THE ISOLATION, IDENTIFICATION, AND OPTIMIZATION OF ENZYME PRODUCTION CONDITIONS FOR CELLULASE-PRODUCING STRAIN LJ-10 FROM CHILI PEPPER STRAW

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Abstract. Cellulose-degrading fungi can secrete cellulase to degrade cellulose in straw. One strain of cellulase-producing fungus was isolated and screened from pepper straw and named LJ-10. Morphological and molecular analyses identified strain LJ-10 as belonging to *Fusarium equiseti*. The results of the single-factor experiment indicated that, for strain LJ-10, the optimal carbon source, carboxymethyl cellulose sodium, was 20 g/L; the optimal nitrogen source, peptone, was 2 g/L; the optimal pH value was 7; the optimal liquid volume was 50/150 mL; and the optimal inoculation volume was 2%. The response surface optimization showed that enzyme production was optimal when the nitrogen source peptone was 0.217% (2.17 g/L), the carbon source CMC-Na was 2.044% (20.44 g/L), and the inoculation dosage was 2.14%, resulting in 44.52 ± 0.22 U/mL of enzyme activity, which was close to the predicted value of 45.781 U/mL. Moreover, the CMC enzyme activity of strain LJ-10 increased 2.18-fold compared with that before optimization. On the 40th day of cultivation, the degradation rate of chili straw by strain LJ-10 was 41.93%. After optimization, the CMC enzyme activity of strain LJ-10 increased significantly, providing a reference for the subsequent development and application of cellulose-degrading bacteria.

Keywords: *cellulose-degrading fungi, Fusarium equiseti, carboxymethyl cellulase, single-factor optimization, responsive surface optimization*

Introduction

China is the world's largest vegetable producer, ranking first in global vegetable output. In China, the vegetable cultivation industry holds a position second only to the grain crops, and is characterized by not only high yields but also significant economic benefits (Tang et al., 2023). Post-harvest commercialization processes can significantly enhance vegetable quality and increase market value. Despite their benefits, these post-harvest processes generate considerable waste, including inedible parts (roots, stems, leaves) and packaging materials like vegetable trays. This residual biomass is commonly referred to as 'tail vegetables' or 'vegetable waste.' (Szymańska-Chargot et al., 2017; Zhang and Dong, 2022). With their substantial production volume and high moisture content, tail vegetables can lead to serious environmental damage if not managed properly, whether through uncontrolled disposal or unprocessed field incorporation (Hasan-Beikdashti et al., 2012; Szymańska-Chargot et al., 2011). Vegetable waste is rich in structural components such as cellulose and hemicellulose, among which cellulose degradation represents a critical step in tail vegetable treatment

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(Wang et al., 2024). Cellulose, in particular, possesses a dense and highly stable molecular structure. This stability arises from the tight packing of glucan chains through extensive non-covalent interactions, forming a robust crystalline architecture that exhibits inherent recalcitrance to enzymatic and microbial degradation (Sruthy et al., 2025).

Extensive research has demonstrated that cellulolytic microorganisms exhibit significant efficacy in cellulose decomposition (Chukwuma et al., 2021). Several studies have demonstrated that cellulolytic microorganisms originate from diverse ecological niches. Cellulolytic microbes are present in materials such as min pigs manure (Li et al., 2020), silkworm excrement (Li et al., 2023), soil (Ma et al., 2024), leaves (Khosravi et al., 2022), and humus soil (Zhang et al., 2023). The functional roles of cellulolytic microorganisms are multifaceted. Notably, these microbes act as efficient biocatalysts that significantly accelerate lignocellulosic biomass degradation, including agricultural residues such as crop straw (Gao et al., 2023), these microorganisms function as effective composting accelerators, enhancing the decomposition rate of organic matter during the composting process (Soto-Paz et al., 2022). Furthermore, cellulolytic microbes can be synergistically combined with complementary microbial consortia to enhance organic matter degradation efficiency. This co-cultivation approach not only accelerates biomass decomposition but can also be optimized for value-added bioproducts such as bioalcohols through controlled fermentation processes (Fu et al., 2024). The taxonomic and functional diversity of cellulolytic microorganisms has been extensively characterized, with robust experimental evidence confirming the cellulose-degrading capacity of bacterial species including "*Bacillus cereus*" (Wang et al., 2024), "*Bacillus subtilis*" (Zhang et al., 2023), "*Trichoderma longibrachiatum*" (Rani and Dhoble, 2023), and "*Penicillium oxalicum*" (Waghmare et al., 2021). Cellulolytic microorganisms preferentially metabolize the structural cellulose component of crop residues through enzymatic depolymerization, breaking down the *β-1,4*-glycosidic bonds to yield oligosaccharides and glucose monomers. This biochemical transformation not only accelerates residue decomposition but also mineralizes organic matter, thereby liberating bioavailable nutrients essential for soil fertility (Lu et al., 2025).

Fusarium spp. is widely recognized as phytopathogenic fungi, with extensive literature documenting their capacity to infect diverse plant hosts and induce disease pathogenesis. Numerous studies have characterized the molecular mechanisms underlying *Fusarium's* pathogenicity, including mycotoxin production and vascular tissue colonization (Kim et al., 2012). The pathogenic success of *Fusarium* spp. stems from their ability to enzymatically degrade plant structural barriers and suppress host defense mechanisms, thereby facilitating host invasion and tissue colonization, this process involves the coordinated secretion of cell wall-degrading enzymes (CWDEs) including cellulases, xylanases, and pectinases, which compromise the plant's physical defenses while creating pathways for hyphal penetration (Ali et al., 2012; Christakopoulos et al., 1996; Huang et al., 2015).

