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Enhanced Inulin Saccharification by Self-Produced Inulinase from a Newly Isolated *Penicillium* sp. and its Application in D-Lactic Acid Production

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Abstract In order to find an alternative for commercial inulinase, a strain XL01 identified as *Penicillium* sp. was screened for inulinase production. The broth after cultivated was centrifuged, filtered, and used as crude enzyme for the following saccharification. At pH 5.0 and 50 °C, the crude enzyme released 84.9 g/L fructose and 20.7 g/L glucose from 120 g/L inulin in 72 h. In addition, simultaneous saccharification and fermentation of chicory flour for D-lactic acid production was carried out using the self-produced crude inulinase and *Lactobacillus bulgaricus* CGMCC 1.6970. A high D-lactic acid titer and productivity of 122.0 g/L and 1.69 g/(L h) was achieved from 120 g/L chicory flour in 72 h. The simplicity for inulinase production and the high efficiency for D-lactic acid fermentation provide a perspective and profitable industrial biotechnology for utilization of the inulin-rich biomass.

Keywords Inulinase · Chicory flour · *Penicillium* sp. · D-Lactic acid · Simultaneous saccharification and fermentation

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Introduction

Nowadays, renewable materials, especially lignocellulosic biomass, are considered as an attractive feedstock for the production of biochemicals due to their abundance and low cost. However, complicated pretreatment process must be employed for sugar hydrolysis, which would generate various inhibitory compounds and require detoxification before fermentation [1]. Compared with structural polysaccharides, storage polysaccharides can be easily hydrolyzed and fermented. Inulin is just this kind of reserve carbohydrate existed in the roots and tubers of Jerusalem artichoke, chicory, dahlia, and burdock. In China, many regions have recently initiated the large-scale cultivation of Jerusalem artichoke and chicory because these plants grow well in poor soil, show resistance to plant diseases, and do not interfere with the food chain [2, 3]. Inulin consists of linear chains of β-2,1-linked D-fructofuranose residues terminated by a glucose residue through a sucrose-type linkage at the reducing end. It can be hydrolyzed into fructose by the fructofuranosyl hydrolases, including the most common inulinase, levanase, and certain invertase [4–6]. Fructose is not only a safe alternative sweetener in the food or beverage industry but also a versatile carbon source that can be converted into different kinds of biochemicals, such as bioethanol, single cell oil, 2,3-butanediol, and L-lactic acid etc. [3, 7–10].

Because of the lack of strains possessing ability to both inulin hydrolysis and fructose conversion into value-added chemicals, commercial inulinase is often added for inulin prehydrolysis. However, the commercial enzymes are expensive and not suitable for large-scale inulin hydrolysis (Sigma Product ID I6285, Novozym 960 from *Aspergillus niger*, $350 for 250 mL). Dao et al. found a commercial glucoamylase (GA-L New, Genencor, Wuxi, China) that exhibited high inulinase activity and used it for inulin hydrolysis and fermentation [4]. However, its optimum pH for inulin hydrolysis is 4.0, which do not match with the simultaneous saccharification and fermentation (SSF) process because most microbes preferred near-neutral pH environment. Therefore, easy production of appropriate and effective inulinase on a relatively large scale and at low cost is of significant importance for inulin industry. Currently, microbial production of inulinase has been proposed as the most promising approach for industrial production of inulinase. Inulinases are encountered in fungi, yeasts, and bacteria. Among them, *Aspergillus* sp., *Penicillium* sp., and *Kluyveromyces* sp. are apparently the most reported strains. It was reported that the production of inulinase from *A. ficuum* JNSP5-06 reached 25 IU/mL after 5 days of fermentation [11]. Mansouri et al. reported that the produced inulinase reached 47.7 IU/mL by a newly isolated of *P. subrubescens* [12]. Strategies for improvement of inulinase production had also been investigated. A maximum inulinase activity of 728 IU/mL was obtained from *K. marxianus* in exponential fed-batch mode [13]. Although scholars had widely studied inulinase production, most of the literatures only focused on improvement of inulinase activity and lacked application in inulin hydrolysis. It is important to identify the produced inulinase meeting the industrial standards, such as matched reaction conditions with SSF, vigorous and stable activities, and excellent substrate and product tolerance.

