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IMPROVED ETHANOL PRODUCTION FROM SWEET SORGHUM THROUGH PRETREATMENT WITH ENZYMES FROM ITS ENDOPHYTES

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ABSTRACT

Sweet sorghum is an essential economic renewable energy crop for ethanol production through fermentation. The endophytes residing in plants decompose the plant cell walls using cellulase to access nutrients for their growth. Therefore, isolating the endophytes with high cellulase-producing capability from *Sweet sorghum* could be a potential approach for better hydrolysis of sweet sorghum, thereby increasing industrial ethanol production. In this study, forty-eight cellulase-producing endophytes were isolated from fresh *sweet sorghum* and screened by the Congo red staining method. Of them, strain X-25 with better hydrolysis activity was identified as *Bacillus* sp. through 16S rDNA sequencing. The alcohol production process was optimized by the orthogonal experiment, and the ethanol content reached a maximum of 10.84 g/100 mL. The Scanning electron microscopy (SEM) micrograph results demonstrated that cellulase in endophytic bacteria could effectively disrupt or diminish the cell wall structure of sweet sorghum straw. Overall, this study will provide a novel approach to production of ethanol from sweet sorghum straw.

Keywords: *Sweet sorghum*; Endophyte; Cellulase; Ethanol; *Bacillus*;

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INTRODUCTION

Fossil fuels are the primary energy source of humanity, but their escalating use has contributed to significant environmental and social concerns, such as greenhouse gases emission, the oil crisis, global warming, etc. (Li *et al.* 2013). Recent increase in fossil fule consumption rate, the worldwide fossil fuel reserves are projected to be exhausted within the next 40-50 years (Mohapatra *et al.* 2017). Recently, the Chinese government has a key policy objective of promoting alternative energy sources to reduce reliance on oil imports (Fu *et al.* 2019; Schmid and Xiong 2021). In this regard, bioethanol produced from plants and other materials has emerged as a promising substitute for fossil fuel and has become the research hotspot owing to its characteristics of renewable, pure, and carbon-neutral energy (Chen *et al.* 2022a; Chen *et al.* 2022b; Wen *et al.* 2022).

Sweet sorghum is a typical C4 plant that grows quickly with a life cycle of around 120-150 days (Mathur *et al.* 2017; Xie and Xu 2019) and has a high biomass yield ranging from 6.0 to 7.5 t dry matter (Hu *et al.* 2017). Furthermore, sweet sorghum has excellent water-use efficiency, using just half the water of corn and one-third of sugarcane (Hu *et al.* 2017; Xie and Xu 2019). Sweet sorghum is distributed worldwide due to its easy adaptability to various settings and less fertilizer requirement. However, it is highly suitable for agricultural cultivation in northern China (Regassa and Wortmann 2014; Hu *et al.* 2017). Therefore, sweet sorghum has gained immense recognition as the most suitable plant for biofuel production (Mathur *et al.* 2017).

The stems of sweet sorghum yield sweet juice rich in sucrose content, but the remaining solid contains a lignocellulosic residue, which is unsuitable for food application. Therefore, this solid is commonly used as a raw material for ethanol production due to its high sugar content (Cai *et al.* 2016; Castro *et al.* 2017). The polymeric sugars/lignocellulosic biomass are solubilized by pretreatment, enzymatic hydrolysis, and microbial fermentation to obtain ethanol. *Saccharomyces cerevisiae* is commonly used in fermentation for ethanol production, and numerous studies have been conducted to enhance ethanol yield through yeast fermentation. (Sasaki *et al.* 2015; Nuanpeng *et al.* 2018; Kasegn *et al.* 2023). However, transforming intricate cellulose and hemicellulose compounds into individual sugar units through biodegradation is still challenging, limiting their application further (Behera *et al.* 2014).

Organic solvents are often used for the pretreatment of sweet sorghum during ethanol production from lignocelluloses (Jafari *et al.* 2016); However, this method has drawbacks, including prolonged extraction times, excessive consumption, high energy input, and high pollution chances, affecting product quality (Zhang

et al. 2017). Therefore, recently, the pioneering facilities for ethanol production from the lignocellulosic agricultural residues have been subjected to enzymatic hydrolysis for biomass depolymerization (Lynd *et al.* 2017), which might establish a promising method for extracting bioactive compounds effectively and selectively.