Cellulolytic microorganisms demonstrate dual agricultural applications, serving both as composting accelerators and as bioinoculants for in-situ crop residue management through straw incorporation. This application accelerates the decomposition of straw, thereby improving soil conditions. Simultaneously, the process converts the resulting nutrients into nourishment for subsequent crops. Furthermore, cellulolytic microbes are instrumental in the production of various biotechnologically valuable products, presenting a broad spectrum of potential applications.

Materials and methods

Sampling location and main culture media

The pepper stalk samples were obtained from the production base of Shenyang Normal University and stored at 4°C for subsequent analysis.

Potato Dextrose Agar (PDA): potatoes 200 g/L, glucose 20 g/L, agar 20 g/L.

Potato Dextrose Broth (PDB): potatoes 200 g/L, glucose 20 g/L.

Hutchinson's liquid medium: The medium formulations are based on the paper by Gao et al. (2023).

Screening medium: sodium carboxymethyl cellulose (CMC-Na) 20 g/L, $MgSO_4$ 0.5 g/L, K_2HPO_4 1 g/L, NaCl 0.5 g/L, agar 20 g/L, natural pH.

Liquid enzyme production medium (CMC): CMC-Na 10 g/L, $MgSO_4 \cdot 7H_2O$ 0.2 g/L, K_2HPO_4 1 g/L, yeast extract 3 g/L.

Starch-free filter paper strips: 1 × 6 cm (50 ± 0.5 mg) filter paper strips were soaked in glacial acetic acid for 12 h to obtain starch-free filter paper strips.

The aforementioned culture media were sterilized at 121°C for 30 min.

Methods

Isolation and screening of cellulose-degrading fungi

The fungal isolation procedure commenced with extensive washing of pepper stalks under continuous water flow to eliminate surface contaminants. After air-drying on sterile filter paper in a biosafety cabinet, samples were mechanically disintegrated into uniform 0.5 cm segments. These fragments were subsequently transferred to CMC for selective growth of cellulolytic fungi. Distinct colonies were subsequently isolated on PDA through three-phase streaking and maintained at 28°C (Guo et al., 2021), a control group was established by spreading 100 μL of sterile water on a PDA plate. Upon initial fungal colony emergence on PDA plates, hyphae were isolated and successively transferred to fresh PDA plates until consistent hyphal and spore morphology was observed under an optical microscope (10×40 magnification). Purified fungal isolates were cryopreserved in 30% glycerol (v/v) at -80°Cfor long-term storage (Sanjay et al., 2016).

Initial and subsequent screening of cellulose-degrading fungi

The purified strains were cultured on PDA medium at 25°C for 5-7 days (inverted position). Activated cultures were then transferred to screening medium plates using a 9-mm cork borer and incubated at 25°C for 3-5 days. Selected strains with vigorous growth were stained with Congo red (1 g/L, 30 min) and subsequently destained with 1 M NaCl (30 min). The hydrolytic activity was quantified by measuring the diameters of the clear zone (D) and the corresponding colony (d), with results expressed as the D/d ratio (Zhang et al., 2023).

Fungal strains demonstrating chromogenic halos were aseptically inoculated into Potato Dextrose Broth (PDB) and incubated at 25°C with orbital shaking (120 rpm) for 72 h to prepare seed cultures. A 2% (v/v) inoculum (1.4 mL) of the seed culture was aseptically transferred to two separate media: (1) 70 mL of liquid enzyme production medium and (2) 70 mL of Hutchinson's liquid medium containing a strip of starch-free filter paper. The cultures were then incubated at 25°C with orbital shaking at 120 rpm for 72 h. Following cultivation, 2 mL aliquots of culture broth were centrifuged (8000 rpm, 10 min, 4°C) to obtain cell-free supernatants. The resulting supernatants, serving as crude enzyme preparations, were subsequently analyzed for cellulolytic activity using the 3,5-dinitrosalicylic acid (DNS) method. Specifically, carboxymethyl cellulase (CMCase) and filter paperase (FPAase) activities were quantified colorimetrically. he degradation of filter paper strips in Hutchinson's liquid medium was monitored (Fu et al., 2024).

Glucose standard solutions (0.0, 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 mg/mL) were prepared, and 3.0 mL of DNS reagent was added to each tube. The mixtures were heated in a boiling water bath (100°C) for 5 min, followed by cooling to room temperature. Distilled water was added to adjust the total volume to 15 mL, followed by thorough mixing. Absorbance at 540 nm (OD_{540}) was then measured to generate the standard curve (Mansour et al., 2016).

Enzyme activity (U/mL) was defined as the amount of cellulase required to liberate 1 mg of glucose from carboxymethyl cellulose (CMC-Na) per minute per mL of enzyme solution under standard conditions (pH 4.8, 50°C) (Zhang et al., 2023).

$$\text{Enzyme activity (U/mL)} = \frac{X \times N \times 1000}{W \times T} \qquad \text{(Eq.1)}$$

where X represents the glucose content (mg) obtained from the glucose standard curve; N represents the dilution factor of the enzyme solution; W represents the volume of enzyme solution added in the reaction (mL); and T represents the enzymatic reaction time (min).