D-Lactic acid is a value-added chemical and important building block, which can be converted into multiple useful chemicals such as lactide, in particular [14]. The cyclic lactides are then polymerized to polylactic acid (PLA), a kind of promising biodegradable and biocompatible polymer. As poly DL-lactic acid (PDLLA), based on polymerization of D- and L-lactic acid, is known to show superior thermal stability to each homopolymer alone, which

creates a huge market demand for D-lactic acid. In our previous study, we used the commercial inulinase for inulin saccharification and then fermented to D-lactic acid by *Lactobacillus bulgaricus* CGMCC 1.6970 [15]. In this study, the inulinase was biosynthesized by a newly isolated *Penicillium* sp. strain to replace the commercial one. The culture conditions and fermentation process of *Penicillium* sp. were optimized for inulinase production. Then, in order to compare with the commercial inulinase, the home-grown inulinase was utilized for inulin saccharification and a higher concentration of monosaccharide was obtained. In addition, SSF was carried out using the home-grown inulinase and *L. bulgaricus* CGMCC 1.6970 for high-titer D-lactic acid production from chicory flour. The results provided a practical way of bio-chemical production from inulin-rich biomass at low cost and high convenience.

Materials and Methods

Materials

The chicory flour from chicory tuber and inulin were supplied by Qinghai Weide Biotech Co., Ltd. (Qinghai, China). Yeast extract was purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA). Bacteriological peptone was purchased from Oxoid (Basingstoke, Hampshire, England). Beef extract was purchased from Aoboxing Biotech Co., Ltd. (Beijing, China). All other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Screening for Inulinase-Producing Strains

The soil samples near the roots of Jerusalem artichoke in Jiangsu province were used as natural isolation sources. After multiplication enrichment culture and serial dilution, the soil samples were spread on agar plates and incubated at 30 °C for 7 days to obtain enough colonies. Medium for aimed strain enrichment comprised (g/L) inulin 20, $NaNO_3$ 2, $MgSO_4 \cdot 7H_2O$ 1, KH_2PO_4 0.5, and $FeSO_4 \cdot 7H_2O$ 0.1. Agar was added (1.8% [*w*/*v*]) for the preparation of the agar plates. Each microbial isolate was previously transferred to 30 mL of seed medium, which comprised (g/L) glucose 5, bacteriological peptone 5, beef extract 3, and NaCl 5, in a 150-mL Erlenmeyer flask and cultivated at 30 °C in a temperature-controlled shaker (MAXQ 4000; Thermo SCIENTIFIC, Waltham, Massachusetts, USA) for 24 h by shaking at 200 rpm. After that, 2% (*v*/*v*) of each inoculum was incubated to a 250-mL flask containing 50 mL of inulinase production medium for re-screening. The inulinase production medium comprised (g/L) inulin 20, beef extract 20, $(NH_4)_2SO_4$ 5, NaCl 5, and $MgSO_4 \cdot 7H_2O$ 0.5, pH 5.0. After cultivated at 200 rpm and 30 °C for 7 days, the cultures were centrifuged and the supernatants were filtered through a 0.22-μm Millipore filter, which was used as the crude enzyme to determine the inulinase activities. The activity assay method was described in our previous study [15]. One unit (IU) of inulinase activity was defined as the amount of enzyme that liberated 1 μmol of fructose equivalents per minute from inulin under the assay conditions.

18S rDNA Gene Sequencing and Analysis

Genomic DNA was isolated according to the protocol of EZgene™ Fungal gDNA Miniprep Kit (Biomega, Guangzhou, China). Universal fungal consensus primers, EF3 (5′-TCCT

CTAAATGACCAAGTTTG-3′) and EF4 (5′-GGAAGGGRTGTATTTATTAG-3′), were used for the PCR amplification of 18S rDNA gene [16]. The PCR-amplified product was cloned into pEASY-Blunt cloning vector (TransGen Biotech, Beijing, China) and sequenced by BGI Tech. (Shanghai, China). The sequence was compared to sequences from the GenBank by Blastn.

Effect of Nitrogen and Carbon Sources on Inulinase Production

Five kinds of nitrogen sources, including tryptone, yeast extract, beef extract, bacteriological peptone, and soya peptone, were selected to seek out the best for inulinase production. The media comprised (g/L) inulin 15, one kind of nitrogen sources 15, $(NH_4)_2SO_4$ 5, NaCl 5, and $MgSO_4 \cdot 7H_2O$ 0.5. After cultivated at 200 rpm and 30 °C for 6 days, the produced inulinase activities were measured. Then, concentration of the best nitrogen source was further optimized from 10 to 30 g/L using the above culture conditions. Selection of carbon sources was similar. Five kinds of carbon sources, including inulin, sucrose, glucose, starch, and fructose, were chosen as candidates. Their effects on inulinase production were investigated by incorporating 15 g/L one kind of carbon source and 20 g/L beef extract as nitrogen source. The inulinase activities were determined after 6 days to screen the optimal carbon source, and concentration of the best carbon source was also further optimized from 10 to 30 g/L.