Plant endophyte refers to a microorganism that resides in the intercellular space or cells of healthy plant tissue and organ for at least a part of its life cycle without interfering with the plant tissue (Zhang *et al.* 2020). These microorganisms include bacteria, fungi, actinomycetes, etc., which can be isolated by histological methods. The endophytes in sweet sorghum can break down the plant cell walls by cellulase to obtain nourishment for their growth (Zhang *et al.* 2017). Therefore, the present study sought to isolate a specific endophytic strain from sweet sorghum with high cellulase activity, to increase ethanol production.

Based on the above considerations, a feasibility test was conducted on the cellulase degrading enzymes by optimizing the relevant conditions to improve industrial ethanol production. Overall, this study will provide a theoretical basis for using new endophytic strains with proprietary enzymes to improve ethanol production from lignocelluloses of sweet sorghum.

MATERIALS AND METHODS

Experimental material: Sweet sorghum straw was collected and provided from Wuwei City, Gansu Province, identified by Dr. Yang Lin of Lanzhou University of Technology, and stored in a refrigerator at 4 °C. Commercially cellulase purchased from the Solarbio Bioengineering Co., Ltd (12 units/mg), and other reagents were acquired from GuangFu Reagent Company, China and are analytical grade.

Isolation and culture of endophytes: The stems, leaves, and roots of fresh samples were thoroughly washed under running water for 5-10 minutes and sectioned into 1 cm-sized fragments. Then, these samples were subjected to 1-minute treatment with 75% ethanol, followed by immersion in 2% NaClO solution for different durations: 5 minutes for roots, 4 minutes for stems, and 3 minutes for leaves. Later, the surface fungicide was removed by rinsing the samples six times using sterile water. Finally, the last rinsing water was collected and used to evaluate the effectiveness of sterilization (Miyamoto *et al.* 2004).

Under aseptic conditions, the surface-disinfected plant materials were kept in an ice bath before being cultured on medium I (Glucose 10g, Peptone 5g, KH_2PO_4 1.0g, $MgSO_4$•$7H_2O$ 0.5g, Bengal Red 0.2g, 15-20 g agar, 1000ml distilled water, pH 7.0). The plates were subjected to incubation at 28°C for 7-10 days. Additionally, 0.1 mL of rinsing water was incorporated into the medium A plate (Glucose 10.0g, Peptone 5.0g, KH_2PO_4 1.0g, $MgSO_4$•$7H_2O$ 0.5g, Bengal Red 0.2g, 15-20 g agar, 1000ml distilled water, pH 7.0) during the final rinse and cultured under identical conditions by evaluating the sterilization efficiency of control (Davila-Gomez *et al.* 2011).

Screening of endophytic bacteria producing cellulase: The cellulase-producing endophytes were screened by the Congo red staining plate method. Briefly, the endophytic colonies with different forms isolated by the streak plate method were inoculated into solid screening medium II (CMC-Na 20.0 g, AGAR 20.0 g, $(NH_4)_2SO_4$ 1.0g, KH_2PO_4 1.0g, Na_2HPO_4 1.0g, $MgSO_4$ 0.5 g, Congo red 0.2 g and 1000 mL water, pH 7.0). Congo red can be used as a cellulose degradation tracer in AGAR medium due to its significant affinity for cellulose, establishing a groundwork for rapid and sensitive screening of cellulose-degrading bacteria. Cellulase activity can be determined according to the diameter of the transparent zone (Hendricks *et al.* 1995; Gupta *et al.* 2012). The endophytic bacterial colonies exhibiting significant cellulase activity were selected for subsequent analyses.

Preparation of cellulase: The different isolate was inoculated with approximately 100 mL of liquid medium III (Peptone 10.0g, CMC-Na 10.0g, NaCl 5.0g, and 1000 mL water, pH adjusted to 7.0) at 37 °C (Zhang *et al.* 2017). The fermentation broth was subjected to continuous shaking (Thermostatic oscillator, SHZ-82; Changzhou Guohua Electric Appliance Co, China) at 37 °C for 48 hours and centrifuged (Centrifuge, TGL-16; Hunan Xiangyi Laboratory Instrument Development Co, China) at 5000 rpm for 20 minutes. Then, the liquid portion of the sample was collected as a crude enzyme solution for further use and stored at 4 °C. The commercial cellulase was accurately measured and then mixed with a phosphate buffer at pH 7.0 to create an enzyme solution with an activity of 10 U/mL as the control group. Meanwhile, the enzyme solution was divided into two parts, one of which was treated in a boiling water bath as a negative control.

