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

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Research Article

Keywords: comparative genomics, secretome, lignocellulose, bamboo, bioethanol

Posted Date: March 19th, 2021

DOI: https://doi.org/10.21203/rs.2.17974/v2

Version of Record: A version of this preprint was published at Archives of Microbiology on April 1st, 2021.
See the published version at https://doi.org/10.1007/s00203-021-02306-6.

Abstract

Bacillus is an excellent organic matter degrader, and it has exhibited various abilities required for lignocellulose degradation. Several B. velezensis strains encode lignocellulosases and however their usefulness based on their ability to transform biomass has not been appreciated. In the present study, genomic, comprehensive comparative genomic and secretomic analyses were used to clarify the lignocellulose-degrading potential of these bacteria. The complete genomes of 20 B. velezensis strains and an endophytic strain, B. velezensis LC1, were analysed to find common and unique genes encoding carbohydrate-active enzymes (CAZymes) and evaluate their potentials to degrade lignocellulose. By comparative whole genomic and CAZyomic analyses of all 21 strains, we identified genes coding for lignocellulolytic enzymes with the potential to degrade cellulose and hemicellulose. Further identification of the secretome of B. velezensis LC1 by liquid chromatography-tandem mass spectrometry (LC-MS/MS) confirmed that a considerable number of proteins in the culture medium are involved in lignocellulose degradation, including endoglucanase, hemicellulases, and other related proteins. Moreover, assays of the activities of several lignocellulolytic enzymes show that these enzymes are more active in bamboo powder compared to glucose substrates. After a 6-day treatment, the degradation efficiencies of cellulose, hemicellulose and lignin from bamboo powder were 59.90%, 75.44% and 23.41%, respectively. The hydrolysate was subjected to ethanol fermentation with Saccharomyces cerevisiae and Escherichia coli KO11, yielding 10.44 g/L ethanol after 96 h. These findings indicate that B. velezensis LC1, efficiently degrades bamboo lignocellulose components, allowing for subsequent ethanol production.

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 [1, 2]. 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 [3, 4].

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 [5,6]. Compared to physical and chemical methods, microbial lignocellulosic degradation is a green process [7]. 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 [8-11]. Several *B. velezensis* strains encode lignocellulosases [12-16]. 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 [17-22]. To prove their potential usefulness, the whole genome sequences of 21 *B. velezensis* strains, including *B. velezensis* LC1 [23], were retrieved from the NCBI website and then submitted to dbCAN database to search carbohydrate-active enzymes (CAZymes), consist of six categories, encoding genes [24]. 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 [24,25]. 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/fifigtree/) were utilized to construct and visualise phylogenetic trees from concatenated blocks using 1000 bootstrap replicates and the maximum-likelihood method. The CAZyome 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 1M NaOH at a concentration of 10% w/v, followed by autoclaving at 121 ℃ for 60 min [26]. Afterwards, the compounds were filtered and centrifuged (10,000 × g for 10 min), the

precipitates were neutralised by 1 M H_2SO_4 and recentrifuged to remove salts formed during neutralisation. Finally, treated samples were washed by distilled water and dried to constant weight at 60 ℃. Van Soest method [27] was performed to determine the cellulose, hemicellulose and lignin of raw and pretreatment materials and following these equations:

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

Cellulose content = ADF − acid detergent lignin (ADL)

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), $(NH4)_2SO_4$ (2 g/L), K_2HPO_4 (1 g/L), KH_2PO_4 (1 g/L), $MgSO_4$ (0.2 g/L), $CaCl_2$ (0.1 g/L), $FeSO_4$•$7H_2O$ (0.05 g/L), and $MnSO_4$•H_2O (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 ℃), 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 ℃, then centrifuging (12,000 rpm, 30 min, 4 ℃), 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 micro gram 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 [28]. Measurements were performed by nanoflow reversed-phase C18 liquid chromatography (EASY nLC, Thermo Scientific) coupled online to a 7-Tesla 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 d. 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 [27]. The glucose and xylose contents of the superficial hydrolysate were measured according to the NREL methods [29]. The following equations were used for calculating degradation efficiency:

$$\text{Degradation efficiency of cellulose} = (1 - \frac{\text{The mass of cellulose in deposit}}{\text{The mass of cellulose in raw material}}) * 100\%$$

$$\text{Degradation efficiency of hemicellulose} = (1 - \frac{\text{The mass of hemicellulose in deposit}}{\text{The mass of hemicellulose in raw material}}) * 100\%$$

$$\text{Degradation efficiency of lignin} = (1 - \frac{\text{The mass of lignin in deposit}}{\text{The mass of lignin in raw material}}) * 100\%$$

