Comparative genomic and secretomic characterisation of endophytic Bacillus velezensis LC1 producing bioethanol from bamboo lignocellulose

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Abstract

Bacillus is an excellent organic matter degrader, and it has exhibited various abilities required for lignocellulose degradation. Several B. velezensis strains encode lignocellulosases, however their ability to efficiently transform biomass has not been appreciated. In the present study, through the comparative genomic analysis of the whole genome sequences of 21 B. velezensis strains, CAZyome related to lignocellulose degradation was identified and their similarities and differences were compared. Subsequently, the secretome of B. velezensis LC1 by liquid chromatography-tandem mass spectrometry (LC–MS/MS) were identified and confirmed that a considerable number of proteins were involved in lignocellulose degradation. Moreover, after 6-day treatment, the degradation efficiency of the B. velezensis LC1 toward cellulose, hemicellulose and lignin were 59.90%, 75.44% and 23.41%, respectively, the hydrolysate was subjected to ethanol fermentation with Saccharomyces cerevisiae and Escherichia coli KO11, yielded 10.44 g/L ethanol after 96 h. These results indicate that B. velezensis LC1 has the ability to effectively degrade bamboo lignocellulose and has the potential to be used in bioethanol production.

Keywords Comparative genomics · Secretome · Lignocellulose; bamboo · Bioethanol

Introduction

Bamboo is a perennial herbaceous plant with high lignocellulose content, which is considered to be an excellent raw material for the production of ethanol, biogas and many other valuable products. The production of bioethanol is divided into raw material pretreatment, saccharification of cellulose and hemicellulose, and fermentation of hydrolysate (Wi et al. 2017; Yuan and Wen 2017). At present, a large number of studies have been devoted to the pretreatment of raw materials to improve the saccharification efficiency. The effective degradation of lignocellulose is a speed-limiting step and a problem that needs to be solved urgently in the conversion of biomass to ethanol (Chen et al. 2017; Mansour et al. 2016).

There are natural microorganisms that can be used for biological treatment of lignocellulose in nature, but the related research work is often ignored. Researchers tend to participate in the study of specific enzymes and degradation
mechanism of lignocellulose degradation. Even studies on microbial degradation systems often focus on strains with rapid growth, high adaptability, and those that are easy to genetically manipulate (Dam et al. 2011; Woo et al. 2014). Compared to physical and chemical methods, microbial lignocellulosic degradation is a green process (Capolupo and Faraco 2016). Species belonging to the genus Bacillus are generally excellent degraders, with specific strains exhibiting various abilities for lignocellulose degradation, including the degradation of cellulose, hemicellulose, and lignin (Gong et al. 2017; Huang et al. 2013; Khelil et al. 2016; Zhu et al. 2014). Several B. velezensis strains encode lignocellulases (Chen et al. 2009; He et al. 2012; Kim et al. 2017b; Liu et al. 2017; Niazi et al. 2014). They are often regarded as biological agents because of their capabilities in promoting growth and anti-pathogenic activity. However, their potential usefulness based on their ability to transform biomass has not been appreciated (Belbahri et al. 2017; Chen.2017; Jin et al. 2017; Kim et al. 2017a; Molinatto et al. 2017; Pan et al. 2017). To prove their potential usefulness, the whole genome sequences of 21 B. velezensis strains, including B. velezensis LC1(Li et al. 2020), were retrieved from the NCBI website and then submitted to dbCAN database to search carbohydrate-active enzymes (CAZymes), consist of six categories, encoding genes (Lombard et al. 2014). Next, we analyzed the secretome of B. velezensis LC1, and tested the degradation efficiency and enzyme activity change of B. velezensis LC1 to the alkali pretreated bamboo powder. Finally, bamboo bioethanol productivity was assessed by a continuous reaction that consist of B. velezensis LC1 hydrolysing lignocellulose, Saccharomyces cerevisiae fermenting glucose, and Escherichia coli KO11 fermenting xylose.

