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High-titer lactic acid production by *Lactobacillus pentosus* FL0421 from corn stover using fed-batch simultaneous saccharification and fermentation

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HIGHLIGHTS

- A LA hetero-fermentative strain was identified as *Lb. pentosus* FL0421.
- FL0421 produced LA with yields of 0.52–0.82 g/g stover from five agro stovers.
- LA titer and yield reached 92.30 g/L and 0.66 g/g stover in fed-batch SSF.
- FL0421 is a good candidate for genetic engineering for high LA titer and yield.

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ABSTRACT

Because the cost of refined sugar substrate and limit of worldwide food availability, lignocellulosic materials are attractive for use in lactic acid (LA) production. In this study, we found *Lactobacillus pentosus* strain FL0421 produced LA with high yields (0.52–0.82 g/g stover) from five NaOH-pretreated and washed agro stovers through simultaneous saccharification and fermentation (SSF). We developed a fed-batch SSF process at 37 °C and pH 6.0 using the cellulase of 30 FPU/g stover and 10 g/L yeast extract in a 5-L bioreactor to produce LA from 14% (w/w) NaOH-pretreated and washed corn stover under non-sterile condition. The LA-titer, yield and productivity reached 92.30 g/L, 0.66 g/g stover and 1.92 g/L/h, respectively; and acetic acid titer and yield reached 34.27 g/L and 0.24 g/g stover. This study presented a feasible process for LA production from agro stovers and provided a candidate strain for genetic engineering for high-titer and -yield lignocellulosic LA production.

1. Introduction

Lignocellulose is one of the most abundant renewable feedstocks and has recently attracted substantial interest for biofuel and biochemical production (Ragauskas et al., 2006). The large-scale use of lignocellulosic biomass would overcome many environmental problems and provide a cost-effective feedstock that does not compete with other food sources. Among the various forms of biomass, agro residues (corn stover, wheat straw, rice stover, sugarcane bagasse, and corncob) are easily gathered after grain harvesting, which therefore provides a source of cost-effective lignocellulosic biomass. For example, the main agro residue, corn stover, contains 37.5% cellulose, 22.4% hemicellulose, and 17.6% lignin, which can be hydrolyzed into hexose and pentose for fermentation (Zhu et al., 2007). One of these biochemicals, lactic acid (LA), is an important fermentation product owing to its great potential for use in the production of biodegradable and biocompatible polylactic acid (PLA) polymers, which provide a sustainable alternative to petroleum-derived products (Madhavan Nampoothiri et al., 2010). However, there are at least 2 major technical obstacles to the production of LA from lignocellulosic

Abbreviations: LA, lactic acid; LAB, lactic acid bacteria; SSF, simultaneous saccharification and fermentation; SHF, separate hydrolysis and fermentation; FPU, filter paper unit.

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residues: biomass pretreatment and hydrolysis, and efficient fermentation of pentose from the hydrolysates into LA. Pretreatments are employed to reduce the effects of biomass recalcitrance and enhance the accessibility of cell wall polysaccharides to enzymes, thus making the conversion rates of polysaccharides to monomeric sugars more efficient (Wyman et al., 2005). The pretreatments used with agro stovers include physical pretreatments (size reduction), physicochemical pretreatments (liquid hot water, steam explosion, and ammonia fiber explosion), chemical pretreatments (acid, alkaline, alkaline/oxidative, wet oxidation, and ozonolysis), and biological pretreatments (Talebnia et al., 2010). Alkaline pretreatments (lime, wet-oxidation, and soaking with ammonia) are effective in delignification, resulting in solid residues of cellulose fibers and some hemicellulose (Kim and Lee, 2007; Varga et al., 2003). However, these pretreatments generate aromatic compounds (phenolics) and acetyl derivatives (Toquero and Bolado, 2014), which affect downstream hydrolysis and fermentation steps LA fermentation ability of lactic acid bacteria (LAB) was reported to be inhibited using pretreated agro stovers without detoxification, while the opposite phenomenon was observed using these substrates with washing or bio-detoxification (Hu et al., 2015; Zhao et al., 2013). In addition to the need to overcome inhibitor repression, high-level LA fermentation requires efficient fermentation process with lignocellulosic biomass. Biofuel production and biochemical fermentation based on lignocellulosic biomass can be divided into separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) steps (Olofsson et al., 2008). SSF is a favored method for LA production, since high solid loading can increase LA titer and the glucose released following cellulase activity during SSF is rapidly converted to LA by the fermenting microorganism, which minimizes end-product inhibition of cellulase caused by glucose and cellobiose accumulation (John et al., 2009). During SSF, another abundant sugar (xylose) was released from lignocelluloses and this pentose can be fermented into LA via the pentose phosphate pathway (a homo-fermentation pathway) and the phosphoketolase pathway (a hetero-fermentation pathway). Recently, *Bacillus coagulans* was demonstrated as suitable for LA production due to its thermophilic growth characteristics, strong ability for pentose homo-fermentation, and robust tolerance of inhibitors (Bischoff et al., 2010; Hu et al., 2015; Patel et al., 2006; Zhao et al., 2013). High-titer LA production was also obtained using the *Pediococcus acidilactici* DQ2 and sulfuric acid-pretreated corn stover, which was accompanied by biological detoxification, although this strain cannot utilize xylose (Zhao et al., 2013). Several other LABs, such as *Lactobacillus pentosus* (Bustos et al., 2005) and *Lactobacillus brevis* (Chaillou et al., 1998), are reported to ferment xylose via the phosphoketolase pathway.