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

Fermentation

The glucose-fermenting *Saccharomyces cerevisiae* was pre-cultured in YPD at 30 ℃ for 24 h, while *Escherichia coli* KO11, a xylose-fermenting strain, was pre-cultured in LB at 37 ℃ 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 ℃. 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-d-cultures for assays performed to measure lignocellulolase activities as described by Luo et al. [30]. 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 $mol^{-1}cm^{-1}$). 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 [31]. Bamboo lignocellulose pretreated by alkali has a higher enzymatic digestibility than the raw material [26]. 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 pretreament. 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 [26]. The results indicate that NaOH pretreament 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 [23], was compared with 20 other *B. velezensis* strains [9-22] at whole genomic level. 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 CAZyome 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 [32]. In addition, the coding genes of GH43, GH51 and GH53 families related to the hydrolysis of type arabinogalactan [33], and the CE4 and CE7 families related to the acetyl transfer of xylan [34] were detected, which were all involved in the degradation of hemicellulose. We also identified several non-catalytic modules, including CBM3 and CBM12, which always bind to cellulose or amorphous cellulose [35]. 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 [36], GT19 own lipid A disaccharide synthase [37]. 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

In order 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 three-day cultivation were precipitated with 12% (w/v) trichloroacetic aid (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; Fig. S1; Fig. S2).

In addition, the degradation-related enzymes, including hemicellulases, cellulases, and others, were abundant in bamboo powder medium (Table 2). The hydrolysis 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 [38]. In this study, we found that β-1,3-1,4-glucanases (1384351103) and endoglucanases (1384349288) 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 [39,40].

Hemicellulose is the second most abundant component in lignocellulose. The degradation process of hemicellulose is also the result of a series of enzymes [41]. In this study, four hemicellulases were identified, consisting of one arabinogalactan endo-β-1,4-galactanase (GH53) (1384349889), one β-xylanase (GH43) (1384351325), one glucuronoxylanase (GH30) (1384349284), and one acetyl xylan esterase (CE1) (1384350660) (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 [42].

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 [43]. 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 [44,45].

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 [46]. CBM12 is found among chitinases where the function is chitin-binding.

Moreover, other enzymes involved in starch degradation, plant cell wall modifications, and protein degradation (proteases) were also detected (Table 2).

Degradation effect of *B. velezensis* LC1 on pretreated bamboo powder on different culture medium

A large number of studies have shown that *B. velezensis* strains have application value in agriculture and biotechnology, so they have attracted wide attentions [15-21]. *B. velezensis* LC1, previous isolated from the intestinal microbiome of *Cyrtotrachelus buqueti* and demonstrated the lignocellulose degrading ability through bamboo shoots degrading experiments [23]. In this experiment, we measured cellulase (endoglucanase, β-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, β-glucosidase, and manganese peroxidase in pretreated bamboo powder culture medium was promptly increased after 3 d of treatment. One exception was the rapid increase in exoglucanase activity from the 4 d after treatment.

Then, we measured lignocellulose degradation efficiency using pretreated bamboo powder. After 6 d 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 *B. velezensis* LC1 has a good degradation effect on lignocellulose. Meanwhile, a large number of other studies on *B. velezensis* also support our point of view [8-11].

Hydrolysate fermentation

In the past, the energy conversion methods of bamboo lignocellulosic were mainly chemical and physical pretreatment [3]. 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 [47].

Conclusions

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. 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.

Abbreviations

B. velezensis: *Bacillus velezensis*; **GH**: Glycoside Hydrolase; **GT**: glycosyltransferase; **CE**: carbohydrate esterase; **CBM**: carbohydrate binding domain; **PL**: polysaccharide lyase; **AA**: Auxiliary Activitie; **CAZyme**: carbohydrate-active enzyme; **ABTS**: [2,2'-Azino-bis (3-ethylbenzothiazoline-6- sulfonic acid)]; **BP**: bamboo powder; **YPD**: Yeast Extract Peptone Dextrose Medium; **DNS**: 3,5-dinitrosalicylic acid; **CDS**: Sequence coding for amino acids in protein; **NCBI:** National Centre for Biotechnology Information.