Methods

Mine homologous genes related to lignocellulose degradation

Genomic data of 21 B. velezensis strains were retrieved from the GenBank DNA database, including nucleotide, amino acid, and putative coding sequences (the names of the selected strains are shown in Table S1). The specific domain of each CAZyme family with selected CDD model (position-specific scoring matrix) from GenBank is identified by hmmsearch instruction, and then the unique hidden Markov model (HMM) of each CAZyme family based on multiple sequence alignment was generated. For the CAZyme families without CDD models, the full-length proteins sequences in GenBank can be manually edited to create HMMs. HMMs are used to analyze whether there are CAZyme genes in these sequences. dbCAN database contains a large number of known HMMs. Submit the retrieved genomic sequences of 21 B. velezensis strains to dbCAN, set E-value < 1e-5 (alignment length > 80 amino acids) or E-value < 1e-3 (alignment length ≤ 80 amino acids) and then CAZymes were annotated (Lombard et al. 2014; Yin et al. 2012). Finally, the homologous families were analysed by OrthMCL software (http://orthomcl.org/orthomcl/) and annotated manually.

Phylogenetic analysis

Whole genome phylogenomic analysis was performed among 21 B. velezensis strains. Mugsy was used to align the genomic data, and bx-python tool kit (https://bitbucket.org/james_taylor/bx-python) was used to concatenate homologous blocks. RAxML version 7.2.8 (24) and FigTree version 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/) were utilized to construct and visualise phylogenetic trees from concatenated blocks using 1000 bootstrap replicates and the maximum-likelihood method. The CAZyme phylogenomic analysis was performed on homologous CAZy genes.

Pretreatment of bamboo materials and calculation of lignocellulose content

The bamboo materials were sampled from the bamboo botanical garden of the Bamboo Diseases and Pests Control and Resources Development Key Laboratory of Sichuan Province, China. The bamboo powders samples were treated with 3% (w/w) Tween-80 to enhance alkali pretreatment efficiency. Then, dried BPs were added in 1 M NaOH at a concentration of 10% w/v, followed by autoclaving at 121 °C for 60 min (Sun et al. 2014). Afterwards, the compounds were filtered and centrifuged (10,000xg for 10 min), the precipitates were neutralised by 1 M H2SO4 and recentrifuged to remove salts formed during neutralisation. Finally, treated samples were washed by distilled water and dried to constant weight at 60 °C. Van Soest method was performed to determine the cellulose, hemicellulose and lignin of raw and pretreatment materials and following these equations (Van Soest et al. 1991):

Hemicellulose content = neutral detergent fibre (NDF) − acid detergent fibre (ADF).

Cellulose content = ADF − acid detergent lignin (ADL) - ash content

Lignin content = ADL − ash content

Secretome preparation and analysis

The B. velezensis LC1 was cultured in liquid medium at pH 7.2, 37 °C and 200 rpm. The medium consists of pretreated bamboo shoot powder (10 g/L), (NH4)2SO4 (2 g/L), K2HPO4
(1 g/L), KH$_2$PO$_4$ (0.2 g/L), CaCl$_2$ (0.1 g/L), FeSO$_4$·7H$_2$O (0.05 g/L), and MnSO$_4$·H$_2$O (0.02 g/L). Cultures of *B. velezensis* LC1 growing in bamboo powder media were harvested on the 3rd day. First, the culture was filtered, then the supernatant was obtained by centrifugation (5000 rpm, 10 min, 4 °C), followed by another round of filtration (0.2 µm membranes). Secretome proteins were harvested by precipitating the filtrate using 12% (w/v) trichloroacetic acid overnight at 4 °C, then centrifuging (12,000 rpm, 30 min, 4 °C), and finally washing for three times with 96% ethanol (v/v). The dried secretome protein pellets were resuspended in a solution containing 8 M urea and 4% (w/v) 3-[(3-cholamidopropyl)dithiothreitol. Twenty-five microgram of protein samples of different concentration were loaded into a 12% SDS–polyacrylamide gel for electrophoresis.