Effects of Temperature and pH on Inulin Saccharification

The crude enzyme was used for inulin saccharification. To determine the optimum temperature of crude inulinase, reactions were performed at various temperatures (40–80 °C) in sodium acetate buffer (pH 4.8). The optimal pH for enzyme activity was investigated in the range from 3.5 to 7.0 using citrate phosphate buffer at 55 °C. Other conditions were the same as the standard assay. One hundred twenty grams per liter inulin was hydrolyzed at 50 °C, pH 5.0 in sodium acetate buffer, and 80 rpm for 72 h with an inulinase dosage of 30 IU/g inulin. Every 12 h, 1 mL of the reaction mixture was taken and incubated at 100 °C for 5 min to inactivate the enzyme for sugar analysis.

Simultaneous Saccharification and Fermentation

L. bulgaricus CGMCC 1.6970 inoculum was prepared as our previous report [15]. SSF process for D-lactic acid production was performed in 250-mL Erlenmeyer flasks containing 100 mL of medium at 42 °C and pH 5.2 without shaking. The fermentation medium contained (g/L) chicory flour 120, peptone 10, beef extract 10, yeast extract 5, K_2HPO_4 2, CH_3COONa 2, $MgSO_4 \cdot 7H_2O$ 0.58, $MnSO_4 \cdot H_2O$ 0.18, ammonium citrate dibasic ($C_6H_{14}N_2O_7$) 2, and $CaCO_3$ 80. The dosage of crude enzyme was 30 IU/g chicory flour. Samples were collected periodically to determine the biomass, the concentration of sugars, and the produced D-lactic acid.

Analytical Methods

The cell density was monitored by reading the absorbance of culture broth samples at 600 nm, using an ultraviolet spectrophotometer (Spectrumlab752s; LengGuang Tech, Shanghai, China). The concentrations of glucose, fructose, sucrose, and fructooligosaccharide (FOS) were assayed using high-performance anion exchange liquid chromatography (HPAEC; DIONEX ICS-5000$^+$SP, Thermo SCIENTIFIC) with a CarboPac™ PA10 column (250 × 2 mm) at

30 °C. Gradient elution was applied using three solvents: water, 200 mM NaOH solution, and 500 mM NaAc solution, as described by our previous studies [17]. D-Lactic acid was assayed by high-performance liquid chromatography (HPLC; Agilent Technologies 1200 series, Waldbronn, Germany) using a Bio-Rad Aminex HPX-87H column (300 × 7.8 mm) and a refractive index detector. HPLC was performed with a mobile phase of 5 mM H_2SO_4 at a flow rate of 0.6 mL/min. The column temperature was maintained at 55 °C [15].

Results and Discussion

Isolation and Identification of an Inulinase-Producing Strain

In total, 61 strains were isolated from the agar plates that contained inulin as the sole carbon source. These isolates were further screened based on their inulinase activities. Among them, eight strains showed distinct positive results and the highest inulinase activity was 13.4 IU/mL. Taxonomic characterization of the best performing strain was carried out by 18S rDNA sequence. A fragment of 1.5 kb was amplified, sequenced, and compared to sequences in GenBank database. It displayed above 99% identity with sequences of several *Penicillium* sp. strains. Thus, this strain was identified as *Penicillium* sp., named *Penicillium* sp. XL01 and registered (catalog no. CCTCC M20160355) in the China Center for Type Culture Collection (CCTCC, Wuhan, China). Up to now, many studies on inulinases from *Penicillium* sp. had been reported, which mainly aimed at improving the inulinase activities by different strategies or gene cloning and heterogenous expression [18–20]. In this study, we focused on the saccharification of inulin and application in D-lactic acid production.