Declarations

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.

Funding

This work was supported by Sichuan science and technology program (2019YFG0139) and Scientific Research Foundation of Leshan Normal University (XJR19001).

Compliance with ethical standards

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.

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.

Acknowledgements

The data were analyzed on the free online platform of Majorbio Cloud Platform (www.majorbio.com).

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Tables

Table 1. Annotated common genes encoding lignocellulose-degrading enzymes of 21 *B. velezensis* strains

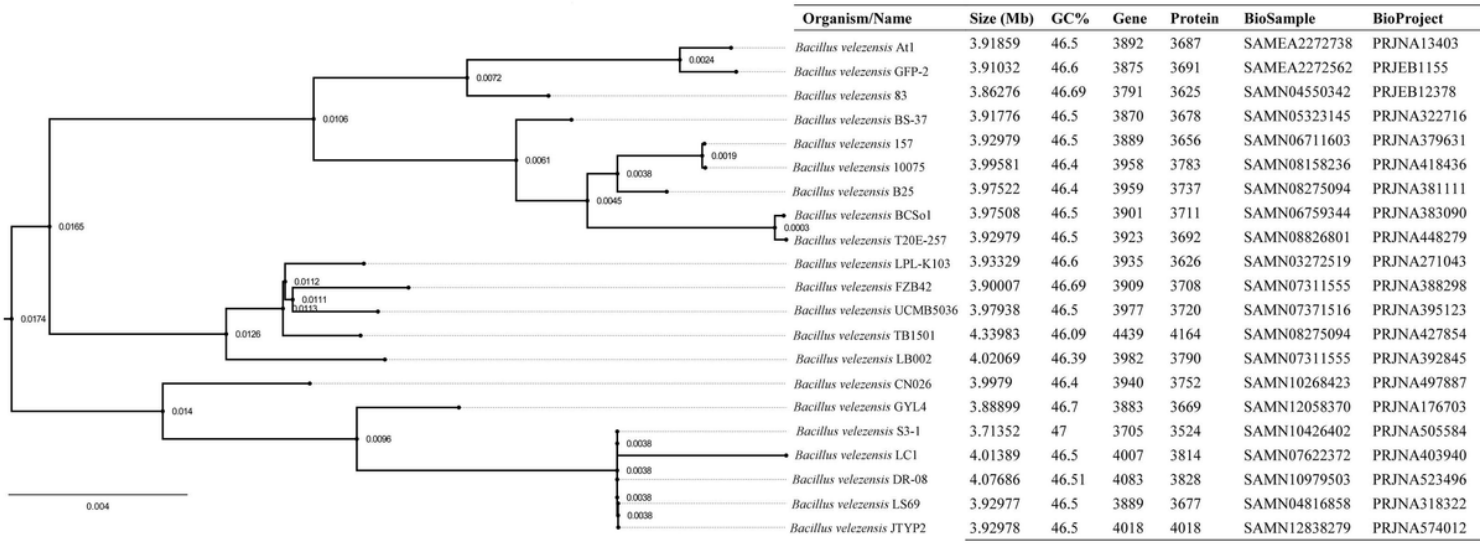
Classification	CAZy	Predicted function	EC number
Cellulose-related	GH5	endo-1,4-β-glucanase	EC 3.2.1.4
	GH30	glucan endo-1,6-β-glucosidase	EC 3.2.1.-
	GH4	6-phospho-β-glucosidase	EC 3.2.1.86
	CH4	6-phospho-α-glucosidase	EC 3.2.1-
	GH4	alpha-glucosidase/alpha-galactosidase	EC:3.2.1.20
	GH4	6-phospho-β-glucosidase	EC 3.2.1.86
	GH1	6-phospho-beta-galactosidase	EC:3.2.1.85
	GH1	aryl-phospho-beta-D-glucosidase	EC:3.2.1.86
	GH1	6-phospho-β-galactosidase	EC 3.2.1.85
	GH16	β-glucanase	EC 3.2.1.-
	GH32	sucrose-6-phosphate hydrolase	EC 2.4.1.-
	GH32	levanase	EC 3.2.1.65
	GH13	α-glucosidase	EC 3.2.1.20
	PL1	pectate lyase	EC 4.2.2.-
	PL9	pectate lyase	EC 4.2.2.2
Hemicellulose-related	GH43	arabinan endo-1,5-α-L-arabinosidase	EC 3.2.1.-
	GH43	arabinoxylan arabinofuranohydrolase	EC 3.2.1.-
	GH43	1,4-β-xylosidase	EC 3.2.1.37
	GH51	α-N-arabinofuranosidase	EC 3.2.1.55
	GH30	glucuronoxylanase	EC 3.2.1.-
	CE 7	acetylxylan esterase	EC 3.1.1.72
	CE 3	acetylxylan esterase	EC 3.1.1.72
	GH53	arabinogalactan endo-1,4-β-galactosidase	EC 3.2.1.89