LC–MS/MS analysis was implemented at Shanghai OE Biotech. Co., Ltd. as previously described (Goldman et al. 2019). Measurements were performed by nanoflow reversed-phase C18 liquid chromatography (EASY nLC, Thermo Scientific) coupled online to a 7-T linear ion trap Fourier-Transform ion cyclotron resonance mass spectrometer (LTQ FT Ultra, Thermo Scientific). Proteome Discoverer 2.3 (Thermo Scientific) was used to identify and quantify proteomic spectra, and then map the peptides to the annotated *B. velezensis* LC1 genome (data not shown) using the default settings (Table S4).

Pretreated bamboo powder degradation by *B. velezensis* LC1

To obtain fermentable sugar from bamboo, *B. velezensis* LC1 was used to degrade the bamboo powder. The *B. velezensis* LC1 was cultured as described above for 6 days. After centrifuging (13,000 rpm, 10 min), the supernatant hydrolysate and pellet were collected. The supernatant hydrolysate was terminated by heating at 100 °C for 30 min. The pellet was dried and weighed to determine the levels of cellulose using the Van Soest method. The glucose and xylose contents of the superficial hydrolysate were measured according to the NREL methods (Sluiter et al. 2012). The following equations were used for calculating degradation efficiency:

\[
\text{Degradation efficiency of cellulose} = \left(1 - \frac{\text{The mass of cellulose in deposit}}{\text{The mass of cellulose in raw material}}\right) \times 100\%
\]

\[
\text{Degradation efficiency of hemicellulose} = \left(1 - \frac{\text{The mass of hemicellulose in deposit}}{\text{The mass of hemicellulose in raw material}}\right) \times 100\%
\]

\[
\text{Degradation efficiency of lignin} = \left(1 - \frac{\text{The mass of lignin in deposit}}{\text{The mass of lignin in raw material}}\right) \times 100\%
\]

The bamboo shoot supernatant hydrolysate was sterilised by 0.22 µm filter and stored at -20 °C until it was used for ethanol fermentation.

Fermentation

The glucose-fermenting *Saccharomyces cerevisiae* was pre-cultured in YPD at 30 °C for 24 h, while *Escherichia coli* KO11, a xylose-fermenting strain, was pre-cultured in LB at 37 °C for 24 h. *S. cerevisiae* (50 g/L) and *E. coli* KO11 (100 g/L) cells were prepared after centrifugation of the pre-cultured cells. The initial cell concentrations were 0.33 g/L (*S. cerevisiae*) and 1.0 g/L (*E. coli* KO11) at the beginning of fermentation. 100 mL of hydrolysate was used for ethanol fermentation in 250 mL serum bottles under anaerobic conditions at 37 °C. The fermentation system was shaken at 200 rpm for 96 h. Starting from 48 h of fermentation, ethanol production was monitored per 12 h. The ethanol concentration was determined via High Performance Liquid Chromatography (HPLC). All reactions were repeated three times.

Enzyme assays

Two millilitres of supernatant hydrolysate were obtained daily from 6-day cultures for assays performed to measure lignocellulolase activities as described by Luo et al (2018). In brief, carboxymethyl cellulose (CMC), microcrystalline cellulose (MCC), and salicin were used as substrates to measure the activities of endoglucanase, exoglucanase, and β-glucosidase, respectively. Veratryl alcohol (VA), 2,2′-azino-bis (ABTS), and xylan were used as substrates to determine LiP, laccase, and xylanase activities, respectively. Manganese peroxidase (MnP) activity was measured spectrophotometrically by monitoring the oxidation of 2,6-DMP at 469 nm (ε469 = 49,600 mol⁻¹cm⁻¹). All the experiments were repeated five times.
Results and discussion