Optimization of Inulinase Production Conditions by *Penicillium* sp. XL01

Firstly, effect of nitrogen source on inulinase production was investigated. Previous studies reported that various nitrogen sources, including peptone, yeast extract, and beef extract [21], could be the best nitrogen source for inulinase production in different microbes. In this study, five different nitrogen sources were selected to determine their effects on inulinase production of *Penicillium* sp. XL01. The results showed that beef extract was the best nitrogen source, giving an inulinase activity of 30.5 IU/mL (Fig. 1a). Effect of beef extract concentration was further studied. Figure 1b showed that beef extract at 20 and 25 g/L exhibited higher activity, and 20 g/L was selected for all other experiments. Effect of carbon source on inulinase production was next investigated. In previous studies, inulin or sucrose was often employed as the preferred carbon source [21]. For *Penicillium* sp. XL01, it is obvious that inulin was the best choice, giving an inulinase activity of 33.0 IU/mL (Fig. 1c). Then, the effect of inulin concentration on inulinase production was carried out. The results showed that 20 g/L was the optimal inulin concentration, and the resultant inulinase activity increased to 38.7 IU/mL (Fig. 1d).

Time course of inulinase production by *Penicillium* sp. XL01 was investigated under the above optimum conditions. In Fig. 2, the inulinase activity increased fast during the first 7 days and then slightly. The obtained inulinase activity was 46.2 IU/mL at the seventh day. Currently, *Aspergillus* species are the predominant inulinase producers, including the commercial inulinase from Sigma, which was produced by *A. niger*. In order to assess the ability to hydrolyze inulin, the inulinase produced by *Penicillium* sp. XL01 was compared with the commercial inulinase in the following experiments.

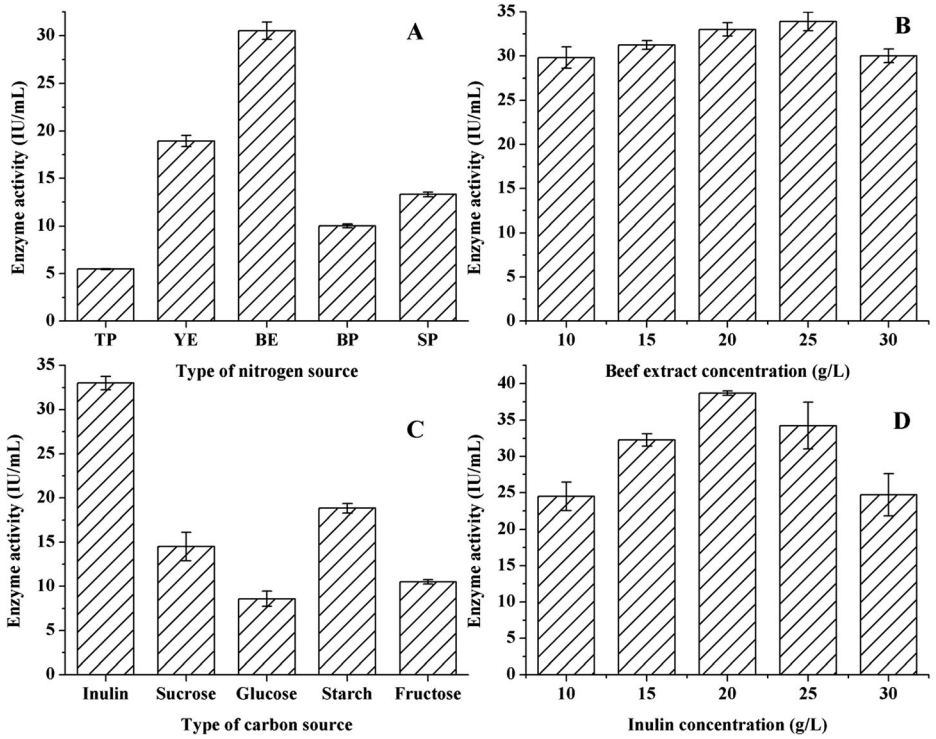

Fig. 1 Effects of nitrogen and carbon sources on inulinase production. (All conditions not listed in the figure were as follows: **a**, **b**, 15 g/L inulin; five nitrogen sources, tryptone (TP), yeast extract (YE), beef extract (BE), bacteriological peptone (BP), and soya peptone (SP). **c**, **d**, 20 g/L beef extract. For **a**, **b**, **c**, and **d**, inoculum volume, 1.5% (*v/v*); fermentation time, 6 days; shaker speed, 200 rpm; temperature, 30 °C; and pH, 5.0). All experiments were performed in triplicate