Table 2. Identification of the main CAZy proteins in the secretome of *B. velezensis* LC1 in bamboo powder (BP) culture

Locus tag	Description	Peptides	Unique Peptides	Protein mass (kDa)	Isoelectric point	Score	CAZy
1384350573	Bifunctional penicillin-binding protein 1C	2	2	88.3	9.88	3.92	GT51
1384350643	Putative multimodular carbohydrate-active enzyme	3	3	92.9	6.65	8.73	GT51
1384351304	ATP synthase subunit alpha	4	4	56	5.15	12.9	GT4
1384351201	Glycosyltransferase 2	3	3	55	6.58	4.67	GT2
1384349288	Endoglucanase	3	3	55.2	8.1	2.87	GH5
1384349889	Arabinogalactan endo-beta-1,4-galactanase	8	8	41.3	8.56	12.32	GH53
1384351325	Beta-xylanase	5	5	42.2	6.98	11.07	GH43
1384347997	Sucrose-6-phosphate hydrolase	10	10	56.1	5.69	54.68	GH32
1384351193	Sucrose-6-phosphate hydrolase	4	4	66.2	7.46	2.18	GH32
1384349284	Glucuronoxylanase	10	10	47.8	8.29	28.43	GH30
1384351103	Endo-beta-1,3-1,4-glucanase	5	5	26.7	6.92	15.06	GH16
1384348333	Alpha-glycosidase	12	12	119	4.88	38.21	GH13
1384347992	Alpha-amylase	7	7	72.4	6.49	29.61	GH13
1384351150	6-phospho-beta-glucosidase	5	5	48.6	5.4	4.16	GH13
1384350269	Beta-amylase	2	2	55.1	5.48	2.73	GH13
1384348933	Serine aminopeptidase	9	9	8.4	4.96	2.75	CE1
1384350660	Acetylxylan esterase	2	2	35.5	6.11	4.83	CE1
1384351298	Serine hydroxymethyltransferase	2	2	45	6.21	5.5	CBM12
1384350476	FAD-binding oxidoreductase	2	2	50.1	9	4.16	AA7
1384350268	NAD(P)H-dependent	4	4	25.6	5.97	9.25	AA6

	oxidoreductase						
1384350569	NAD(P)-dependent oxidoreductase	2	2	31.2	5.05	3.01	AA6