Screening and Evaluating cellulase for Sweet sorghum bagasse (SSB) pretreatment: Sweet sorghum stalks were collected from Wuwei/Minqin County, Gansu Province, and subjected to drying at 60°C until constant weight. The sample was crushed through a 40-mesh sieve for later use. Approximately 1.0 g of powdered sweet sorghum was mixed with 9 mL of water to a 50 mL Erlenmeyer flask and sterilized with moist heat at 121°C for 20 minutes. The cellulase with equivalent enzyme activity of 200 U/mL from different strains was added to the flask; then the mixture was treated by shaking at 37 °C and 150 rpm for 48 h. The hydrolysate was centrifuged at 5000 rpm for 20 min. The liquid supernatant and the precipitation of constant weight at 60

°C were collected to analyze the fermentable sugar concentration and cellulose content.

Simultaneous Saccharification and Fermentation (SSF) for Alcohol Production with Sweet sorghum bagasse (SSB): Bioethanol was produced using the SSF process. (Zhao *et al.* 2011). The impacts of various factors on the SSF experiment were evaluated as follows: different nitrogen sources (potassium nitrate, ammonium sulfate, soybean meal, urea, sodium nitrate) and their contents (0.5%, 1%, 1.5%, 2%, 2.5%), carbon sources (SSB crushed through a 40-mesh sieve of 0.81%, 1.01%, 1.21%, 1.41%, 1.6%), potassium hydrogen phosphate (0.5%, 0.6%, 0.7%, 0.8%, 0.9%), and calcium chloride (0.05%, 0.1%, 0.15%, 0.2%, 0.25%) were added and sterilized at 121°C for 20 minutes. Then, 0.1mol/L of ferrous sulfate (0%, 0.05%, 0.1%, 0.15%, 0.2%) and 10 U/g of the prepared cellulose were added after sterilization with a 0.22 μm microporous filter membrane. *Saccharomyces cerevisiae* was inoculated with 1 % and fermented for 96 hours at 37 °C and 120 rpm. The ethanol content was further analyzed. Moreover, a five-factor four-level orthogonal experiment was designed and employed based on the results of the single-factor experiments, and the final optimized process was obtained after analyzing and comparing the ethanol content. Analysis of variance (ANOVA) was performed in SPSS 17.0 software and comparisons with $p \leq 0.05$ were considered as significantly different (Mishra *et al.* 2019)

Analytical methods: The cellulase activity was determined and defined according the previously reported method (Zhang *et al.* 2017). The 3,5-dinitrosalicylic acid (DNS) method was employed to quantify the reducing sugar content in the hydrolysate (Lindsay 1973). The cellulase content was determined using the previously reported potassium dichromate iodometric titration method (Wang *et al.* 2014). Briefly, the cellulose content of a sample is determined by measuring the amount of potassium dichromate consumed during the reaction. Ethanol concentrations were determined according to the standard method of GB-T 12288-1990 (ISO 1998).

Scanning electron microscopy (SEM): The impacts of diverse cellulases on the structural integrity of sweet sorghum were investigated by SEM (JSM-5600LV; JEOL Ltd, Japan). The sweet sorghums were supplied with a non-enzyme control or endophyte-derived cellulase. The sample was secured to a gold-coated black tape, coated with gold and then subjected to an acceleration using 20 kV voltage (100 μm, 100× magnification;10 μm, 2000× magnification) under high vacuum conditions to observe local detail features(Zu *et al.* 2009; Li *et al.* 2012).

Strain identification: Genomic DNA extraction from X-25 was carried out using the Ezup column DNA Extraction Kit specifically designed for bacterial genomic DNA, as per the manufacturer's instructions. (Sangon Biotech, Shanghai, China). The extracted DNA was amplified by Polymerase chain reaction PCR to obtain 16S RNA. PCR was performed in 50 μL of reaction mixture (5 μL of 10× PCR buffer, 4 μL of 2.5 mmol / L dNTPs, 1 μL of 5 μmol/L of reverse primer, 1 μL of 5 μmol/L of positive electrode material, 0.5 μL of Taq enzyme, 1 μL of template) and added 50 μL of double distilled water. The reaction conditions were as follows: denaturation at 94 ° C for 3 minutes, then denaturation at 94 ° C for 30 seconds, annealing at 56 ° C for 30 seconds, extension at 72 ° C for 50 minutes at the end of the cycle, 7 minutes at 72 ° C, and finally cooling to 4 ° C (Hu *et al.* 2021a). The 16S rDNA gene sequences were performed using GenBank database (http://www.ncbi.nlm.nih.gov/). The retrieved reference sequences from GenBank were aligned and compared with the already determined sequences using the multiple sequence alignment program CLUSTAL X version 1.81. Using the molecular evolution genetic software for analysis MEGA version 3.1 program, a phylogenetic tree was generated (Zhang *et al.* 2017).