Saccharification of Inulin by Inulinase from *Penicillium* sp. XL01

Considering the operation simplicity and cost, crude enzyme was used for inulin saccharification. First, effect of temperature and pH on enzyme activity and stability was determined. For most inulinases from fungi, the optimum temperature was 40–60 °C, especially 50–55 °C [21]. However, in the present study, the optimum temperature was 65 °C (Fig. 3a), higher than the commercial one (Xu et al., 2016a). Generally, a relatively high temperature was favorable in the industrial application for avoiding microbial contamination and permitting high sugar concentrations. Thermal stability of inulinase was also studied. When incubated at 50 °C for 48 h, 86% of the initial activity was kept, while incubated above 60 °C, the activity lost rapidly, and only about 40% of the initial activity was kept after 0.5 h. Figure 3b exhibited that the inulinase preferred weak acidic conditions, and the maximum activity was obtained at pH 5.0. It is worth noting that the optimum pH (pH 5.0) is close to the optimum pH for D-lactic acid production (pH 5.2) by *L. bulgaricus* CGMCC 1.6970 [15]. After incubated at various pH values for 2 h, over 92% of the maximum activity was retained in a pH range of 4.0–6.0, and 87% of the maximum activity was retained at pH values of 3.0 and 7.0. These results indicated that the inulinase produced by *Penicillium* sp. XL01 was very stable at appropriate pH values.

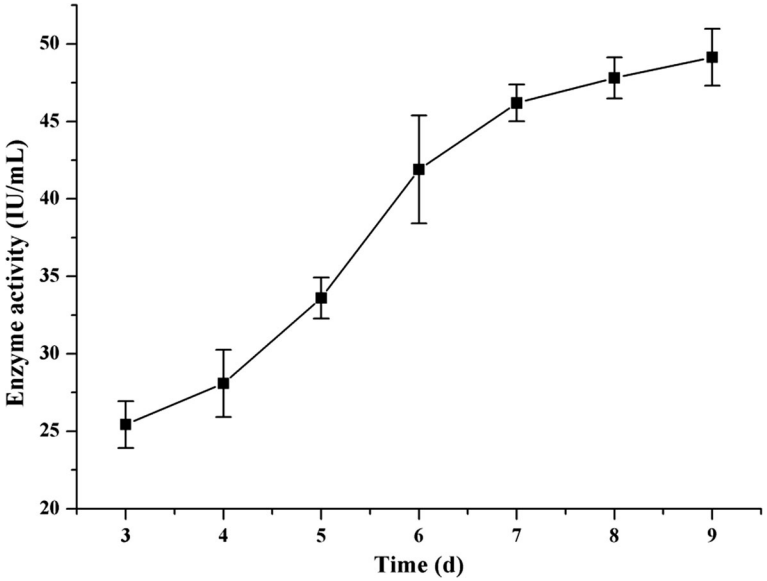

Fig. 2 Time course of inulinase production by *Penicillium* sp. XL01. (All conditions not listed in the figure were as follows: carbon source, 20 g/L inulin; nitrogen source, 20 g/L beef extract; inoculum volume, 1.5% (*v/v*); shaker speed, 200 rpm; temperature, 30 °C; and pH, 5.0). The experiment was performed in triplicate

Saccharification of inulin by inulinase from *Penicillium* sp. XL01was carried out at 50 °C, pH 5.0. Figure 4 showed the distribution of soluble sugars in the liquid at different times. The FOS observed at 0 h was partly due to the partial degradation of inulin as the samples were incubated at 100 °C to inactivate the inulinase before analysis [17]. Both the inulin and FOS were hydrolyzed quickly, and the concentration of FOS was below the detection limit after 24 h. Meanwhile, a significant increase of fructose and glucose was observed. Approximately 84.9 g/L fructose and 20.7 g/L glucose were detected after 72 h. Compared with the