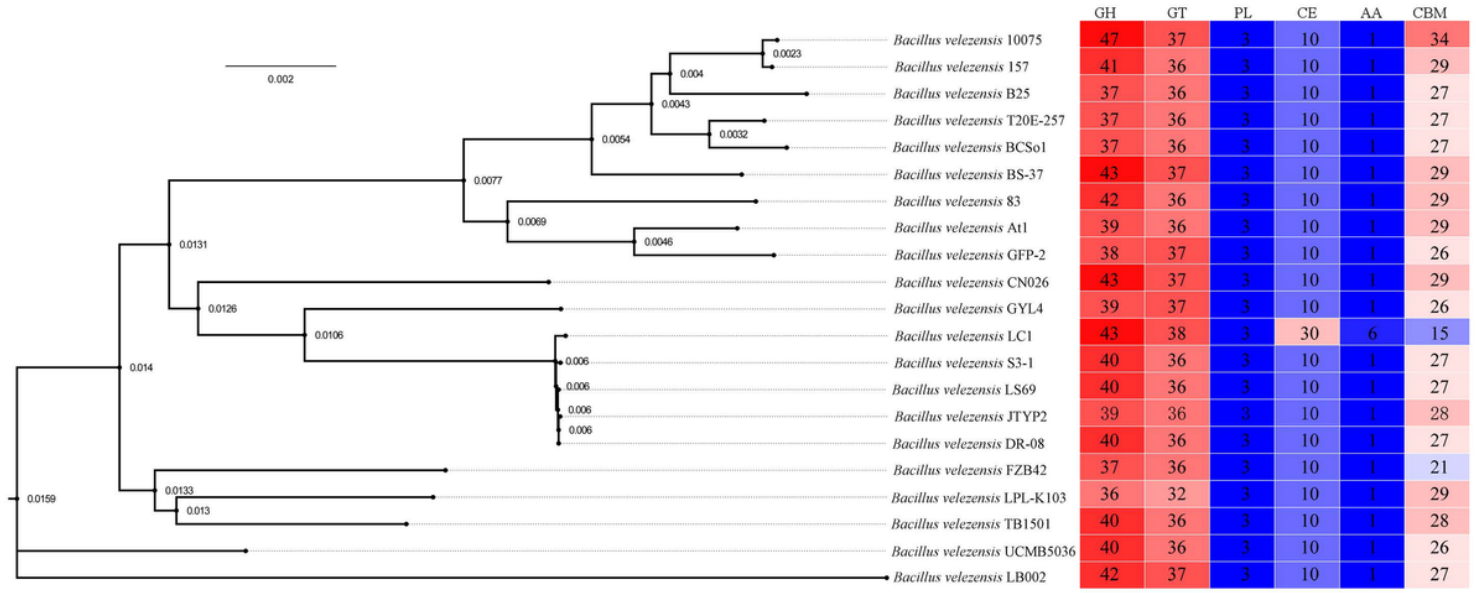
Figures



Organism/Name	Size (Mb)	GC%	Gene	Protein	BioSample	BioProject
Bacillus velezensis At1	3.91859	46.5	3892	3687	SAMEA2272738	PRJNA13403
Bacillus velezensis GFP-2	3.91032	46.6	3875	3691	SAMEA2272562	PRJEB1155
Bacillus velezensis 83	3.86276	46.69	3791	3625	SAMN04550342	PRJEB12378
Bacillus velezensis BS-37	3.91776	46.5	3870	3678	SAMN05323145	PRJNA322716
Bacillus velezensis 157	3.92979	46.5	3889	3656	SAMN06711603	PRJNA379631
Bacillus velezensis 10075	3.99581	46.4	3958	3783	SAMN08158236	PRJNA418436
Bacillus velezensis B25	3.97522	46.4	3959	3737	SAMN08275094	PRJNA381111
Bacillus velezensis BCSo1	3.97508	46.5	3901	3711	SAMN06759344	PRJNA383090
Bacillus velezensis T20E-257	3.92979	46.5	3923	3692	SAMN08826801	PRJNA448279
Bacillus velezensis LPL-K103	3.93329	46.6	3935	3626	SAMN03272519	PRJNA271043
Bacillus velezensis FZB42	3.90007	46.69	3909	3708	SAMN07311555	PRJNA388298
Bacillus velezensis UCMB5036	3.97938	46.5	3977	3720	SAMN07371516	PRJNA395123
Bacillus velezensis TB1501	4.33983	46.09	4439	4164	SAMN08275094	PRJNA427854
Bacillus velezensis LB002	4.02069	46.39	3982	3790	SAMN07311555	PRJNA392845
Bacillus velezensis CN026	3.9979	46.4	3940	3752	SAMN10268423	PRJNA497887
Bacillus velezensis GYL4	3.88899	46.7	3883	3669	SAMN12058370	PRJNA176703
Bacillus velezensis S3-1	3.71352	47	3705	3524	SAMN10426402	PRJNA505584
Bacillus velezensis LC1	4.01389	46.5	4007	3814	SAMN07622372	PRJNA403940
Bacillus velezensis DR-08	4.07686	46.51	4083	3828	SAMN10979503	PRJNA523496
Bacillus velezensis LS69	3.92977	46.5	3889	3677	SAMN04816858	PRJNA318322
Bacillus velezensis JTYP2	3.92978	46.5	4018	4018	SAMN12838279	PRJNA574012

Figure 1

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. A maximum-likelihood phylogenetic tree with 1000 bootstrap replicates was constructed for the concatenated blocks of the aligned sequence using RAxML version 7.2.8 (24) and was visualised using FigTree version 1.3.1 (http://tree.bio.ed.ac.uk/software/fifigtree/).