Morphological observation and molecular identification of fungi

The selected fungal isolates were inoculated onto PDA plates and incubated at 25°C for 72 h. Hyphal fragments and spores were aseptically collected from colony margins using a sterile inoculation needle and transferred to glass slides containing lactophenol cotton blue stain. After applying a coverslip, hyphal and spore morphology was examined microscopically. Genomic DNA was subsequently extracted from the fungal strain using the Solarbio Fungal DNA Extraction Kit following the manufacturer's protocol. The internal transcribed spacer (ITS) region was amplified by polymerase chain reaction (PCR) using universal fungal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Kumar and Shukla, 2005). PCR products were commercially sequenced (Sangon Biotech, Shanghai), and the resulting sequences were compared with NCBI database entries using BLAST analysis. Phylogenetic analysis was conducted using MEGA11.0 software with the neighbor-joining algorithm (Saitou and Nei, 1987). Bootstrap analysis with 1000 replicates was performed to assess node support, and the resulting tree was used to determine the phylogenetic position of the strain. The sequence was subsequently uploaded to the NCBI website to obtain the strain's accession number.

Single-factor optimization and response surface optimization

The effects of carbon sources (Lactose, Maltose, Potato extract, CMC-Na, Starch, Sucrose, Glucose, Mannitol), nitrogen sources (Urea, $NaNO_3$, Tryptone, $(NH_4)_2SO_4$, Peptone, Beef extract, Yeast extract), pH (3, 4, 5, 6, 7, 8, 9, 10, 11), inoculum size (1%, 2%, 3%, 4%, 5%, 6% of the culture medium volume), and liquid volume (50, 60, 70, 80, 90/150 mL) on the CMCase production of the strain were determined using the single-factor method. The optimal concentration ranges for the carbon and nitrogen sources, which exhibited the most significant impact on enzyme activity, were set at 5, 10, 15, 20, and 25 g/L, and 1, 2, 3, 4, 5, 6, and 7 g/L, respectively. The CMCase activity of LJ-10 was measured to determine the optimal concentrations of the most suitable carbon and nitrogen sources.

Building upon the single-factor optimization of the culture medium formulation, nitrogen source (*A*), carbon source (*B*), and inoculum size (*C*) were selected as influencing factors. The carboxymethyl cellulase (CMC) enzyme activity of LJ-10 served as the evaluation index. A three-factor, three-level response surface analysis was conducted using a *Box-Behnken* design within Design-Expert 8.0.6 software. The optimal culture conditions, determined through a combination of model prediction and experimental validation, were subjected to three parallel trials to verify the model's accuracy.

The degradation efficiency of cellulose-degrading fungi

Pepper straw samples were collected from the production base of Shenyang Normal University. The samples were initially cut into 1-cm segments. They were subsequently dried at 65°C until a constant weight was achieved. Exactly 1.0 g of the dried pepper straw was accurately weighed and subjected to degradation treatments according to the protocols established both before and after response surface optimization. According to the culture protocols before and after the optimization of the response surface, the medium was prepared and the strains were inoculated. The conical flask was incubated by orbital vibration at a speed of 120 rpm at 25°C. The dry weight of chili stems in the culture medium was measured every 10 days to determine the degradation rate.

The degradation rate (%) of the pepper straw was calculated using the following formula:

$$\text{Degradation rate (\%)} = \frac{W_0 - W_1}{W_0} \times 100 \qquad \text{(Eq.2)}$$

where W_0 represents the initial dry weight of the pepper straw in the medium before inoculation. W_1 represents the dry weight of the pepper straw remaining after the incubation period and subsequent drying.

Data processing

All the above experiments were repeated three times. The results of the single-factor experiment were processed using IBM SPSS Statistics 27. The mean and standard deviation were tested using the single-factor *ANOVE* test, and the significant differences between treatments were tested using the graph basis test. The final results were expressed in the form of mean ± standard deviation, with lowercase letters indicating significant differences. Different letters represent groups with statistically significant differences, while the same letters indicate no significant differences. Import the calculation results into Origin version 2024 for plotting. The response surface optimization calculation was carried out using Design-Expert 8.0.6, and the response surface image was optimally drawn.

Results

Isolation and screening of cellulose-degrading fungi

From thirty-three fungal strains initially isolated from chili pepper (Capsicum annuum) stalks, twelve cellulolytic isolates (designated LJ-1 to LJ-12) were selected based on their ability to form clear hydrolysis zones on Congo Red-stained plates following NaCl decolorization. Strains LJ-4, LJ-7, LJ-10, and LJ-11 demonstrated superior cellulolytic activity, with hydrolysis zone-to-colony diameter ratios (D/d) of 1.99 ± 0.45, 1.85 ± 0.11, 2.64 ± 0.60, and 2.01 ± 0.20, respectively. LJ-10 exhibited the most pronounced activity (*Table 1*). Filter paper degradation assays demonstrated significant cellulose hydrolysis by strains LJ-7, LJ-10, and LJ-11, with complete structural collapse and gelatinization of Whatman No.1 filter paper within 7 days of incubation at 30°C, indicating superior cellulase production. Subsequently, CMCase and FPAase activities were determined for these four fungal strains. Glucose concentrations were calculated using the standard curve equation $y = 1.7415x + 0.0265$ (R2 = 0.999), where y represents the OD540 of the crude enzyme solution, and x represents the glucose concentration. The calculated CMCase activities for LJ-4, LJ-7, LJ-10, and LJ-11 were 9.37 ± 0.30 U/mL, 19.44 ± 0.21 U/mL, 22.20 ± 0.18 U/mL, and 18.49 ± 0.33 U/mL, respectively. The FPAase activities for these four strains were 42 ± 0.21 U/mL, 5.33 ± 0.45 U/mL, 5.67 ± 0.17 U/mL, and 4.21 ± 0.38 U/mL, respectively. Based on the above results, an increased ratio of transparent circles following Congo red staining indicates enhanced cellulose degradation. Higher CMCase and FPAase activities also signify stronger cellulose-degrading capability. Therefore, strain LJ-10 demonstrated superior cellulolytic performance through Congo Red assay (*D/d* = 2.64), complete filter paper degradation, and highest CMCase/FPAase activities, establishing it as the target strain.