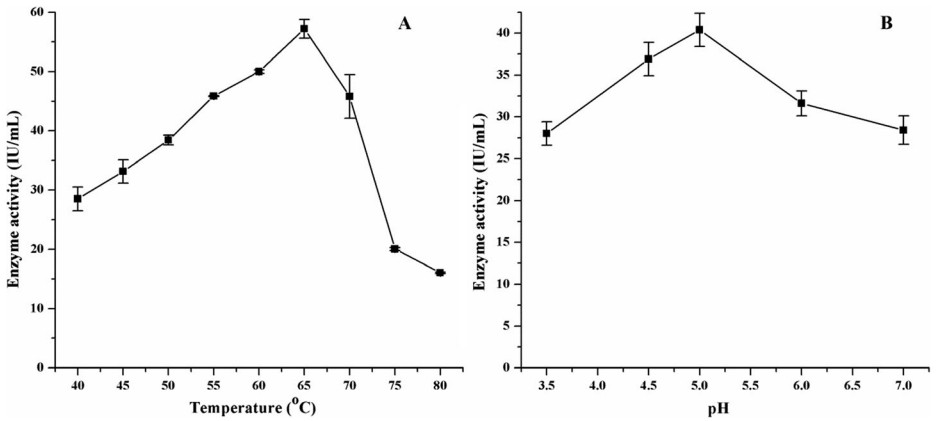

Fig. 3 Effects of temperature and pH on inulinase activity. All conditions not listed in the figure were as follows: **a** sodium acetate buffer (pH 4.8); **b** citric phosphate buffer (pH 3.5–7.0); temperature, 55 °C; **a**, **b**, enzyme, crude enzyme of the seventh day; substrate solution, 5% (*w/w*) inulin; reaction time, 10 min). All experiments were performed in triplicate

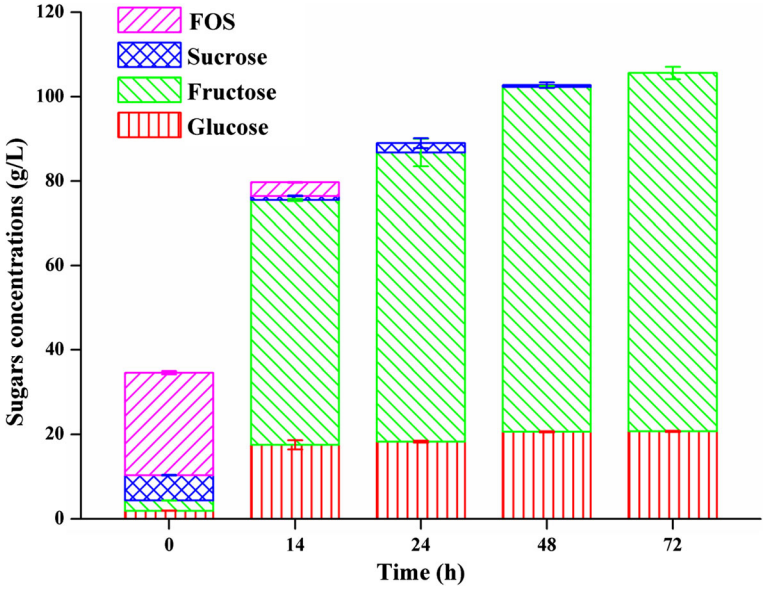

Fig. 4 Time course of inulin saccharification by inulinase from *Penicillium* sp. XL01. (All conditions not listed in the figure were as follows: inulin, 120 g/L; inulinase dosage, 30 IU/g inulin; temperature, 50 °C; and sodium acetate buffer, pH 5.0). All experiments were performed in triplicate