RESULTS

Endophyte isolation and cellulase-producing microorganism screening: In screening medium B, a total of 129 endophytes exhibiting diverse morphologies were successfully obtained from the leaves, stems, and roots of sweet sorghum using Congo red staining. This method yielded 48 isolates, which were numbered X1-X48. As shown in Table 1, the cellulase activity of 10 strains was ≥30 U/mL, among which the cellulase activities of strains X-19 and X-25 reached 66.679 and 57.586 U/mL, respectively. Furthermore, the strain X-25 with better hydrolysis activity was subjected to 16S rDNA sequencing. The sequence search and homology comparison were performed by BLAST at National Center of Biotechnology Information (NCBI) (http://blast.ncbi.nlm.nih.gov). The phylogenetic tree was constructed using MEGA 7.0 software, and a UPGMA-type phylogenetic tree was selected with a bootstrap value set to 1000 iterations. (Fig. 1). In the database, the X-25 strain exhibited a sequence similarity of 100% with the known *Bacillus pumilus*, the X-25 strain was tentatively classified as *Bacillus sp*.

Screening and Evaluating cellulase for Sweet sorghum bagasse (SSB) pretreatment: The total sugar and cellulose contents were measured, and the summarized data can be found in Table 1. As indicated in Table 1, compared with commercial cellulase, the enzymes produced by the strains X-7, X-19, X-16, and X-25 had better sugar hydrolysis and cellulose degradation yields. Of them, the enzyme produced by strain X-25 showed the highest sugar production of 433.19 μg/mL and cellulose

degradation yield of 86.16%. So strain X-25 was preserved for further research.

Optimization of the alcohol production process of SSF in SSB by an orthogonal method: The results of the effects of different factors on SSF alcohol production in SSB are depicted in Fig. 2. As shown in Fig. 2A, compared with other nitrogen sources, soybean meal showed the best effect in producing alcohol and can be used as a high-quality nitrogen source. Furthermore, adding 1.5% soybean meal as a nitrogen source in different amounts (as shown in Fig. 2B) achieved the highest alcohol production. Upon utilizing sweet sorghum as the carbon source, the highest alcohol yield was obtained at the addition of 5-6 g/100 mL (Fig. 2C). The effects of adding different ions, including potassium, calcium, and iron, on alcohol production showed a bell-shaped distribution, and the optimum addition amounts of K_2HPO_4, $CaCl_2$, and $FeSO_4$ were 0.7%, 0.15%, and 0.1%, respectively (Fig. 2D, 2E, 2F).

According to the single-factor test results, a five-factor four-level orthogonal test was developed, and the results are summarized in Table 2. The results of analysis data are shown in Table 3. Ferrous ion concentration had less relevance and was further set as the error term. An orthogonal array test was performed, revealing that nitrogen concentration had a significant influence on ethanol content. Based on the R-value obtained by the range analysis method, the effects of various factors on alcohol production were in the order of nitrogen concentration > potassium ion concentration > calcium ion concentration > ferrous ion concentration > carbon content. Based on the orthogonal experiment results, the optimal combination for alcohol production was determined as A2B2C1D4E3. According to the orthogonal experiment results, the final optimized alcohol production process included: the carbon source of 6%, the nitrogen source of 1.5%, and the addition amounts of 0.6% K_2HPO_4, 0.2% $CaCl_2$, and 0.15% $FeSO_4$, respectively. The ethanol content reached a maximum of 10.84 g/100 mL under the above conditions.