Table 1. *Hydrolysis circle diameter (D), colony diameter (d) and their ratios of cellulose-degrading fungal*

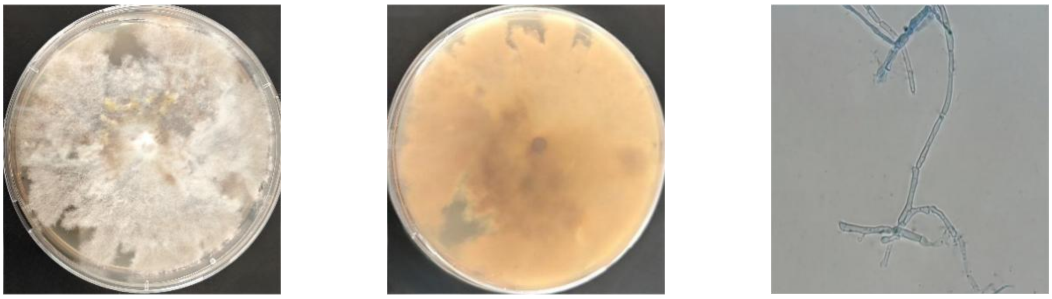
	Diameter of transparent zone	Diameter of colony	Ratio (*D/d*)	The degree of disintegration of the filter paper
LJ-1	2.27 ± 0.06	1.93 ± 0.06	1.17 ± 0.06e	+
LJ-2	5.33±0.15	3.00±0.10	1.78±0.11bc	+
LJ-3	3.27±0.06	2.37±0.15	1.38±0.07de	+
LJ-4	4.43±0.12	2.30±0.46	1.99±0.45bc	++
LJ-5	4.57±0.06	2.77±0.12	1.65±0.09bcd	++
LJ-6	5.57±0.06	3.33±0.15	1.67±0.06bcd	++
LJ-7	5.47±0.06	2.97±0.15	1.85±0.11bc	+++
LJ-8	3.87±0.21	3.50±0.10	1.10±0.04e	—
LJ-9	4.73±0.15	3.87±0.23	1.23±0.08e	+
LJ-10	3.40±0.10	1.33±0.32	2.64±0.60a	+++
LJ-11	2.53±0.06	1.27±0.12	2.01±0.20b	+++
LJ-12	3.00±0.06	1.87±0.06	1.63±0.08d	++

—: Indicates no visible change in the filter paper; +: Indicates swollen in the filter paper; ++: Indicates the filter paper becoming deformed; +++: Indicates the filter paper forming an aggregated, paste like structure

Morphological characteristics and molecular biological identification of strain LJ-10

The LJ-10 strain demonstrated rapid radial colony expansion from the inoculation point, exhibiting irregular morphology with lobate margins. Within 120 h of incubation at 25°C, the hyphal network achieved complete substrate colonization on PDA plates (90 mm diameter). Following 5-day incubation on PDA at 25°C, colonies exhibited a beige obverse surface and white reverse pigmentation. Progressive browning developed in central regions of the reverse side after extended cultivation (7-10 days). The periphery showed diffuse margins with dense aerial hyphae forming concentric growth zones (*Fig. 1A, B*). Microscopic observation revealed long, septate hyphae, with no observed spores (*Fig. 1C*).

BLAST analysis of the ITS sequence from strain LJ-10 revealed 99.8% identity with *Fusarium equiseti*. Phylogenetic reconstruction using the neighbor-joining method in MEGA11.0 (1000 bootstrap replicates, Tamura-Nei model) further supported its taxonomic position within the *F. equiseti* clade, albeit with moderate bootstrap support (93%). Based on this phylogenetic analysis and morphological characteristics, the LJ-10 strain was identified as *F. equiseti* (*Fig. 2*), the NCBI accession number for the strain is *PV082970*.



(A) (B) (C)

Figure 1. *Colony and mycelium morphology of strain LJ-10. A: Positive morphology of colonies. B: Back of colony. C: Mycelium (10 × 40)*

