types of renewable feedstocks: starch-based biomass and lignocellulosic biomass. Although the use of lignocellulosic biomass has significant advantages, particularly by consuming existing agricultural wastes, several barriers still exist to its commercialization (Houghton et al., 2006). The price of the cellulase and the cellobiase enzyme complex that depolymerizes cellulose fiber for fermentation is higher than that for the enzymes to degrade starch biomass. Another barrier is the heterogeneity of lignocellulosic biomass composition. Thermochemically pretreated and hydrolyzed lignocellulose contains mixed sugars (hexoses and pentoses) as well as potential growth inhibitors such as furfural and phenolic compounds from lignin. Given pentoses can represent up to 50% of total sugar in a lignocellulosic hydrolysate, complete utilization of pentose sugars is crucial for economic fermentation of lignocellulosic materials. In addition, concurrent consumption of those mixed sugars is needed when considering product yield, productivity and, particularly, fermentation process design. However, the sequential utilization of sugars by microorganisms, a result of carbon catabolite repression (CCR), prevents the simultaneous utilization of sugars and makes the design of overall process difficult and inefficient. To get around the problems caused by the mixed sugar, many researchers have searched for, or engineered, microorganisms which possess pentose utilization and CCR negative (CCR^-) phenotypes (Dien et al., 2004, 2002, 2000; Ho et al., 1998; Nichols et al., 2003, 2001; Qian et al., 2003; Sibirny et al., 2003; Zhang et al., 1995). Recently, *L. brevis* was shown to lack an apparent CCR and was able to ferment pentose sugars with glucose simultaneously, albeit the ethanol yield was still relatively low (Kim et al., 2009).

Lactic acid and ethanol are promising as potential sustainable platform chemicals and fuels. Currently, ethanol is used as an energy source instead of, or mixed with, gasoline. Ethanol is also used to make ethyl lactate (EL) by esterification with lactate (Benedict et al., 2003). EL is an FDA approved organic solvent that currently is drawing much attention due to the potential applications in the food and pharmaceutical industries (Garlotta, 2001; Hirakawa et al., 1988; Nikles et al., 2001). Another important chemical from lactate is poly-lactate (PLA) (Jacobsen et al., 1999). PLA is a biodegradable plastic synthesized from lactic acid. PLA is a plastic material but is degraded in nature or *in vivo* to lactic acid which can then be further metabolized in most biological systems (Duda and Penczek, 2003; Jacobsen et al., 1999). The application of PLA is widespread from textiles and disposable utensils to drug deliv-

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ery vehicles due to its favorable properties (Datta et al., 1995; Garlotta, 2001).

Lactobacillus pentosus has been studied for the production of lactic acid and surface-active compounds from hemicellulose or lignocellulosic biomass (Portilla-Rivera et al., 2008, 2009; Zhu et al., 2007). As an effective host strain for bio-based chemical production from lignocellulosic biomass, we isolated *L. pentosus* JH5XP5 through a series of selection and enrichment procedures. *L. pentosus* JH5XP5 has a unique CCR^- phenotype showing simultaneous utilization of mixed sugars. As facultative heterofermentative lactobacilli, JH5XP5 ferments a wide range of substrates, and, interestingly, produced a significant amount of ethanol. Here, we examined the fermentation characteristics of *L. pentosus* JH5XP5 which overcome the current barriers for the cost effective utilization of lignocellulosic biomass. Enzymatic hydrolysis of cellulose fiber and simultaneous utilization of mixed sugars were integrated and a new fermentation scheme was evaluated using acid-pretreated rice straw as a carbon source.

2. Methods

2.1. Bacterial strains, culture conditions and media

L. pentosus JH5 was isolated as a contaminant from a *L. brevis* culture obtained from a national collection. Once the strain was isolated, the species identity was determined by 16S rDNA sequencing. *L. pentosus* NRRL B-227 was obtained from the Agricultural Research Service culture collection (Peoria, IL, USA). Modified MRS medium (15 g l^{-1} bactopeptone, 5.0 g l^{-1} yeast extract, 2.0 g l^{-1} ammonium citrate, 5.0 g l^{-1} sodium acetate, and 2.0 g l^{-1} dipotassium phosphate) with 20 g l^{-1} of glucose was used for the cell maintenance. In this study, glucose was not included in modified MRS unless stated. Carbon sources were prepared separately and mixed with inoculums initially. The initial pH of the medium was set at 6.0 and temperature was maintained at 37 °C. Fermentations were initiated by adding a 5% (v/v) inoculum.