Scanning electron microscopy (SEM): The effect of cellulase treatment on cell wall degradation of sweet sorghum straw was evaluated, and the microstructure changes of straw under enzymolysis and non-enzymolysis were compared by scanning electron microscopy. The differences between sweet sorghum and treated enzyme, sweet sorghum and non-enzyme treatments, sweet sorghum and enzyme from endophytes were shown in Fig 3. In the non-enzyme treatment, no ruptures or severe damages to the microstructure were observed. (Fig. 3A). However, subsequent to the application of endophyte cellulase, the cell wall of sweet sorghum underwent thinning, leading to a disorganized microstructure. As depicted in Fig. 3B and C, the sweet sorghum straw's cell wall was significantly destroyed. The electron microscopy results indicate that the cellulase of endophytic bacteria can effectively disrupt or compromise the integrity of the cell wall structure of sweet sorghum straw by releasing more cellulase.

Table 1. The characteristics of cellulase-producing endophytes isolated from *Sweet sorghum*..

No.	Cellulase activity	Sugar content	Cellulose
X-33	36.792	245.037	67.511
X-2	32.703	250.963	69.762
X-35	37.264	252.444	32.880
X-5	33.017	267.259	24.693
X-4	31.444	279.111	28.620
X-7	37.893	317.630	59.077
X-19	66.679	319.110	70.899
X-16	30.343	347.259	72.137
X-25	57.856	433.185	86.159
Commercial cellulase	94.049	276.148	61.715
Control	-	201.356	20.635

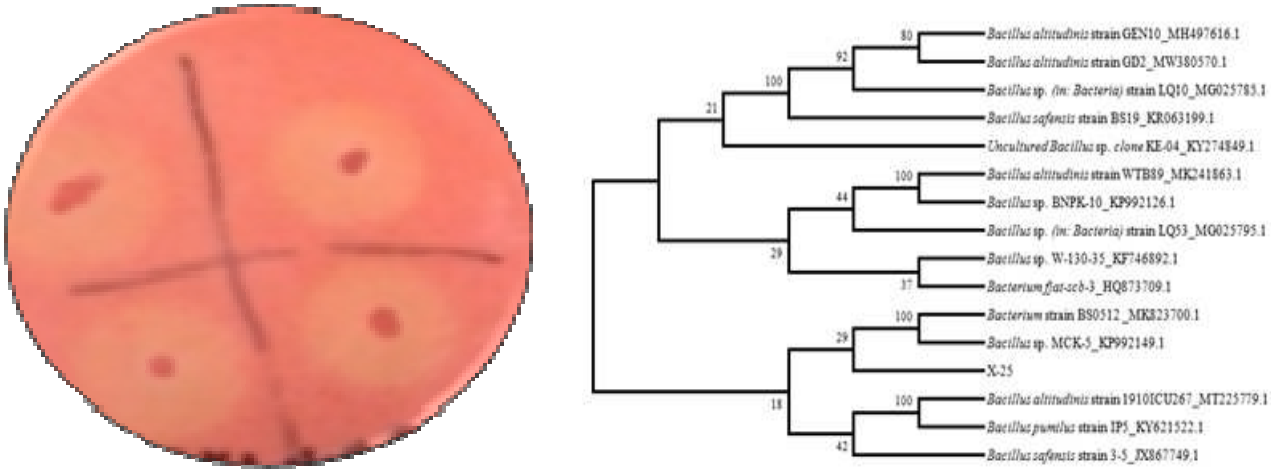
Table 2. Five-factor four-level orthogonal experimental results.

Experimental Number	Experimental conditions and levels					Ethanol content (g/100mL)
	A	B	C	D	E	
	Sweet sorghum (g/100ml)	Soybean meal (%)	K2HPO4 (%)	FeSO4 (%)	CaCl2 (%)	
1	5	1	0.6	0	0.1	7.92
2	5	1.5	0.7	0.05	0.15	10.14
3	5	2	0.8	0.1	0.2	7.36

4	5	2.5	0.9	0.15	0.25	8.89
5	6	1	0.7	0.1	0.25	10.7
6	6	1.5	0.6	0.15	0.2	10.84
7	6	2	0.9	0	0.15	7.78
8	6	2.5	0.8	0.05	0.1	8.48
9	7	1	0.8	0.15	0.15	7.78
10	7	1.5	0.9	0.1	0.1	8.48
11	7	2	0.6	0.05	0.25	8.34
12	7	2.5	0.7	0	0.2	10.14
13	8	1	0.9	0.05	0.2	8.89
14	8	1.5	0.8	0	0.25	10.56
15	8	2	0.7	0.15	0.1	8.61
16	8	2.5	0.6	0.1	0.15	7.64
K1	8.58	8.82	8.69	9.1	8.37	
K2	8.7	10.01	9.79	8.96	8.34	
K3	8.69	8.02	8.55	8.55	9.31	
K4	8.92	8.79	8.51	9.03	9.62	
R	0.34	1.99	1.28	0.55	1.25	