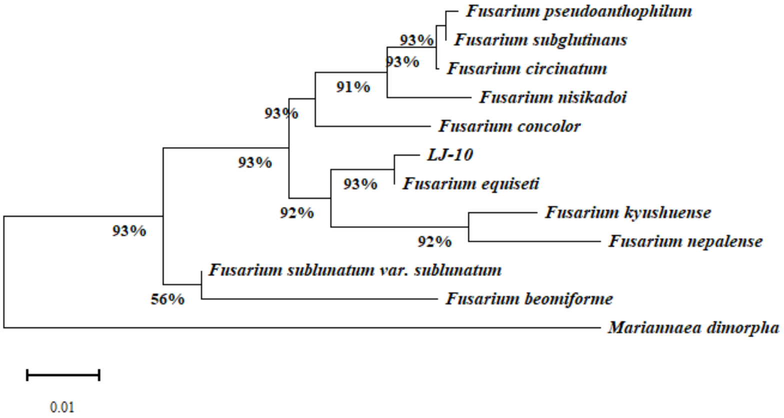


Figure 2. *Molecular identification of cellulose-degrading fungal LJ-10*

Optimization of enzyme production conditions

Effect of different carbon sources on CMCase production by LJ-10

The carbon source plays a crucial role in the production of CMCase by LJ-10. Eight different carbon sources, namely Lactose, Maltose, Potato extract, CMC-Na, Starch, Cane sugar Glucose, and Mannitol, were selected. The LJ-10 strain was inoculated with a 2% inoculum in the enzyme production medium containing different carbon sources and cultured for 3 days. The CMCase production capacity of the LJ-10 strain was then determined, and the results are shown in *Figure 3A*. CMC-Na was identified as the optimal carbon source for the growth of the LJ-10 strain, with CMCase activity reaching 19.19 U/mL. Therefore, CMC-Na can be considered the optimal carbon source for the enzyme production medium of the LJ-10 strain. The CMCase activity of the LJ-10 strain increased with increasing concentrations of CMC-Na. When the concentration of CMC-Na was 20 g/L, the CMCase activity of LJ-10 reached its maximum, at 35.56 U/mL. Further increases in CMC-Na concentration resulted in a decrease in CMCase activity (*Fig. 3B*). Consequently, the optimal carbon source for enzyme production by the LJ-10 strain is CMC-Na, with an optimal concentration of 20 g/L.

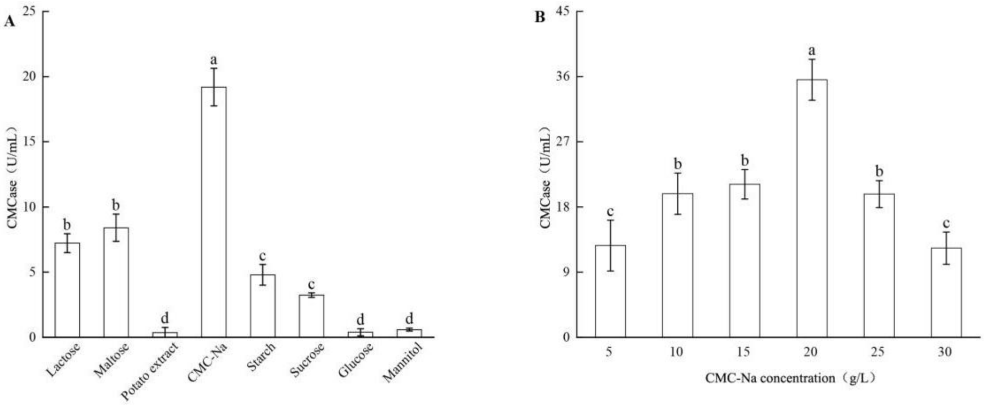


Figure 3. *Single factor optimization of carbon sources and optimal concentration screening of CMC-Na*

The impact of various nitrogen sources on CMCase production by LJ-10

The CMCase production capacity of strain LJ-10 was assessed using various nitrogen sources. The results indicated that peptone, as the sole nitrogen source, yielded the highest CMCase production, reaching 35.58 U/mL (*Fig. 4A*). As illustrated in *Figure 4B*, the maximum CMCase production by strain LJ-10, at 38.41 U/mL, was observed at a peptone concentration of 2 g/L. A gradual decline in CMCase production was noted with increasing peptone concentrations. In conclusion, peptone serves as the optimal nitrogen source for strain LJ-10, with an ideal concentration of 2 g/L.

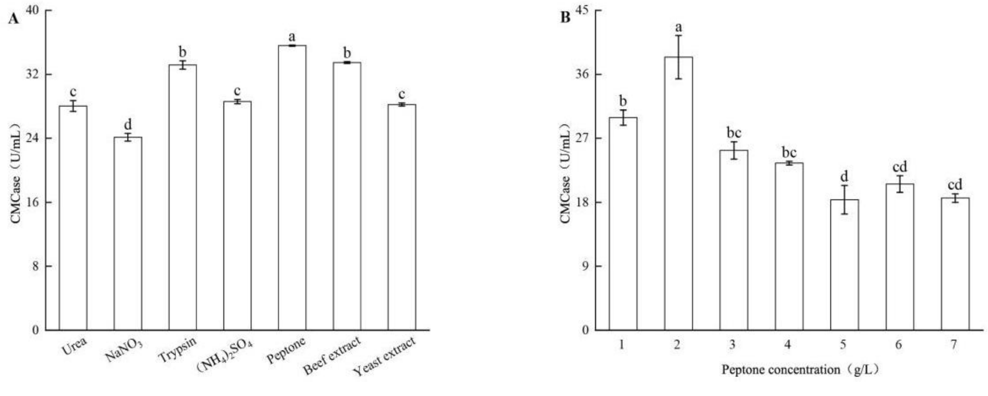


Figure 4. *Single factor optimization of nitrogen sources and optimal concentration screening of peptone*

The impact of liquid volume on CMCase production by strain LJ-10

The working liquid volumes were set at 50, 60, 70, 80, 90, and 100/150 mL. As the liquid volume reached 60/150 mL, the CMCase production capacity of strain LJ-10 gradually decreased. As illustrated in *Figure 5,* the maximum CMCase production by strain LJ-10 was 25.22 U/mL at a liquid volume of 60 mL/150 mL. In conclusion, 60/150 mL is the optimal working liquid volume for CMCase production by strain LJ-10.