	GH	GT	PL	CE	AA	CBM
Bacillus velezensis 10075	47	37	3	10	1	34
Bacillus velezensis 157	41	36	3	10	1	29
Bacillus velezensis B25	37	36	3	10	1	27
Bacillus velezensis T20E-257	37	36	3	10	1	27
Bacillus velezensis BCSo1	37	36	3	10	1	27
Bacillus velezensis BS-37	43	37	3	10	1	29
Bacillus velezensis 83	42	36	3	10	1	29
Bacillus velezensis At1	39	36	3	10	1	29
Bacillus velezensis GFP-2	38	37	3	10	1	26
Bacillus velezensis CN026	43	37	3	10	1	29
Bacillus velezensis GYL4	39	37	3	10	1	26
Bacillus velezensis LC1	43	38	3	30	6	15
Bacillus velezensis S3-1	40	36	3	10	1	27
Bacillus velezensis LS69	40	36	3	10	1	27
Bacillus velezensis JTYP2	39	36	3	10	1	28
Bacillus velezensis DR-08	40	36	3	10	1	27
Bacillus velezensis FZB42	37	36	3	10	1	21
Bacillus velezensis LPL-K103	36	32	3	10	1	29
Bacillus velezensis TB1501	40	36	3	10	1	28
Bacillus velezensis UCMB5036	40	36	3	10	1	26
Bacillus velezensis LB002	42	37	3	10	1	27

Figure 2

Comparative CAZyome analysis of the 21 Bacillus velezensis strains and the amounts of GH, GT, AA, CE, PL and CBM at the whole genome level.