Effect of alkali pretreatment on chemical components of bamboo

Lignocellulose-derived ethanol is widely considered a clean liquid fuel. However, raw lignocellulose has a recalcitrant structure that lowers bioconversion efficiency, making pretreatment necessary to make lignocellulose more vulnerable to enzymes or bacteria for the fermentation of ethanol and other products (Zhao et al. 2012). Bamboo lignocellulose pretreated by alkali has a higher enzymatic digestibility than the raw material (Sun et al. 2014). In the present study, we used sodium hydroxide (NaOH) to pretreat the bamboo powders. Approximately 70% of bamboo solids were recovered after NaOH pretreatments. The hemicellulose and cellulose contents were partially decreased from 22.13 g/100 g and 44.94 g/100 g to 21.13 g/100 g and 37.34 g/100 g after pretreatment, while the lignin significantly decreased from 18.71 g/100 g to 4.84 g/100 g. Furthermore, we calculated the recovery of cellulose, hemicellulose and solubilization of lignin after NaOH pretreatment. The result showed that 95.48% and 83.09% of hemicellulose and cellulose were recovered and the solubilization of lignin was 74.13%. Cellulose remained stable before and after alkaline pretreatment, which is consistent with previous studies (Sun et al. 2014). The results indicate that NaOH pretreatment of bamboo efficiently removes lignin without significantly impacting the cellulose and hemicellulose.

Comparative genomic analysis of CAZymes of B. velezensis strains

The assembled genome of B. velezensis LC1, contains 44 GHs, 38 GTs, 30 CEs, 3 PLs, 6 AAs, and 15 CBMs, was compared with 20 other B. velezensis strains at whole genomic level (Belbahri et al. 2017; Chen et al. 2009; Chen.2017; He et al. 2012; Huang et al. 2013; Jin et al. 2017; Khelil et al. 2016; Kim et al. 2017a; Kim et al. 2017b; Li et al. 2020; Liu et al. 2017; Molinatto et al. 2017; Niazi et al. 2014; Pan et al. 2017; Zhu et al. 2014). The results showed that B. velezensis LC1 was phylogenomically closely related to the B. velezensis DR-08 (Fig. 1), while B. velezensis LC1 strongly resemble B. velezensis S3-1 according to the CAZyme profile (Fig. 2).

The CAZymes coding genes of 21 genomes were detected at the whole genome level, and the results revealed the similarities and differences of lignocellulose degrading genes (Table 1 and Table S2). In terms of genes involved in cellulose degradation, among the enzymes encoded by these genes, GH5 and GH30 families have endoglucanase activity, while enzymes represented by GH4 family usually exhibit β-glucosidase activity and have high frequency in the genome. PL1 and PL9 CAZyme families encode pectate lyase that action on (1→4)-α-D-galacturonan results in the production of oligosaccharides (See-Too et al. 2017). In addition, the coding genes of GH43, GH51 and GH53 families related to the hydrolysis of type I arabinogalactan (Vanholme et al. 2009), and the CE4 and CE7 families related to the acetyl transfer of xylan (Zhang et al. 2011) were detected, which were all involved in the degradation of hemicellulose. We also

![Fig. 1](https://example.com/fig1.png) Comparative genomic analysis of the 21 Bacillus velezensis strains and the genome information at the whole genome level. The genome sequences of 21 B. velezensis strains were retrieved from the National Centre for Biotechnology Information (NCBI; [https://www.ncbi.nlm.nih.gov/]). More detailed information is shown in Table S2.
identified several non-catalytic modules, including CBM3 and CBM12, which always bind to cellulose or amorphous cellulose (Mu et al. 2021). Notably, there are no CAZyme encoding genes detected in other 20 B. velezensis strains, such as GH76, GT19, CE1, CBM12, AA6, AA7, etc., among which GH76 is an α-glucan active enzyme family using the retaining reaction (Thompson et al. 2015), GT19 own lipid A disaccharide synthase (Crowell et al. 1987). The presence of a large number of genes related to cellulose and hemicellulose degradation in B. velezensis strains suggest that they may have potential application in bioethanol production.
Identification of secretomes of *B. velezensis* LC1 grown on medium containing bamboo powder