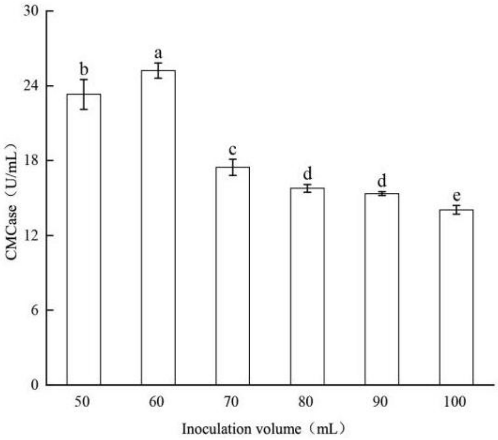


Figure 5. *Effect of different liquid loading amounts on the CMCase of strain LJ-10*

The impact of pH on CMCase production by strain LJ-10

The pH of the culture medium significantly influences both the growth and enzyme production capabilities of the strain. Preliminary experiments were conducted to determine the optimal pH range for strain growth. Strain LJ-10 demonstrated growth within a pH range of 3 to 10. Based on these preliminary findings, the CMCase production capacity of the strain was assessed across the pH range of 3 to 10, with results presented in *Figure 6*. The maximum CMCase activity, 28.39 U/mL, was observed at a pH of 7. At pH values below 7, the CMCase production by LJ-10 generally increased with increasing pH. Conversely, at pH values above 7, the CMCase production by LJ-10 exhibited a general decline with increasing pH. Consequently, a pH of 7 was identified as optimal for maximizing enzyme production by LJ-10.

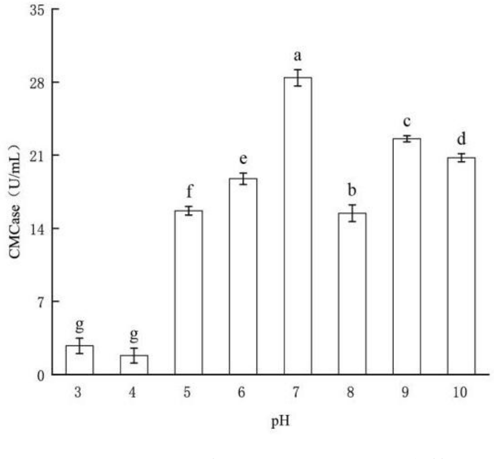


Figure 6. *CMCase of strain LJ-10 at different pH*

Optimization of inoculum size

Using an OD_{600} of 0.5 as the seed culture, varying proportions of spore suspension were added to 70 mL of liquid enzyme production medium, and the results are presented in *Figure 7*. The highest CMCase activity, 27.19 U/mL, was observed when 2% (1.4 mL) of the spore suspension was added. A general decline in CMCase activity was observed with increasing inoculum volume. These findings indicate that the optimal inoculum volume for LJ-10 is 2%.

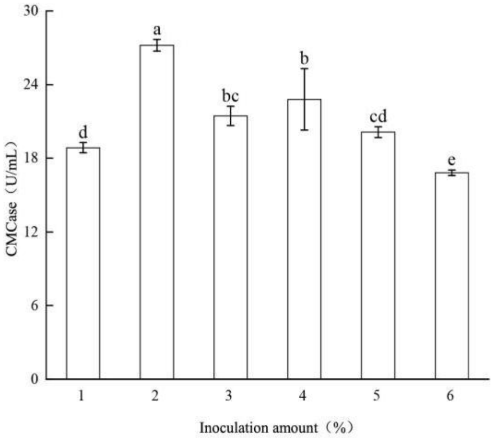


Figure 7. *CMCase production of strain LJ-10 under different inoculation amounts*

Response surface methodology (RSM) experiments were conducted

Carbon sources, nitrogen sources and inoculation amounts play a significant role in the growth and enzyme-producing capacity of microorganisms. Based on the single-factor results, key influencing factors were selected for response surface optimization, including the carbon source CMC-Na, nitrogen source peptone, and inoculum amount, as the three factors. The experimental design and results for the LJ-10 strain response surface are presented in *Table 2*. Using Design-Expert 13 software, a multiple regression analysis of the Box-Behnken model experimental data yielded a quadratic fitting model: $Y = 47.37 + 1.63A - 4.74B + 5.63C + 1.24AB - 5.53AC + 0.6794BC - 9.32A - 9.36A^2 - 9.36B^2 - 9.74C^2$. In this equation, Y represents CMCase activity, A represents the nitrogen source, B represents the carbon source, C represents the inoculum amount, -1 represents the minimum value of the single factor within the experimental range, 0 represents the intermediate value of the single factor within the experimental range, and 1 represents the maximum value of the single factor within the experimental range.

The response analysis results for CMCase production by the LJ-10 strain were obtained using Design-Expert 13 software. The sum of squares of deviations in *Table 3* represents the deviation between the sample values and the mean value. The regression analysis results for the LJ-10 strain indicated that the model for CMCase production by the LJ-10 strain had $P < 0.0001$, $F = 86.79$, lack of fit $P = 0.1695$, and $R^2 = 0.9797$, indicating a good fit between the model and the actual situation. The P values for A, B, C, AC, A^2, B^2, and C^2 were all < 0.05, indicating that these independent variables and interaction combinations significantly affected CMCase production by the LJ-10 strain. Among them, the P values for B, C, A^2, B^2, and C^2 were all < 0.0001. Among the independent variables, C had the largest F value and the smallest corresponding P value, indicating that the inoculum amount had a more significant impact than the carbon and nitrogen sources. In the interaction combinations, B^2 had the largest F value and the smallest corresponding P value, indicating that B^2 had a more significant impact on CMCase production by the LJ-10 strain.

Therefore, the order of influence on CMCase production by the LJ-10 strain was: inoculum amount > carbon source > nitrogen source.