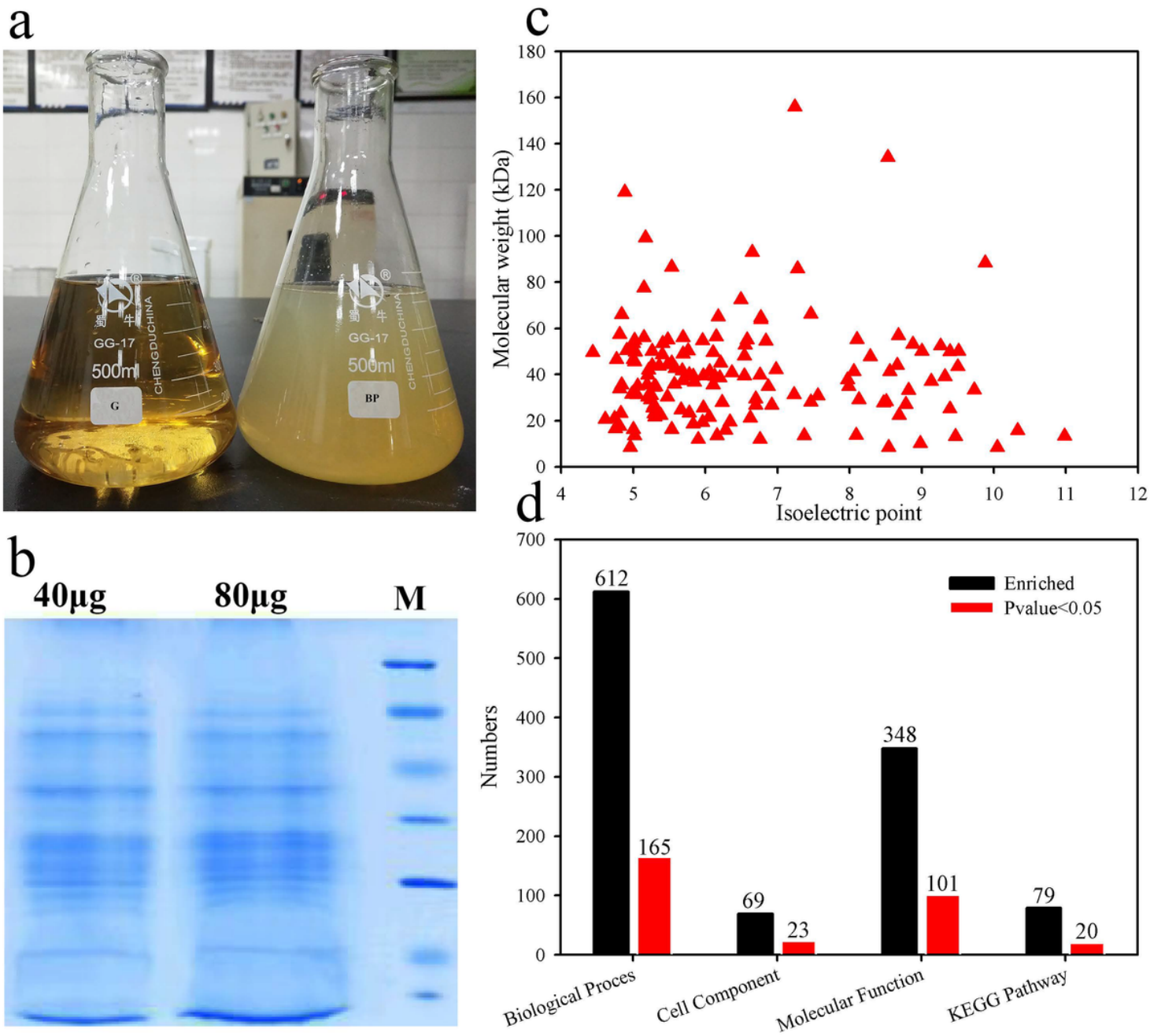


Figure 3

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 focussing 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.