To further confirm expression of lignocellulase systems in *B. velezensis*, we select *B. velezensis* LC1 as the representative and analyzed the secretomes growing on bamboo powder and glucose medium (control). First, the supernatants of a 3-day cultivation were precipitated with 12% (w/v) trichloroacetic acid (TCA) to obtain proteins. Secretomic proteins were detected only in the bamboo powder medium (Fig. 3a). These proteins were analyzed by 1D-PAGE (Fig. 3b) and LC–MS/MS and a total of 142 proteins were identified (Table S3). The basic characteristics of the proteins are shown in Fig. 3c. The pI values of most proteins are in the range from 5.0 to 10.0. These proteins were then functionally annotated and enriched in biological processes (612 proteins), cell components (69 proteins), molecular functions (348 proteins), and KEGG pathway (79 proteins), respectively (Fig. 3d; Figs. S1; Fig. S2).

In addition, the degradation-related enzymes, including hemicellulases, cellulases, and others, were abundant in bamboo powder medium (Table 2). The hydrolysis of identified proteins. Both the molecular weight and isoelectric point were theoretically obtained using Proteome Discoverer v.1.3 beta (Thermo Scientific) during protein identification. d Functional annotation of identified proteins in the *B. velezensis* LC1 secretome by LC–MS/MS

![Secretome identification of *B. velezensis* LC1 in the presence of bamboo powder substrate. a Protein extraction by trichloroacetic acid (TCA). G, glucose substrate, BP, bamboo powder substrate. b 1D-SDS-PAGE of proteomes of *B. velezensis* LC1 grown on bamboo powder substrate. c Molecular weight and isoelectric focusing of identified proteins. e Functional annotation of identified proteins in the *B. velezensis* LC1 secretome by LC–MS/MS](image)
process of cellulose was completed under the synergistic effect of various enzymes. Firstly, endoglucanase acts on amorphous cellulose or soluble cellulose to randomly open the internal bond of cellulose. Then, exoglucanase acts on crystalline compounds such as fiber oligosaccharides and microcrystal fibers and catalyzes the non-reducing or reducing end of polysaccharide chain. Finally, β-glucosidase is used to hydrolyze fiber dextrin and fiber disaccharide to produce glucose (Sharma et al. 2016). In this study, we found that β-1,3–1,4-glucanases (1,384,351,103) and endoglucanases (1,384,349,288) may be involved in cellulose degradation (Table 2). The enzyme as β-1,3–1,4-glucanase in Bacillus was classified into GH16 family and had the ability to degrade β-glucan (McCarthy et al. 2003; Teng et al. 2006).

Hemicellulose is the second most abundant component in lignocellulose. The degradation process of hemicellulose is also the result of a series of enzymes (Adav et al. 2010). In this study, four hemicellulases were identified, consisting of one arabinogalactan endo-β-1,4-galactanase (GH53) (1,384,349,889), one β-xylanase (GH43) (1,384,351,325), one glucuronoxylanase (GH30) (1,384,349,284), and one acetyl xylan esterase (CE1) (1,384,350,660) (Table 2). Among them, arabinogalactan endo-β-1,4-galactanase was found to catalyze the hydrolysis of β-1,4-galactosidic bonds in arabinogalactan and galactose side chains (Torpenholt et al. 2011).

We need to pay special attention to CE1, which is a CAZyme family, associated with hemicellulose degradation, that has been only identified in B. velezensis LC1 through genomic coding gene annotation and secreted protein identification. The CE1 family contains enzymes that target a variety of specific substrates, such as acetyl xylan esterase, cinnamoyl esterase, feruloyl esterase (Mai-Gisondi et al. 2017). During the degradation of hemicellulose, xylanase can hydrolyze β-1–4 bonds in the main chain of xylan, while xylan acetyl transferase can assist other hemicellulose-degrading enzymes to remove side chain residues on the hemicellulose skeleton (Yang et al. 2015; Zhang et al. 2011).