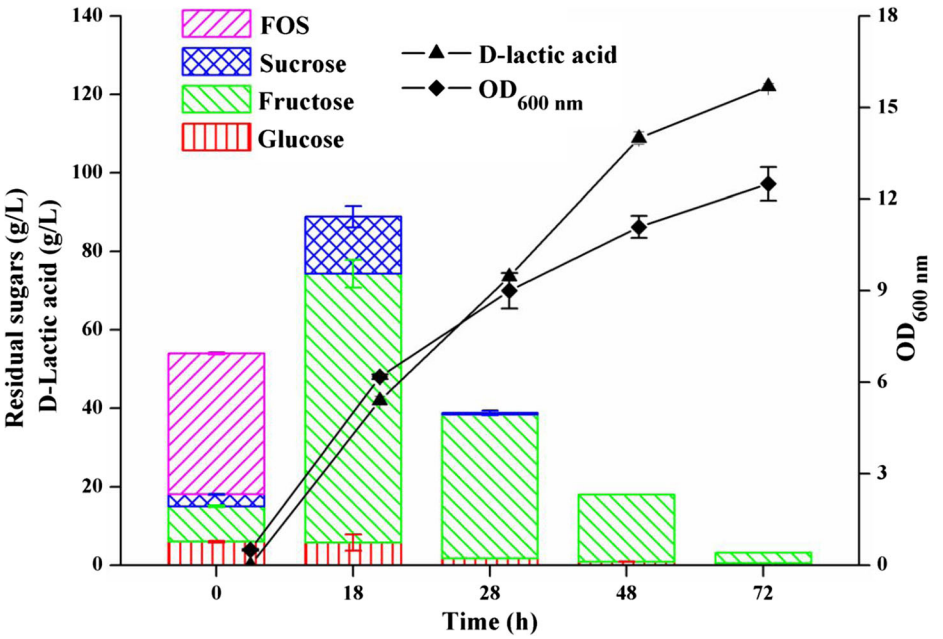

Fig. 5 High-titer D-lactic acid production by SSF using inulinase from *Penicillium* sp. XL01. (All conditions not listed in the figure were as follows: strain, *L. bulgaricus* CGMCC 1.6970; inoculum volume, 10% (*v/v*); temperature, 42 °C; pH, 5.2; chicory flour, 120 g/L; inulinase dosage, 30 IU/g chicory flour; and $CaCO_3$, 80 g/L). All experiments were performed in triplicate

commercial inulinase, which released 23.9 g/L fructose and 16.4 g/L glucose from 120 g/L inulin and quite a lot of FOS was remained after 72 h [15], the inulinase from *Penicillium* sp. XL01 is more efficient and vigorous in inulin hydrolysis.

High-Titer D-Lactic Acid Production by SSF Using Inulinase from *Penicillium* sp. XL01

As our previous study had demonstrated that SSF was superior to separate hydrolysis and fermentation (SHF), SSF process for D-lactic acid production from chicory flour was carried out in this study by employing *L. bulgaricus* CGMCC 1.6970 (Fig. 5). At the beginning of the fermentation, inulin in chicory flour was partially hydrolyzed due to sterilization, resulting in the production of 29.5 g/L of mix sugars, including 20.7 g/L FOS. After 18 h of fermentation, the FOS was exhausted, and it was not detected until the end of the fermentation. During the entire SSF process, the concentration of fructose first increased and then gradually reduced. The concentration and productivity of D-lactic acid were 108.9 g/L and 2.27 g/(L h), respectively, at 48 h. In the end, 122.0 g/L D-lactic acid was achieved with the productivity of 1.69 g/(L h). The results indicated that the inulinase from *Penicillium* sp. XL01 is a good candidate for inulin hydrolysis and value-added chemical production.

Conclusions

In this study, a strong inulinase producer *Penicillium* sp. XL01 was isolated from soil to replace the commercial one. After evaluation, the effects of culture conditions on inulinase production, an optimized fermentation process, which resulted in the production of 46.2 IU/mL inulinase, was obtained. Moreover, compared with the commercial inulinase, the inulinase from *Penicillium* sp. XL01 exhibited much higher saccharification ability. By combination of the crude enzyme and *L. bulgaricus* CGMCC 1.6970, D-lactic acid with high titer (122.0 g/L) was achieved from chicory flour by SSF, which would have perspective and profitable industrial application.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Yu, H., Zhang, M., Ouyang, J., & Shen, Y. (2014). Comparative study on four chemical pretreatment methods for an efficient saccharification of corn stover. *Energy & Fuels, 28*(7), 4282–4287.
2. Wang, L., Xue, Z., Zhao, B., Yu, B., Xu, P., & Ma, Y. (2013). Jerusalem artichoke powder: a useful material in producing high-optical-purity L-lactate using an efficient sugar-utilizing thermophilic *Bacillus coagulans* strain. *Bioresource Technology, 130*, 174–180.