Table 2. *Experimental design and results of strain LJ-10 to optimize the response surface under enzyme production conditions*

No.	Factors			Response value
	A	***B***	***C***	**CMCase**
1	0	0	0	45.7717
2	-1	0	-1	17.135
3	0	0	0	46.6475
4	0	1	-1	16.748
5	-1	0	1	38.2748
6	0	1	1	30.5355
7	1	0	-1	29.4011
8	1	0	1	28.4185
9	1	1	0	27.724
10	1	-1	0	34.9516
11	0	-1	1	38.4345
12	-1	1	0	19.9248
13	0	-1	-1	27.3647
14	0	0	0	47.7967
15	0	0	0	48.6797
16	-1	-1	0	32.1277
17	0	0	0	47.9438

Table 3. *Regression analysis results of strain LJ-10 response analysis test*

	Sum of squares	**Degrees of freedom**	**Mean square**	***F*-value**	***P*-value**	
Model	1852.44	9	205.83	86.79	< 0.0001	Significant
A	21.23	1	21.23	8.95	0.0202	
B	179.99	1	179.99	75.89	< 0.0001	
C	253.29	1	253.29	106.80	< 0.0001	
AB	6.19	1	6.19	2.61	0.1503	
AC	122.35	1	122.35	51.59	0.0002	
BC	1.85	1	1.85	0.7786	0.4069	
A^2	366.10	1	366.10	154.36	< 0.0001	
B^2	368.98	1	368.98	155.58	< 0.0001	
C^2	399.11	1	399.11	168.28	< 0.0001	
Residual	16.60	7	2.37			
Lack of fit	11.30	3	3.77	2.84	0.1695	Not significant
Pure error	5.30	4	1.33			
Cor total	1869.05	16				

Figure 8A illustrates the interactive effects of nitrogen and carbon sources on CMCase activity at a 2% inoculum level. The circular contour lines indicate that the interaction between nitrogen and carbon sources is not significant. *Figure 8B* depicts the interactive effects of carbon source and inoculum level on CMCase activity when the nitrogen source is at 0.2% (2 g/L); the circular contour lines suggest that their interaction is also insignificant. *Figure 8C* shows the interactive effects of nitrogen source and inoculum level on CMCase activity when the carbon source is at 2% (20 g/L). The elliptical contour lines indicate a significant interaction between these two factors. The steepest surface plot for the interaction between nitrogen source and inoculum level suggests that their interaction has the most significant impact on CMCase activity.

The peak CMCase activity is observed at the center of this region when all variables are at their baseline levels, indicating the extremum of CMCase activity. Analysis using Design-Expert 13 software suggests that the optimal conditions for CMCase production by strain LJ-10 are: a nitrogen source of 0.217% (2.17 g/L), a carbon source of 2.044% (20.44 g/L), and an inoculum level of 2.14%, resulting in a maximum CMCase activity of 45.781 U/mL.

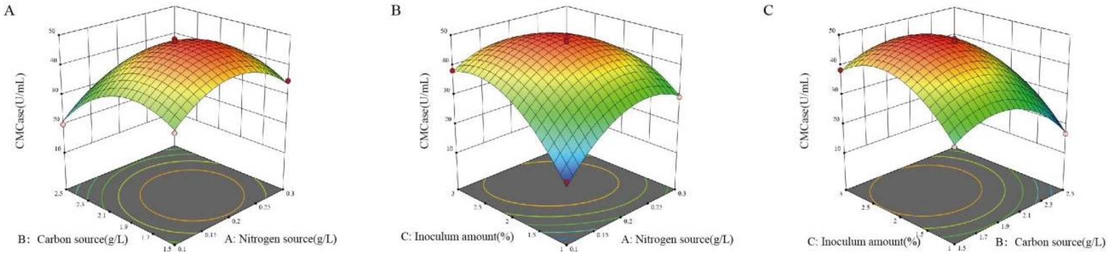


Figure 8. *Response surface plot of the mutual effect on CMCase of strain LJ-10*

Results validation

To validate the reliability of the model's predictive results, verification experiments were conducted using the optimized medium composition and inoculum size. The results demonstrated that the CMCase activity of strain LJ-10 reached 44.52 ± 0.22 U/mL, which closely aligns with the model's predicted value. Through a T-test analysis, the optimized CMCase activity was found to be significantly higher than that prior to optimization, exhibiting a 2.18-fold increase. This indicates the feasibility of the optimized medium and inoculum size derived from the model (*Fig. 9*).

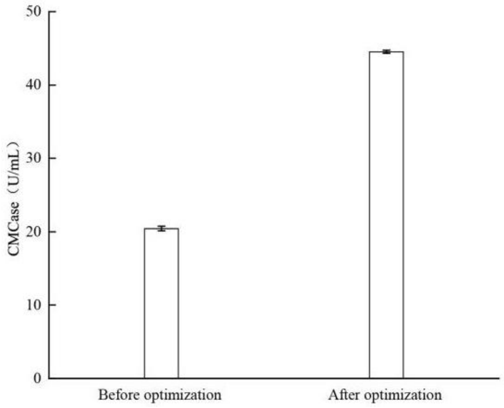


Figure 9. *Response surface optimization verification mass*

The degradation rate of cellulose-degrading bacteria was determined

The degradation rate of pepper stems by strain LJ-10 gradually increased with the extension of the cultivation time. On the 10th day of cultivation, before optimization, the degradation rate of chili straw by strain LJ-10 was 7.37%, and after optimization, it was 11.55%. On the 30th and 40th days of cultivation, the degradation rates of pepper stems by the optimized strain LJ-10 were 41.88% and 41.93% respectively, and the difference between the two was not significant. It is indicated that the degradation ability of strain LJ-10 on chili stems tends to be stable after 30 days. At 40 days, the degradation rate of LJ-10 by the optimized strain increased by 20.54% compared with that before optimization (*Fig. 10*).