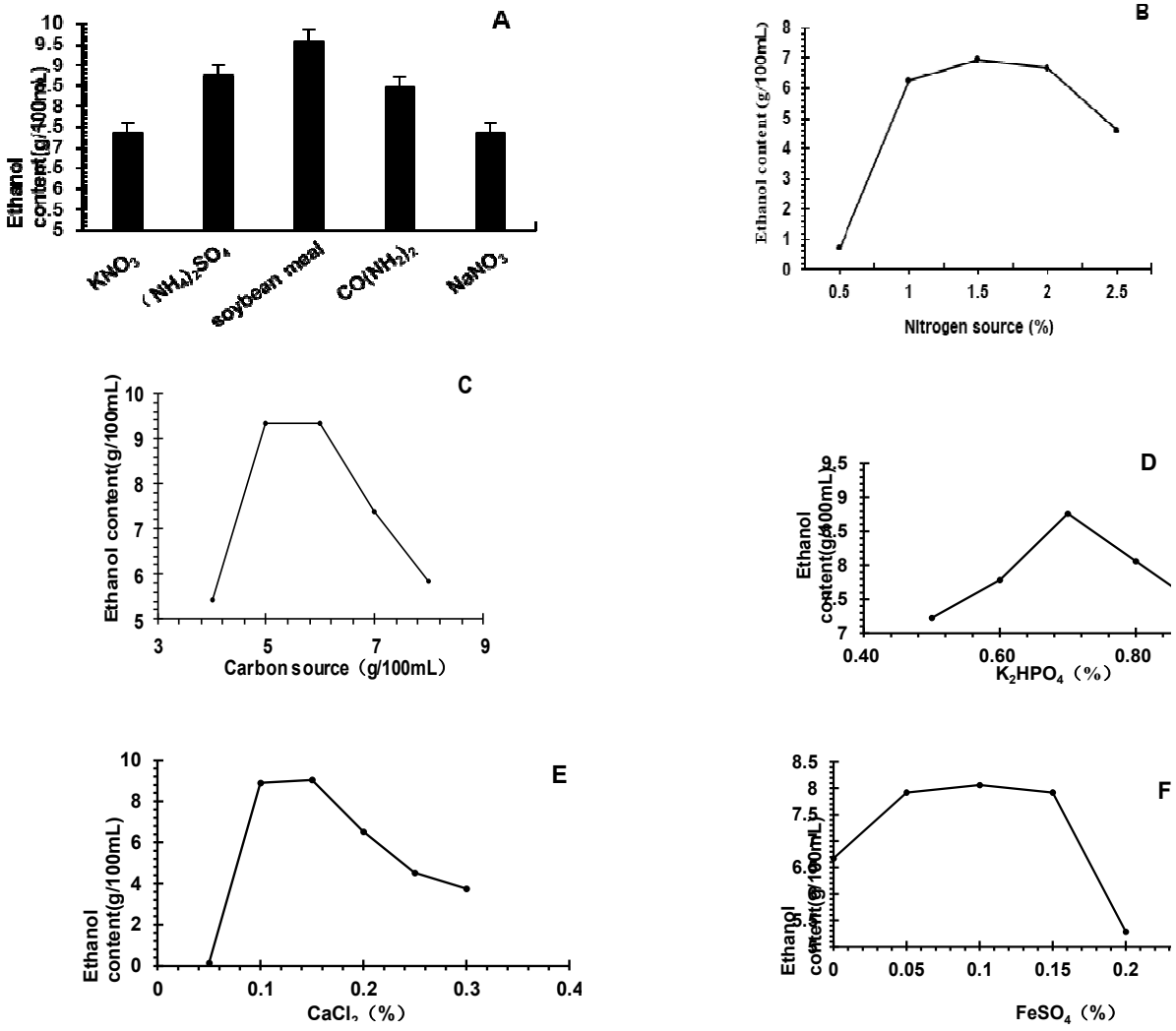
Table 3. ANOVA of simultaneous saccharification and fermentation for alcohol production with sweet sorghum bagasse.

