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Characterization of inulin hydrolyzing enzyme(s) in commercial glucoamylases and its application in lactic acid production from Jerusalem artichoke tubers (Jat)

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HIGHLIGHTS

- Glucoamylase was identified as an inulin hydrolyzing enzyme.
- Commercial glucoamylase was used as inulinase for Jat hydrolysis.
- High lactic acid titer and yield were obtained in the SSF of Jat.

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ABSTRACT

A high inulinase activity was found in three commercially available glucoamylase enzymes. Its origin was investigated and two proteins in the commercial glucoamylases were identified as the potential enzymes showing inulinase activity. One of the commercial glucoamylases, GA-L New from Genencor, was used for Jerusalem artichoke tubers (Jat) hydrolysis and a high hydrolysis yield of fructose was obtained. The simultaneous saccharification and lactic acid fermentation (SSF) of Jat was carried out using GA-L New as the inulinase and *Pediococcus acidilactici* DQ2 as the fermenting strain. A high lactic acid titer, yield, and productivity of 111.5 g/L, 0.46 g/g DM, and 1.55 g/L/h, respectively, were obtained within 72 h. The enzyme cost using the commercial glucoamylase as inulinase was compared to that using the typical inulinase and a large profit margin was identified. The results provided a practical way of Jat application for lactic acid production using cheap commercial glucoamylase enzyme.

1. Introduction

Although current industrial production of lactic acid overwhelmingly uses starch feedstock, future need of lactic acid for biodegradable polylactide acid (PLA) certainly requires various feedstock for replacing petroleum based polymer materials, other than food-based starch only (Nampoothiri et al., 2010; Gao et al., 2011). Among many feedstock options, Jerusalem artichoke tubers (Jat) is a promising one because of its desirable growing traits such as cold and drought tolerance, wind and sand resistance, saline tolerance, strong fecundity, and high pest and disease resistant (Li and Chan-Halbrendt, 2009). Jat contains high carbohydrates (20%, w/w), in which 70–90% (w/w) are inulin, a polysaccharide composed of fructose unit chains and minor glucose unit (Szambelan et al., 2005). Jat had been widely planted in North America, Europe, and Asia countries besides its advantages (Chi et al., 2011; Li et al., 2013). In recent few years in China, Jerusalem artichoke started the large scale cultivation in the drought and coast regions as well as in the polluted regions by oil drilling or coal mining to replace the corn and wheat as feedstock of fermentation industry for production of ethanol, butanol, sugar alcohols and organic acids (Li et al., 2013). Although its total quantity is not comparable to lignocellulose biomass, Jat could be processed easily using the available technologies, or even easier than starch processing (Chi et al., 2011; Li et al., 2013), while lignocellulose processing is still a technology challenge and only used in the small scale demonstration stage (John et al., 2009; Zhao et al., 2013). Therefore, Jat has been considered as a readily available feedstock at present and a promising one in the future for industrial lactic acid production.

Jat can be hydrolyzed into fructose and glucose by inulinase enzyme or acid catalyst (Chi et al., 2011). Inulinase includes two enzymes, endo-inulinase (EC 3.2.1.7) cleaving middle sites of inulin oligos into even smaller oligos, and exo-inulinase (EC 3.2.1.80) cleaving fructose or glucose from the ends of inulin oligos. The recent studies showed that invertase (EC 3.2.1.26) from yeast and fungi also demonstrated strong inulin hydrolysis property (Wang

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and Li, 2013; Guo et al., 2013). However, these inulinase enzymes are expensive and not available as industrial enzyme for Jat hydrolysis at large scale (Sigma Product ID 16285, Novozym 960, $350 for 250 mL). Therefore, the cost reduction of inulinase is a key step for industrial application of Jat for production of biofuels and biochemicals.

In this study, a high inulinase activity was found from a typical commercial glucoamylase GA-L New of Genencor produced by *Aspergillus niger* fermentation. The inulin hydrolyzing enzyme(s) in glucoamylase GA-L New were characterized and the results showed that the activity was from glucoamylase itself, other than other protein components in this mixed commercial enzyme, although the catalytic properties of the two enzymes were different (glucoamylase cleaves 1,4-α-D-glucosidic linkage, while inulinase cleaves 1,2-β-D-fructosidic linkage). Then the glucoamylase was used for Jat hydrolysis and a high yield of fructose from Jat was obtained. The simultaneous saccharification and lactic acid fermentation (SSF) of Jat was carried out using glucoamylase GA-L New as the inulinase and *Pediococcus acidilactici* DQ2 as the lactic acid fermenting strain. A high lactic acid titer, yield, and productivity of 111.5 g/L, 0.46 g/g DM, and 1.55 g/L/h, respectively, were obtained within 72 h. The results provided a practical way of Jat application for lactic acid production using cheap commercial glucoamylase enzyme.

2. Methods

2.1. Enzyme and strain

The commercial glucoamylase enzymes include glucoamylase GA-L New from DuPont Genencor Science, Wuxi, China, (http://biosciences.dupont.com/duponttm-genencorr-science/), amyloglucosidase A107823 from Aladdin Industrial Co., Ltd., Shanghai, China (http://www.aladdin-e.com), and a local product, glucoamylase YY0515 from Shanghai Yuanye Biological Technology Co., Ltd., Shanghai, China (www.shyuanye.com). All these glucoamylases were produced using *A. niger* as the production strain.