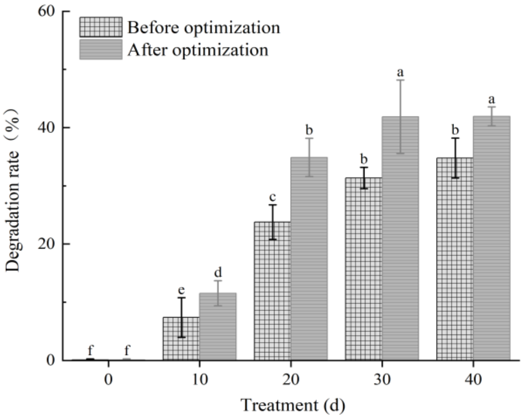


Figure 10. *Degradation efficiency of strain LJ-10*

Discussion and conclusion

Discussion

Currently, research on the biological degradation of cellulose is already widespread. Compared with bacteria, fungi have a larger mycelial coverage area. The branching and growth of hyphae can penetrate into the interior of the cellulose matrix, increasing the contact area between enzymes and substrates, thereby improving degradation efficiency. Meanwhile, fungi can better adapt to different environments, and some fungi can even survive in extreme environments (Datta, 2024). In a study by Waghmare et al., *Penicillium oxalicum* was added to degrade cellulose (Waghmare et al., 2021). In this study, four strains of fungi with high cellulose degradation ability were isolated and screened from healthy chili stalks. In the research by Farshid Khosravi et al., a variety of cellulolytic microorganisms with high-temperature and medium-temperature tolerance were isolated from soil and leaf samples in Kerman Province (Khosravi et al., 2022). Cellulolytic fungi are diverse and have rich sources. Optimizing the enzyme production conditions of cellulolytic fungi can effectively enhance cellulase activity and thus achieve better degradation of cellulose. In the study by Wang et al. (2024) three strains of bacteria with highly efficient cellulose-degrading capabilities were screened out. Under optimal culture conditions, the CMCase activity of one strain of *Bacillus subtilis* reached 15.63 U/mL. In another study, the cellulase activity and β-galactosidase activity of *Aspergillus* reached 3.32 and 196.09 U/g, respectively, under the most favorable culture conditions (35°C, pH = 7.0, cultured for 5 days) (Boondaeng et al., 2024; Kim et al., 2012). believed that the hyphae of plant fungal pathogens can penetrate plant cells and accelerate the degradation of cellulose in plant cell walls (Kim et al., 2012). Meanwhile, Huang et al. (2015) confirmed that *Fusarium* species have a strong ability to degrade cellulose and hemicellulose and identified 147 genes encoding glycoside hydrolases. In addition, numerous experiments have also demonstrated that *Fusarium* species possess certain disease resistance capabilities. Considering the advantages of *Fusarium* species, we selected a strain of *F. equiseti* with the highest cellulase and filter paper enzyme activities as the target strain.

Sukirtha and Lali (2022) investigated the lignin-degrading ability of *Fusarium oxysporum* and optimized the conditions such as temperature, pH, and culture time. They concluded that under the optimal culture conditions (28°C, pH 7.0, and 15 days of cultivation), the activities of lignin-degrading related enzymes produced by *Fusarium oxysporum* were the highest. Similarly, in this study, the culture conditions of the isolated strain were optimized by adjusting the carbon and nitrogen sources and their optimal concentrations, pH, and the volume of culture medium. Under these optimized conditions, the isolated strain secreted more cellulase, thereby significantly enhancing the cellulase activity.

Response Surface Methodology (RSM) was proposed by George E. P. Box and Kempthorne B. Wilson in 1951 and has since gradually evolved into an important statistical analysis tool (Hayato et al., 2011). This method integrates principles of mathematics and statistics and is mainly used to optimize process conditions, understand the interactions between factors in an experiment, and find the best parameter settings that can maximize or minimize the objective function. By constructing a response surface model, researchers can effectively explore the impact of variables on the response, thereby improving the efficiency and accuracy of experiments (Odunayo, 2024). In this model, the sum of squares of deviations reflects the deviation between sample values and the mean value. The smaller the sum of squares of deviations, the smaller the gap between the two. The mean square deviation indicates the degree of deviation between sample values and the mean value. The smaller the mean square deviation, the smaller the degree of deviation and the higher the accuracy. The F-value is a statistical quantity that follows the F-distribution. The larger the F-value, the stronger the significance of the model. The P-value represents the significance probability. When $P > 0.05$, it indicates that the variable has no significant effect on the response value. When $P < 0.05$, it indicates that the variable has a significant effect on the response value. When $P < 0.01$, the effect of the variable on the response value is extremely significant (Sai et al., 2023). Using Design-Expert 13 software for regression analysis of the response analysis of CMCase production by strain LJ-10, the model for CMCase production by the strain has $P < 0.0001$, $F = 86.79$, lack-of-fit $P = 0.1695$, and $R^2 = 0.9797$. These data indicate that the model fits the actual situation well and that the established model can effectively predict the CMCase production by strain LJ-10.

Conclusion

Strain LJ-10 was screened from pepper stalk and identified as *F. equiseti* by ITS gene. *F. equiseti* LJ-10 optimal cellulase production medium formula was nitrogen source 0.217% (2.17 g/L), carbon source 2.044% (20.44 g/L), inoculation amount 2.14%; The verified strain LJ-10 produced 44.52 ± 0.22 U/mL of CMCase, which was 2.18 times higher than that before optimization 20.42 ± 0.42 U/mL. Strain LJ-10 was used to degrade pepper stalk. At the 40th day, the degradation rate of pepper stalk was 41.93%, compared with the degradation rate 40 days before optimization, it increased by 20.54% after optimization. Strain LJ-10 can be used as a cellulose degrading bacterium in straw treatment.

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