3. Chi, Z. M., Zhang, T., Cao, T. S., Liu, X. Y., Cui, W., & Zhao, C. H. (2011). Biotechnological potential of inulin for bioprocesses. *Bioresource Technology, 102*(6), 4295–4303.
4. Dao, T. H., Zhang, J., & Bao, J. (2013). Characterization of inulin hydrolyzing enzyme(s) in commercial glucoamylases and its application in lactic acid production from Jerusalem artichoke tubers (Jat). *Bioresource Technology, 148*, 157–162.
5. Li, L., Chen, C., Li, K., Wang, Y., Gao, C., Ma, C., & Xu, P. (2014). Efficient simultaneous saccharification and fermentation of inulin to 2,3-butanediol by thermophilic *Bacillus licheniformis* ATCC 14580. *Applied and Environmental Microbiology, 80*(20), 6458–6464.
6. Wang, S. A., & Li, F. L. (2013). Invertase SUC2 is the key hydrolase for inulin degradation in *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology, 79*(1), 403–406.
7. Shi, N., Mao, W., He, X., Chi, Z., Chi, Z., & Liu, G. (2017). Co-expression of exo-inulinase and endo-inulinase genes in the oleaginous yeast *Yarrowia lipolytica* for efficient single cell oil production from inulin. *Applied Biochemistry and Biotechnology*. https://doi.org/10.1007/s12010-017-2659-1.
8. Cao, C., Zhang, L., Gao, J., Xu, H., Xue, F., Huang, W., & Li, Y. (2017). Research on the solid state fermentation of Jerusalem artichoke pomace for producing *R*, *R*-2, 3-butanediol by *Paenibacillus polymyxa* ZJ-9. *Applied Biochemistry and Biotechnology, 182*(2), 687–696.
9. Park, J. M., Oh, B. R., Kang, I. Y., Heo, S. Y., Seo, J. W., Park, S. M., Hong, W. K., & Kim, C. H. (2017). Enhancement of 2, 3-butanediol production from Jerusalem artichoke tuber extract by a recombinant *Bacillus* sp. strain BRC1 with increased inulinase activity. *Journal of Industrial Microbiology & Biotechnology, 44*(7), 1107–1113.
10. Petrova, P., Velikova, P., Popova, L., & Petrov, K. (2015). Direct conversion of chicory flour into L(+)-lactic acid by the highly effective inulinase producer *Lactobacillus paracasei* DSM 23505. *Bioresource Technology, 186*, 329–333.
11. Wang, J., Jin, Z., Jiang, B., & Adamu, A. (2003). Production and separation of exo-and endoinulinase from *Aspergillus ficuum*. *Process Biochemistry, 39*, 5–11.
12. Mansouri, S., Houbraken, J., Samson, R., Frisvad, J., Christensen, M., Tuthill, D., et al. (2013). *Penicillium subrubescens*, a new species efficiently producing inulinase. *Antonie Van Leeuwenhoek, 103*(6), 1343–1357.
13. Leelaram, S., Sivanesh, N., Surianarayanan, M., Deepa, P., & Balaje, S. A. (2016). Effect of feeding strategies on inulinase production analyzed in a biocalorimeter. *Process Biochemistry, 51*(6), 692–703.
14. Gao, C., Ma, C., & Xu, P. (2011). Biotechnological routes based on lactic acid production from biomass. *Biotechnology Advances, 29*(6), 930–939.
15. Xu, Q., Zang, Y., Zhou, J., Liu, P., Li, X., Yong, Q., & Ouyang, J. (2016). Highly efficient production of D-lactic acid from chicory-derived inulin by *Lactobacillus bulgaricus*. *Bioprocess and Biosystems Engineering, 39*(11), 1749–1757.
16. Smit, E., Leeflang, P., Glandorf, B., van Elsas, J. D., & Wernars, K. (1999). Analysis of fungal diversity in the wheat rhizosphere by sequencing of cloned PCR-amplified genes encoding 18S rRNA and temperature gradient gel electrophoresis. Applied and Environmental Microbiology, 65, 2614–2621, 6.
17. Xu, Y., Zheng, Z., Xu, Q., Yong, Q., & Ouyang, J. (2016). Efficient conversion of inulin to inulooligosaccharides through endoinulinase from *Aspergillus niger*. *Journal of Agricultural and Food Chemistry, 64*(12), 2612–2618.
18. Rawat, H. K., Chand Jain, S., & Kango, N. (2015). Production and properties of inulinase from *Penicillium* sp. NFCC 2768 grown on inulin-rich vegetal infusions. *Biocatalysis and Biotransformation, 33*(1), 61–68.
19. Singh, R. S., Chauhan, K., Singh, J., Pandey, A., & Larroche, C. (2018). Solid-state fermentation of carrot pomace for the production of inulinase by *Penicillium oxalicum* BGPUP-4. *Food Technology and Biotechnology, 56*(1).
20. Flores-Gallegos, A. C., Morlett-Chávez, J. A., Aguilar, C. N., Riutort, M., & Rodríguez-Herrera, R. (2015). Gene encoding inulinase isolated from *Penicillium citrinum* ESS and its molecular phylogeny. *Applied Biochemistry and Biotechnology, 175*(3), 1358–1370.
21. Kango, N., & Jain, S. C. (2011). Production and properties of microbial inulinases: recent advances. *Food Biotechnology, 25*(3), 165–212.