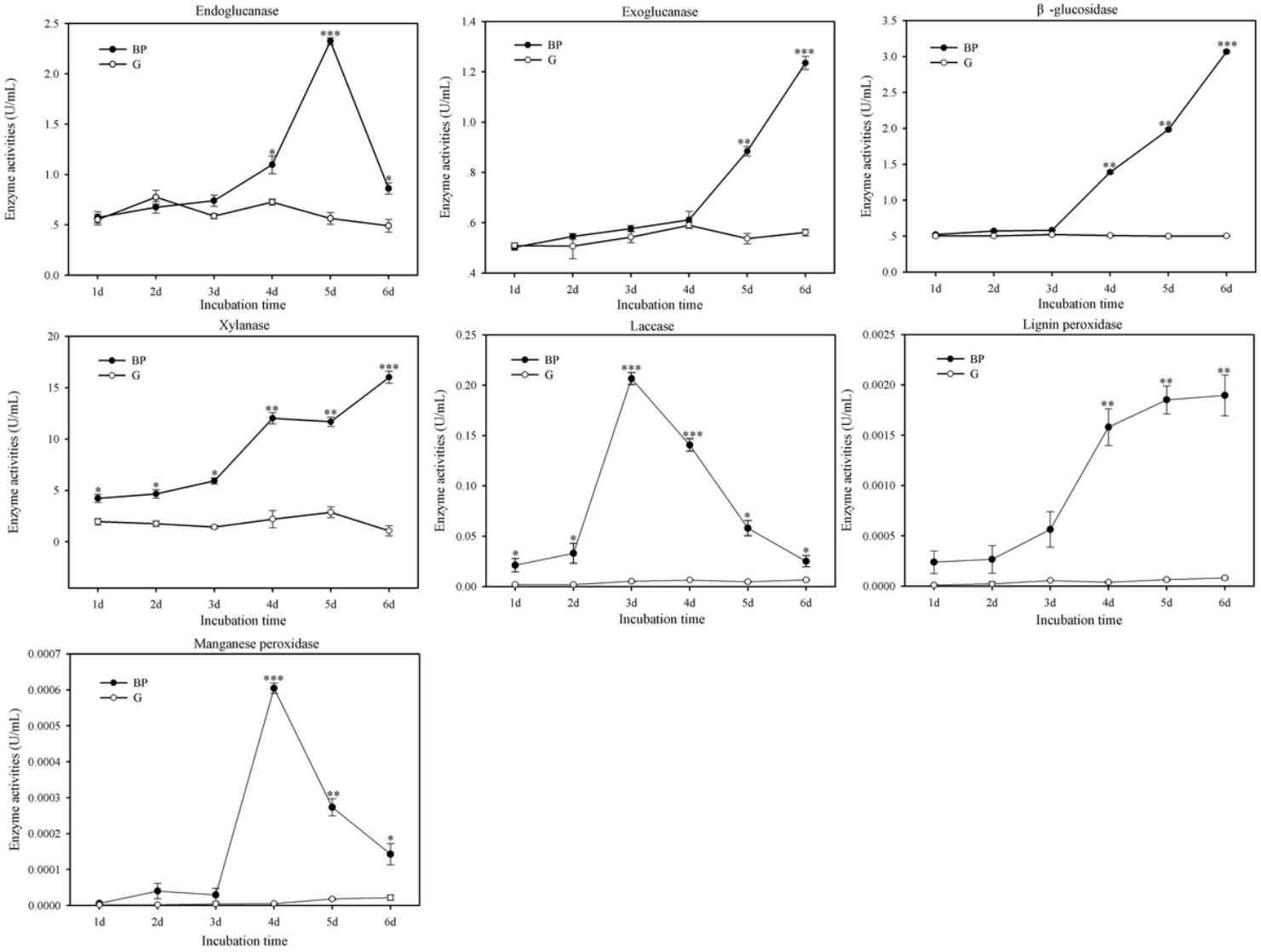


Figure 4

Lignocellulolase activities of B. velezensis LC1. The activities of endoglucanase, β-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: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

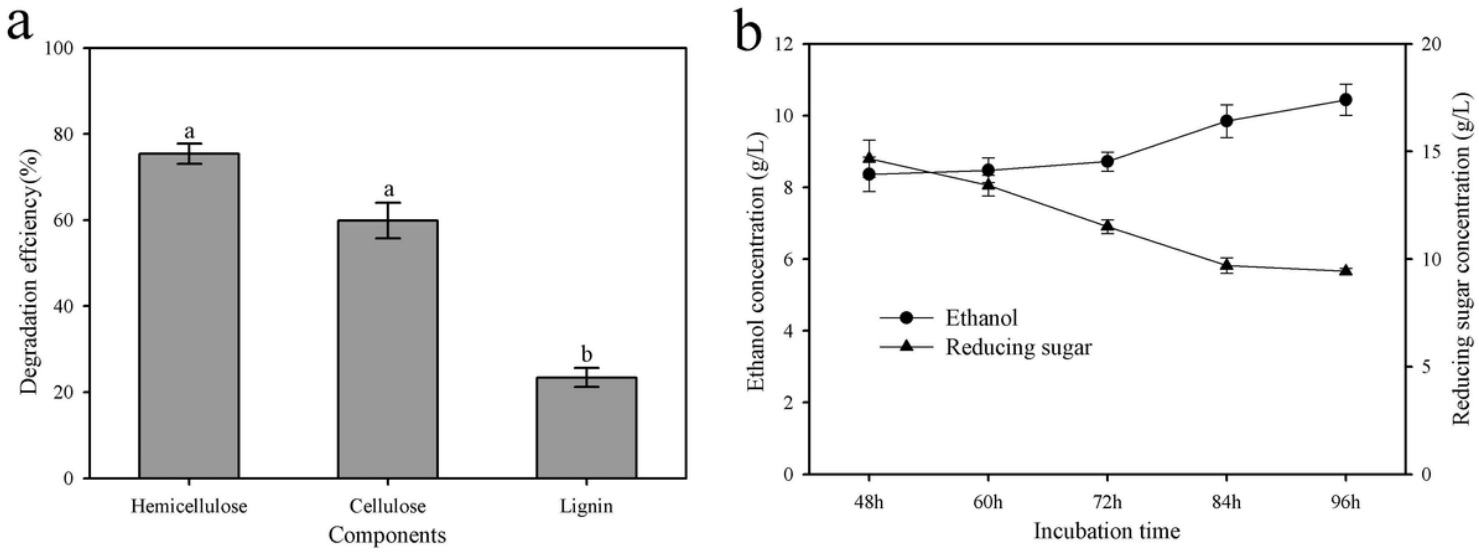


Figure 5

Lignocellulose-degrading efficiency and fermentation performance of of B. velezensis LC1 on bamboo powder. a Lignocellulose degradation efficiency. b Ethanol production and reducing sugar concentrations at 48, 60, 72, 84, and 96 h. The different letters indicate a significant difference in degradation efficiencies of different components at a p value of 0.05 (n= 3).

Supplementary Files

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