In addition, according to the results of comparative genomic analysis among 21 B. velezensis strains and secretomes analysis of B. velezensis LC1, we also found that the CAZymes of the AA families (AA6 and AA7) and the CBM family (CBM12) are unique exist in B. velezensis LC1. Generally, AA6 and AA7 mainly exist in fungi and are related to lignin degradation, which play the functions of oxidoreductase (Pardo et al. 2000). CBM12 is found among chitinases where the function is chitin-binding.

### Table 2

| Locus tag     | Description                                          | Peptides | Unique Peptides | Protein mass (kDa) | Isoelectric point | Score  | CAZy |
|---------------|------------------------------------------------------|----------|-----------------|--------------------|-------------------|--------|------|
| 1,384,350,573 | Bifunctional penicillin-binding protein 1C           | 2        | 2               | 88.3               | 9.88              | 3.92   | GT51 |
| 1,384,350,643 | Putative multimodal carbohydrate-active enzyme       | 3        | 3               | 92.9               | 6.65              | 8.73   | GT51 |
| 1,384,351,304 | ATP synthase subunit alpha                           | 4        | 4               | 56                 | 5.15              | 12.9   | GT4  |
| 1,384,351,201 | Glycosyltransferase 2                                | 3        | 3               | 55                 | 6.58              | 4.67   | GT2  |
| 1,384,349,288 | Endoglucanase                                        | 3        | 3               | 55.2               | 8.1               | 2.87   | GH5  |
| 1,384,349,889 | Arabinogalactan endo-beta-1,4-galactanase            | 8        | 8               | 41.3               | 8.56              | 12.32  | GH53 |
| 1,384,351,325 | Beta-xylanase                                        | 5        | 5               | 42.2               | 6.98              | 11.07  | GH43 |
| 1,384,347,997 | Sucrose-6-phosphate hydrolase                        | 10       | 10              | 56.1               | 5.69              | 54.68  | GH32 |
| 1,384,351,193 | Sucrose-6-phosphate hydrolase                        | 4        | 4               | 66.2               | 7.46              | 2.18   | GH32 |
| 1,384,349,284 | Glucuronoxylanase                                    | 10       | 10              | 47.8               | 8.29              | 28.43  | GH30 |
| 1,384,351,103 | Endo-beta-1,3–1,4-glucanase                          | 5        | 5               | 26.7               | 6.92              | 15.06  | GH16 |
| 1,384,348,333 | Alpha-glycosidase                                    | 12       | 12              | 119                | 4.88              | 38.21  | GH13 |
| 1,384,347,992 | Alpha-amylase                                        | 7        | 7               | 72.4               | 6.49              | 29.61  | GH13 |
| 1,384,351,150 | 6-phospho-beta-glucosidase                           | 5        | 5               | 48.6               | 5.4               | 4.16   | GH13 |
| 1,384,350,269 | Beta-amylase                                         | 2        | 2               | 55.1               | 5.48              | 2.73   | GH13 |
| 1,384,348,933 | Serine aminopeptidase                                | 9        | 9               | 8.4                | 4.96              | 2.75   | CE1  |
| 1,384,350,660 | Acetylxyylan esterase                                | 2        | 2               | 35.5               | 6.11              | 4.83   | CE1  |
| 1,384,351,298 | Serine hydroxymethyltransferase                     | 2        | 2               | 45                 | 6.21              | 5.5    | CBM12|
| 1,384,350,476 | FAD-binding oxidoreductase                           | 2        | 2               | 50.1               | 9                 | 4.16   | AA7  |
| 1,384,350,268 | NAD(P)H-dependent oxidoreductase                     | 4        | 4               | 25.6               | 5.97              | 9.25   | AA6  |
| 1,384,350,569 | NAD(P)-dependent oxidoreductase                      | 2        | 2               | 31.2               | 5.05              | 3.01   | AA6  |
Moreover, other enzymes involved in starch degradation, plant cell wall modifications, and protein degradation (proteases) were also detected (Table 2).