The lactic acid fermentation strain, *P. acidilactici* DQ2 (CGMCC 7471), was isolated in our previous study (Zhao et al., 2013) and stored in China General Microbial Collection Center (CGMCC), Beijing, China. The simplified MRS medium contained 10 g/L of peptone, 5 g/L of yeast extract, 1 g/L of $(NH_4)_2SO_4$, 2 g/L of KH_2PO_4, 0.58 g/L of $MgSO_4{\cdot}7H_2O$, 0.25 g/L of $MnSO_4{\cdot}4H_2O$, 5 g/L of sodium acetate, and 2 g/L of diammonium hydrogen citrate. The medium was autoclaved at 115 °C for 20 min. One vial of *P. acidilactici* DQ2 was inoculated into simplified MRS broth and cultured at 42 °C, 150 rpm for 12 h as inoculum seeds with the inoculum was 10%. pH was maintained by adding 60 g of $CaCO_3$ per 100 g of glucose. All experiments were repeated twice.

2.2. Raw materials chemicals

Jerusalem artichoke tubers (Jat) were purchased from Xinnong Technology Co., Ltd. (Qinghai, China). The Jat was washed, sliced, and dried at 60 °C until constant weight, then ground to fine powder. The composition of the dried Jat was determined using the same method by Kaldy et al. (1980). One gram of the dried Jat composed of 0.75 g inulin (glucose and fructose), 0.03 g cellulose, 0.02 g hemicellulose, 0.02 g protein, 0.01 g fat, and 0.05 g water.

The inulin powder was purchased from Langrui Fine Chemical Co., Ltd. (Shanghai, China). One gram of inulin powder was composed of 0.78 g of fructose and 0.23 g of glucose obtained from 1 g of inulin (dry base) according to the method by Gao et al. (2010).

All chemicals including peptone, yeast extract, $(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4{\cdot}7H_2O$, $MnSO_4{\cdot}4H_2O$, sodium acetate, diammonium hydrogen citrate, were purchased from Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Albumin, Fraction V (CAS Number: PB10056, 69 kDa) was purchased from Beijing Probe bioscience Co., Ltd. (Shanghai, China). Coomassie Brilliant Blue G-250 (CAS Number: 0006192525), Coomassie Brilliant Blue R-250 (CAS Number: 0006104592) was obtained from Sigma–Aldrich.

2.3. Inulinase assay

The inulinase activity in the glucoamylase enzymes was measured using the modified method of Jing et al. (2003). 100 μL of the crude enzyme solution was mixing with 900 μL of 2% inulin powder in a 0.1 M sodium acetate buffer (pH 4.5). The solution was incubated at 60 °C for 10 min, then mixed with 3 mL of 3,5-dinitrosalicylic acid (DNS) solution. The mixture was placed in an ice bath, heated for 5 min at 100 °C to deactivate the enzyme, and the reducing sugars were assayed by observing the change of absorbance at 540 nm. One unit (U) of inulinase activity was defined as the amount of enzymes that produced 1 μmol of reducing sugars per minute from inulin under the assay conditions.

The protein concentration of the three commercially available glucoamylases was measured using Bradford method.

2.4. Purification of glucoamylase proteins

The crude glucoamylase GA-L New solution was filtered through a 0.22 μm membrane and purified using a chromatography column packed with Sephadex G-100 (Life Sciences, Uppsala, Sweden). The column was pre-equilibrated and eluted with citrate buffer (0.1 M, pH 5.0) at a flow rate of 1.0 mL/min and the eluent protein monitored at 280 nm. The SDS–PAGE electrophoresis of the fractions were performed with 12% polyacrylamide denaturing SDS gel and stained with Coomassie Brilliant Blue R-250. The inulinase activity of the fractions was measured using the method above in Section 2.5.

2.5. SSF bioreactor and its operation

SSF operation was carried out in 5-L bioreactor with pH and temperature control. A specially designed helical ribbon impeller was installed for well mixing at high Jat solids loading as described by Zhang et al. (2010) and Zhao et al. (2013). The SSF operation continued to 72 h and samples were withdrawn at regular intervals.

2.6. Analytical methods

Glucose, fructose and lactic acid was analyzed using HPLC (LC-20AD, refractive index detector RID-10A, Shimadzu, Japan) with a Bio-Rad Aminex HPX-87H column at 65 °C. The mobile phase setup 5 mM H_2SO_4 with the rate of 0.6 mL/min. All samples were diluted and filtered before analysis.

3. Results and discussion

3.1. Characterization of inulin hydrolyzing enzyme(s) in commercially available glucoamylases

To find a cheap inulin hydrolyzing enzyme for replacement of expensive inulinase, various available industrial hydrolase enzymes were screened, and a high inulinase activity was found from a typical glucoamylase GA-L New, a product of Genencor widely used in starch processing industry (www.genenco.cn). The inulinase activity data of glucoamylase GA-L New were using inulin powder as substrate in Table 1, indicates that the inulinase activity of