2.2. Isolation of carbon catabolite repression negative (CCR^-) strains

L. pentosus JH5 was grown in 100 ml of the modified MRS with 20 g l^{-1} of xylose for growth substrate and 5 g l^{-1} of 2–deoxyglucose (2-DG) as a CCR inducer. For enrichment, 10% of the initial volume of media was used as inoculums for fresh media containing 2-DG and xylose. After five consecutive cultivations, culture broth was spread on MRS-xylose-2-DG agar plates and several colonies were isolated. Each isolated colony was inoculated and cultivated for 24 h in 100 ml of modified MRS with 10 g l^{-1} of glucose and 10 g l^{-1} of xylose. Cell growth and simultaneous utilization of glucose/xylose were monitored every 4 h. The strain was selected based on the specific cell growth rate and sugar utilization rates. Once the identity of species was determined by 16S rDNA sequencing, the selected strain was designated *L. pentosus* JH5XP5.

2.3. Fed-batch fermentation with mixed sugars

The fermentation was carried out in a BioFlo 3000 Bioreactor (New Brunswick Scientific, Edison, NJ, USA). Temperature was maintained at 37 °C and pH was controlled at 6.0 by addition of 10 N NaOH during the fermentation. Agitation was maintained at 100 rpm without aeration. Modified MRS medium and the carbohydrate(s) were prepared separately to make the final volume of 2.5 l. Initial concentration of mixed sugars were 30 g l^{-1} of glucose, 10 g l^{-1} of xylose and 10 g l^{-1} of arabinose. When sugars were depleted, a concentrated mixed-sugar solution was supplemented reaching initial concentrations of sugars.

2.4. Acid-pretreated rice straw

Acid-pretreated rice straw was prepared by BC International (now Verenium Corporation: Cambridge, MA, USA). Rice straw was cleaned, chopped, and then hemicellulose was hydrolyzed by diluted sulfuric acid. After drying, the acid-pretreated rice straw has a soil like texture properties with dark brown color (Fig. 1a). The resulting mixture contained cellulose fiber, xylose and arabinose from hemicelluloses, and phenolic compounds from lignin. The total carbohydrate content in hydrolyzed rice straw was approximately 50% (w/w) based on dry substrate mass. Remaining 50% dry mass (w/w) of acid-pretreated rice straw was ashes, mostly silica particle (Fig. 1b). Due to the cellulose fiber and lignin, resuspended acid-pretreated rice straw was a mud-like paste with high viscosity (Fig. 1b). This high viscosity limited the initial substrate concentration to 100 g-dry mass l^{-1} (172 g-wet mass l^{-1}) above which agitation could not be maintained to ensure proper mixing.

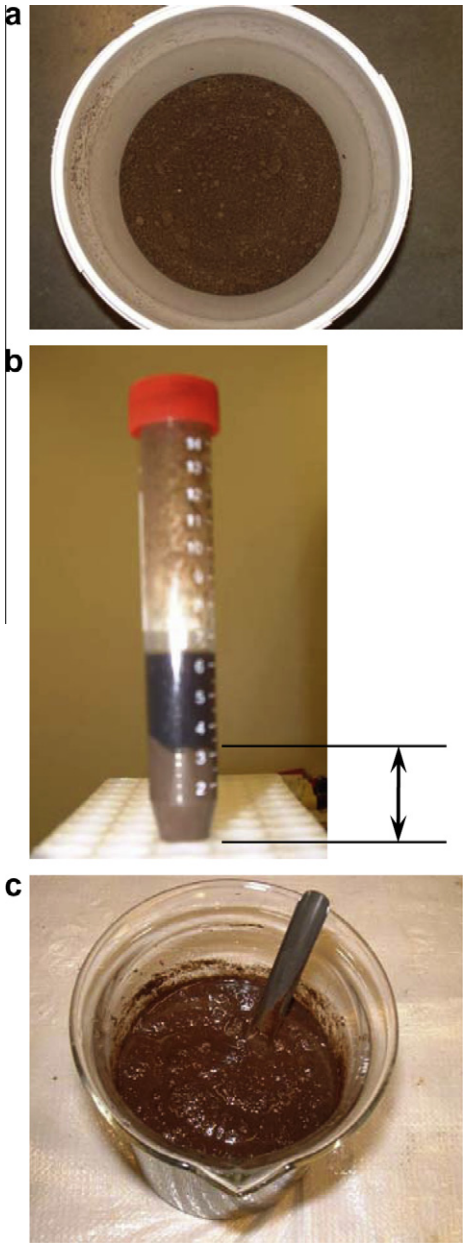

Fig. 1. Acid pretreated rice straw (a) pretreated rice straw prepared by BC International. (b) after fermentation. Volume indicated by arrow is silica beads from rice straw. (c) resuspended in the medium. The concentration is 100 g-dry mass l^{-1}.