Degradation effect of \textit{B. velezensis} LC1 on pretreated bamboo powder on different culture medium

A large number of studies have shown that \textit{B. velezensis} strains have application value in agriculture and biotechnology, so they have attracted wide attentions. \textit{B. velezensis} LC1, previous isolated from the intestinal microbiome of \textit{Cyrtotrachelus buqueti} and demonstrated the lignocellulose degrading ability through bamboo shoots degrading experiments (Li et al. 2020). In this experiment, we measured cellulase (endoglucanase, \( \beta \)-glucosidase and exoglucanase), hemicellulase (xylanase), and ligninase (laccase, lignin peroxidase and manganese peroxidase) activities, the results showed that the activities of these enzymes for pretreated bamboo powder were significantly higher than those for glucose (Fig. 4). The enzyme activity of endoglucanase, \( \beta \)-glucosidase, and manganese peroxidase in pretreated bamboo powder culture medium was promptly increased after 3 days of treatment. One exception was the rapid increase in exoglucanase activity from the 4 days after treatment.

Then, we measured lignocellulose degradation efficiency using pretreated bamboo powder. After 6 days of culture, degradation products were collected for analysis, and the results showed degradation efficiency of cellulose, hemicellulose, and lignin in bamboo powder were 59.90\%, 75.44\%, and 23.41\%, respectively (Fig. 5a). Relevant experiments show that \textit{B. velezensis} LC1 has a good degradation effect on lignocellulose. Meanwhile, a large number of other studies on \textit{B. velezensis} also support our point of view (Gong et al. 2017; Huang et al. 2013; Khelil et al. 2016; Zhu et al. 2014).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{Lignocellulose activities of \textit{B. velezensis} LC1. The activities of endoglucanase, \( \beta \)-glucosidase, exoglucanase, xylanase, lignin peroxidase, laccase, and manganese peroxidase are listed in a–g, respectively. Data are presented as the means and standard deviations of five experiments. Statistical significance: \(*P < 0.05, \quad **P < 0.01, \quad ***P < 0.001\)}
\end{figure}
Hydrolysate fermentation

In the past, the energy conversion methods of bamboo lignocellulosic were mainly chemical and physical pretreatment (Mansour et al. 2016). But now a large number of fungi (Saccharomyces cerevisiae) and some bacteria (Escherichia coli) are considered suitable for bioethanol production because they can take advantage of the abundant cellulose found in bamboo. In our study, the ethanol productivity of bamboo was assessed by the process comprised of B. velezensis LC1 hydrolysed lignocellulose, S. cerevisiae fermented glucose, and E. coli KO11 fermented xylose. The bamboo-based ethanol production process is shown in Fig. 5b, the results revealed that when ethanol yield grows to 10.44 g/L at 96 h, the content of reducing sugar also gradually decreases, which indicates that B. velezensis LC1 has potential in bioethanol conversion, although its bioethanol yield is still lower than that of commercial enzyme-promoted bioethanol (Yuan et al. 2018).

Conclusions

Through the comparative genomic analysis of the whole genome sequences of 21 B. velezensis strains, CAZyme related to lignocellulose degradation was identified and their similarities and differences were compared. Next, proteins related to lignocellulose degradation were identified from secretome of B. velezensis LC1 when grown in alkaline pretreated bamboo powder medium. The proteins degradation efficiency for cellulose, hemicellulose and lignin were 59.90%, 75.44% and 23.41%, respectively. Finally, the hydrolysate was subjected to subsequent ethanol fermentation process, results showed that the ethanol yield was 10.44 g/L at 96 h. These findings indicated that B. velezensis LC1 efficiently bioconvert bamboo lignocellulose components to reducing sugar for ethanol fermentation.

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Author contributions CBL designed and performed the experiments; HT, LZ and YQL wrote the manuscript; LZ, LL, YQL, XWY and HT analyzed the data. All authors read and approved the final manuscript.

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Availability of data and material The sequence reads from this article have been deposited at the NCBI Sequence Read Archive under the accession PRJNA574012. The assembly data set supporting the results of this article has been deposited at GenBank under the accession CP044349. The version described in this paper is CP044349.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval This study does not include any experimental procedure performed on humans or animals.
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