Although efforts are being made to achieve high-yield and high-titer LA production from lignocellulosic biomass, a feasible method to ferment agro residues needs to be developed and a robust LA-producing bacterium needs to be identified. Here, we developed a fed-batch SSF process for LA fermentation using corn stover as carbon source by *Lb. pentosus* FL0421, and found this strain produced LA with 92.30 g/L and 0.66 g/g stover; and produced acetic acid with 34.27 g/L and 0.24 g/g stover under non-sterile condition from NaOH-pretreated and washed corn stover. Thus, strain FL0421 was a good candidate for genetic engineering for high-yield and titer lignocellulosic LA production.

2. Materials and methods

2.1. Raw materials, enzymes, and medium

The lignocellulosic biomasses were cleaned, dried, and sieved using a 100-mesh screen and pretreated at 75 °C for 3 h with 5% NaOH, using a 20% (w/w) loading. Part of the resulting slurry was washed with water until the pH decreased to 8.0, and then filtered to a moisture content of 20% (w/w). National Renewable Energy Laboratory (NREL) analytical methods were followed to determine raw and pretreated material composition in terms of structural carbohydrates and lignin (Sluiter et al., 2010). The cellulase used in this study was Cellic CTec2 (Novozymes, Denmark), which contains cellulase, β-glucosidase, and xylanase activities. YEX medium (10 g/L xylose and 10 g/L yeast extract) was used for seed culturing.

2.2. Genomic characterization of Lb. pentosus FL0421

Strain FL0421 was isolated and maintained by the Fermentation Engineering Laboratory at Huazhong Agricultural University. The genomic DNA of strain FL0421 was extracted using the Axygen Genomic DNA Miniprep Kit (Axygen, Hangzhou, China). The 16S ribosomal RNA gene sequence was amplified by PCR using the universal primers 27f and 1492r (Lane, 1991). PCR products were analyzed by agarose gel electrophoresis, purified using the Axygen PCR Clean-up Kit (Axygen, Hangzhou, China), and sequenced by Invitrogen (Shanghai, China). The 16S rDNA sequence of strain FL0421 was deposited in GenBank [GenBank:KT215616]. Genomic DNA was fragmented to ~300 bp using a Bioruptor UCD-200 (Diagenode). DNA fragments were repaired, modified enzymatically to have A overhangs, ligated with sequencing adaptors, and amplified to obtain the final sequencing library. Gene assembly was performed using SOAPdenovo (Li et al., 2010). The assembled, sequenced DNA was checked using known DNA fragments for validation purposes. The ORFs were predicted separately using Glimmer (Delcher et al., 2007).

2.3. Fermentation of pure sugars to produce LA in flasks

Lb. pentosus FL0421 seed culture was cultured in YEX medium at pH 6.0 and 37 °C for 24 h. Fermentations were performed in glucose, xylose, or cellobiose media (50 g sugar/L, 10 g yeast extract/L) at 37 °C for 48 h, or in glucose medium at 32 °C, 37 °C, 42 °C, or 47 °C for 48 h using 1% (v/v) seed culture in flasks, with a pH of 6.0 by feeding of 12 M NaOH solution every 6 h. To test the effect of the pH, fermentations were performed in glucose medium with initial pH values of 5.0, 5.5, 6.0, or 6.5, and these pH values were adjusted every 6 h by feeding of 12 M NaOH solution. The effect of nitrogen sources on LA fermentation were tested in glucose medium with 10 g/L yeast extract, tryptone, beef extract, acid hydrolyzed casein, or corn steep powder at 37 °C and pH 6.0. Residual sugars, LA, and acetic acid were detected using high-performance liquid chromatography (HPLC). All experiments were conducted in triplicate.

2.4. SSF of LA from NaOH-pretreated lignocellulosic materials

2.4.1. LA production by fermentation of different lignocellulosic materials

Five percent (w/w) NaOH-pretreated and washed lignocellulosic materials (miscanthus stover, wheat stover, corn stover, corncob, or rice stover), 10 g/L yeast extract, and Cellic CTec2 (at a concentration of 30 FPU/g stover) were inoculated with seed culture (10% of the fermentation volume) in 100-mL fermentation cultures in a 150-mL flask. Fermentations were performed at 37 °C for 72 h, and the pH was adjusted to 6.0 every 6 h using 12 M NaOH solution. After fermentation, LA and acetic acid in the culture were detected by HPLC, and the yields were calculated. All experiments were conducted in triplicate.