Source	Sum of squares	Degree of freedom	Mean square	F	P
Modified model	20.266	12	1.689	6.792	0.071
Intercept	1270.031	1	1270.031	5107.921	0
A	1.812	3	0.604	2.429	0.243
B	8.037	3	2.679	10.775	0.041
C	5.276	3	1.759	7.073	0.071
E	5.141	3	1.714	6.892	0.074
Pure error	0.746	3	0.249		
Total	1291.044	16			
Revised total	21.012	15			
R^2	0.965				

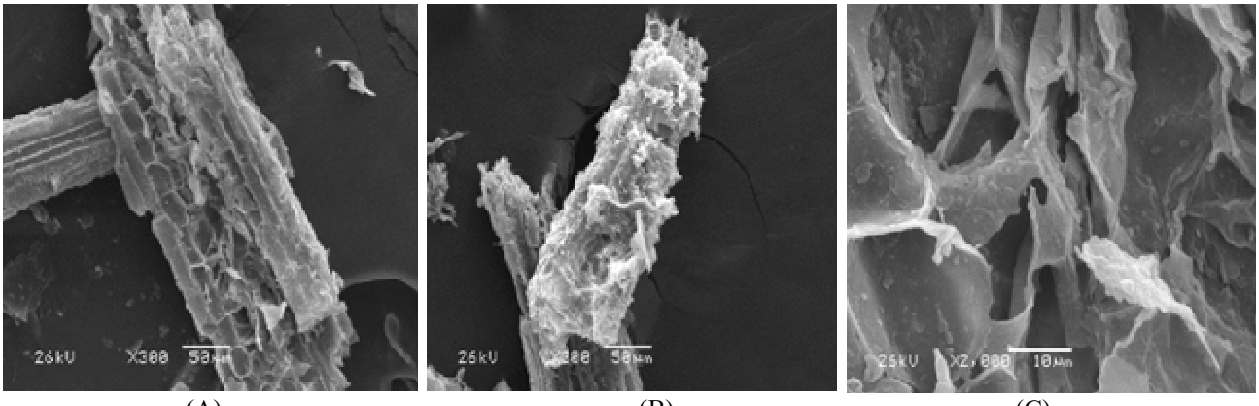


(a) (b)

Figure 1 The Cellulase-producing strain isolated from *Sweet sorghum.* (a) Cellulase-producing strains screened by the Congo red staining method; (b) The phylogenetic tree of X-25 strain based on 16S rRNA gene sequencing.



BFigure 2 The effects of different factors on production of alcohol by SSF in SSB. (A) Different nitrogen sources; (B) Nitrogen source (%); (C) Carbon source (g/100mL); (D) K_2HPO_4 (%); (E) $CaCl_2$ (%); (F) $FeSO_4$ (%).



(A) (B) (C)

Figure3 The microscopic morphology of Sweet sorghum under different treatment processes by SEM analysis. (A) The non-enzyme treatment; (B, C) Endophytes cellulase treatment at 300× and 2000×magnification.

DISCUSSION

Energy is the driving force of social development. In recent years, the escalating energy crisis and environmental deterioration have amplified the need to discover a secure and dependable energy source. Sweet sorghum, classified as an annual C4 herbaceous plant of the Gramineae sorghum genus, can reach a height of more than 3 m. Currently, it is regarded as a highly advantageous energy crop as the sugar in the stalk can be quickly converted into clean and safe fuel ethanol through fermentation, which can effectively alleviate the energy supply crisis, the ecological environment deterioration and promote sustainable development of the economy. Accumulating studies have focused on the agronomic performance of sweet sorghum, such as planting, growth, and harvest time, and ethanol production (the transformation of soluble sugar into juice) (Fernandes *et al.* 2014; Sasaki *et al.* 2014; Wang *et al.* 2015a; Oyier *et al.* 2017; Teixeira *et al.* 2017). However, research on the utilization of residual cellulose is rare (Forte *et al.* 2017; Ammar *et al.* 2020). In the present study, the SSB was hydrolyzed using the endophytic cellulase to produce ethanol, resulting in a high ethanol yield of 10.84 g/100 mL. This yield increased by 49.7% compared to the conventional extraction method without enzymes, which yielded 7.24 g/100 mL.

Potato glucose agar (PDA) is the most widely utilized medium for endophytic bacteria isolation. In a previous study, a PDA medium was utilized for isolating cellulase from different plant endophytes, including *Sauvignon Cabernet* (Liu *et al.* 2016), *Dendrobium nobile* and *Dendrobium chrysanthum* (Chen *et al.* 2012), *Cannabis sativa* (Gautam *et al.* 2013), and seven oil production plant species (Han *et al.* 2013). In this study, the CMC AGAR medium containing cellulase as the sole carbon source was employed to obtain the cellulose-producing endophytes. The cellulase-producing endophytes were screened rapidly and accurately using Congo red staining, and the ability of the isolated strain to produce cellulase was confirmed by comparing the sizes of the transparent circles. This method plays a crucial role in the screening of cellulase-producing endophytic bacteria. Accumulating studies have reported the potential of endophytic cellulase isolated from sweet sorghum to increase sweet sorghum ethanol production (Infanzón-Rodríguez *et al.* 2020). These findings indicated that the enzyme extracted from endophytic plant X-25 could significantly improve the yield of bioethanol. The superior chimerism between plant cell walls and endophytic cellulases significantly contributes to the formation of the cellulose reticular skeleton (Payne *et al.* 2015; Hu *et al.* 2021b). The mechanism underlying ethanol production by cellulase activity is as follows: the increased cellulase activity promotes the cleavage efficiency of the β1-4 glycosidic bond, increasing ethanol production. Several studies have reported that commercial cellulase could not increase the efficiency of glycyrrhizic acid extraction from *Radix Glycyrrhizae* (Jia *et al.* 2022), paeoniflorin from *Paeonia lactiflora* Pall, or paeonol from Cortex Moutan Radicis compared to cellulase derived through conventional processing techniques (Liu *et al.* 2015). Therefore, the suitability of commercial cellulase for enzymatic hydrolysis of plant cell walls to extract various plant active components may not always be optimal. However, the cellulase produced by the endophytes isolated from sweet sorghum possesses a strong cellulolytic ability and undergoes process optimization to produce ethanol. In the present study, the use of sweet sorghum residue after the production of ethanol was evaluated using electron microscopy.

Feng *et al.* (2007) screened out a cellulolytic strain with stable enzyme production and mixed it with *S. cerevisiae* to ferment the corn straw to obtain fuel ethanol. As a result, the process effectively eliminated the inhibition of product feedback of cellulolytic bacteria and high sugar on yeast fermentation and obtained a final ethanol yield 16 g/100 g after fermentation. Wang *et al.* (2015b) screened *Bacillus licheniformis* Z4 and *Bacillus licheniformis* S5 and found that they have a stronger ability to decompose cellulosic substances. The co-inoculation of these two bacteria significantly increased the speed of decomposing cellulosic substances than that of any single strain, indicating that the decomposition of cellulosic substances requires the combined action of multiple microorganisms. Therefore, these processes can be adopted in future studies for further optimization to obtain a high alcohol yield from sweet sorghum. However, to ensure the economic viability of large-scale commercial bioconversion processes, new and effective approaches are highly necessitated to decrease the cost of cellulolytic enzymatic cocktails (Maehara *et al.* 2018).

Sweet sorghum is composed of lignin, cellulose, and hemicellulose, which interconnect to form the constituents of the plant cell wall structure. Under conventional practices, acid or alkali hydrolysis degrades cellulose or hemicellulose into oligosaccharides, which are then fermented to produce ethanol. This method has a low sugar conversion rate, high corrosiveness to equipment, and high equipment investment and operating costs. In contrast, the newly developed enzyme technology is mild and eco-friendly. Studies have reported significant genetic differences between different sweet sorghum varieties, such as juice yield, total sugar content, fermentation capacity, and production of ethanol (Mocoeur *et al.* 2015; Ekefre *et al.* 2017; Uchimiya *et al.* 2017). Therefore, the effect of this newly developed process on different varieties of sweet sorghum needs further exploration. Additionally, the reasonable and effective utilization of straw residue after fermentation still needs solid research, such as nutrient-rich cattle and

sheep feed, heat source fuel, and organic fertilizer raw materials.

In conclusion, sweet sorghum has gained global attention as an energy crop and has been extensively investigated as a valuable feedstock for ethanol production. The present study results demonstrate that the enzyme-assisted method can achieve high extraction rates of polysaccharides, which is beneficial for industrial ethanol production. This study provides an innovative method for broadening the application of sweet sorghum straw in terms of ethanol production.

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Conflicts of Interest: There are no conflicts to declare.

Ethical Compliance: The study does not include human and animal experiments.

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