Postprint: Mutagenic Breeding and Fermentation Condition Optimization of High-Yield Cellulase-Producing Trichoderma koningii

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Abstract

This study aimed to investigate the mutation breeding and fermentation condition optimization of *Trichoderma koningii* for high-yield cellulolytic enzyme production. Through mutagenesis of *T. koningii* via ultraviolet (UV), diethyl sulfate (DES), and combined UV-DES treatments, a mutant strain with enhanced cellulolytic enzyme production was obtained, and its enzyme production fermentation conditions and straw hydrolysis capacity were investigated. The results showed that one mutant strain, designated *T. koningii* UH-1, was screened from the wild-type strain following UV, DES, and combined UV-DES mutagenesis. Compared with the wild-type strain, the mutant exhibited a 42.7% increase in xylanase activity and a 78.5% increase in cellulase activity. The optimal solid-state fermentation conditions for enzyme production by *T. koningii* UH-1 were: corn straw to wheat bran ratio of 7:3, ammonium sulfate concentration of 2%, inoculum size of 1.1 mL, medium moisture content of 67%, fermentation time of 84 h, and potassium dihydrogen phosphate concentration of 1%. Under these conditions, filter paper activity, cellulase activity, and xylanase activity reached 0.86 U/g, 99.81 U/g, and 1150.32 U/g, respectively, whereas the wild-type *T. koningii* exhibited filter paper activity, cellulase activity, and xylanase activity of 0.23 U/g, 46.38 U/g, and 677.86 U/g, respectively. This study demonstrates that combined UV-DES mutagenesis is an effective approach for the mutation breeding of *T. koningii*, significantly improving its enzyme production capacity and straw hydrolysis ability.

Full Text

Mutagenesis Breeding and Fermentation Condition Optimization of High-Yield Fiber-Degrading Enzyme Producing *Trichoderma koningii*

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Abstract

This study investigated the mutagenesis breeding and fermentation condition optimization of *Trichoderma koningii* for high-yield fiber-degrading enzyme production. High cellulolytic enzyme-producing mutants were obtained through mutagenesis (UV irradiation, diethyl sulfate treatment, and UV-diethyl sulfate compound mutagenesis) of *T. koningii*, and their enzyme production fermentation conditions and straw hydrolysis capacity were evaluated. The results showed that a mutant strain *T. koningii* UH-1 was successfully screened following UV, diethyl sulfate, and compound mutagenesis of the original strain. Compared with the original strain, the xylanase activity of the mutant increased by 42.7%, while cellulase activity increased by 78.5%. The optimal solid-state fermentation conditions for *T. koningii* UH-1 were: corn straw to wheat bran ratio of 7:3, ammonium sulfate concentration of 2%, inoculum size of 1.1 mL, culture medium moisture content of 67%, fermentation time of 84 h, and potassium dihydrogen phosphate concentration of 1%. Under these conditions, filter paper enzyme activity reached 0.86 U/g, cellulase activity reached 99.81 U/g, and xylanase activity reached 1,150.32 U/g. In contrast, the original *T. koningii* strain exhibited filter paper enzyme activity of 0.23 U/g, cellulase activity of 46.38 U/g, and xylanase activity of 677.86 U/g. This study demonstrates that UV-diethyl sulfate compound mutagenesis is an effective method for breeding *T. koningii* mutants with improved enzyme production and straw hydrolysis capabilities.

Keywords: *Trichoderma koningii*; ultraviolet mutagenesis; chemical mutagenesis; screening; fermentation conditions

Introduction

China is a major corn-producing country, generating enormous quantities of corn straw annually, which reached 900 million tons in 2017. Due to the lack of practical processing and utilization technologies, corn straw burning has become a serious problem, with over 200 million tons burned each year, causing

resource waste and severe environmental pollution. Corn straw is rich in energy value and can serve as a supplementary energy feed resource if scientifically processed. The conversion of corn straw using various fiber-degrading enzymes produced by microorganisms represents a current research hotspot. Filamentous fungi such as *Aspergillus niger*, *Trichoderma*, and white-rot fungi are recognized as producers of relatively complete cellulase and partial hemicellulase systems, making them ideal strains for straw fiber decomposition.

Trichoderma koningii is commonly used for biodegrading corn straw and can efficiently degrade its fiber components. Chang found that inoculating corn straw with *T. koningii* significantly reduced cellulose and hemicellulose content, while Shi identified *T. koningii* as a strain with strong cellulose decomposition ability, showing increased degradation rates for neutral detergent fiber, acid detergent fiber, cellulose, and hemicellulose in bovine rumen over time. Because corn straw contains various complex chemical bonds that require synergistic action of multiple enzymes for hydrolysis, *T. koningii*' s ability to secrete both cellulase and substantial xylanase is crucial for efficient corn straw hydrolysis. Li reported that the maximum activities of cellulase and xylanase produced by *T. koningii* significantly affected its corn straw degradation capacity, and Feng screened a *T. koningii* strain with high xylanase production capability. These findings indicate that the types and activities of fiber-degrading enzymes significantly influence efficient straw fiber hydrolysis, and obtaining strains with complete enzyme profiles and high activity is key to biological corn straw processing.

The complex chemical structure of corn straw requires not only enzymatic proteins but also non-enzymatic protein factors (such as hydrogen bond-breaking enzymes, lytic polysaccharide monooxygenases, and expansins) for efficient cellulose hydrolysis. Compared with single enzymes produced by genetically engineered strains in liquid fermentation, the multi-enzyme complex system produced by wild strains in solid-state fermentation is more suitable for straw hydrolysis, offering not only a rich enzyme profile but also various auxiliary factors that facilitate opening complex fiber structures. However, low enzyme activity and high costs from wild strain solid-state fermentation limit its application in crop straw hydrolysis. Therefore, improving enzyme production capacity and growth rate of wild strains is crucial for straw biological enzymatic processing technology. Mutagenesis screening is a common method to enhance microbial enzyme production and reduce enzyme preparation costs. UV and chemical mutagenesis have become effective methods for rapid microbial genome mutation due to their good results, simple operation, and low equipment requirements. In *Trichoderma* breeding, Ike used UV mutagenesis to screen two high cellulase-producing strains, Li obtained seven high-yield mutants after UV and microwave mutagenesis of *Trichoderma viride*, and Li used UV, diethyl sulfate, and sodium nitrite compound mutagenesis to obtain high-yield mutants. These studies demonstrate that compound mutagenesis, as an efficient biological mutation method, has promising application prospects in strain improvement. Therefore, this study employed UV, chemical, and UV+chemical compound mutagenesis methods to mutate *T. koningii*, using microcrystalline cellulose as the

sole carbon source to screen for fast-growing, high enzyme-producing mutants. Through optimization of solid-state fermentation conditions for enzyme production, this research aimed to obtain high-yield fiber-degrading enzyme strains and their solid-state fermentation process parameters to provide guidance for efficient biological conversion of corn straw.

Materials and Methods

1.1 Original Strain

The original strain used in this experiment was *Trichoderma koningii* 13006, obtained from the China Center of Industrial Culture Collection.

1.2 Culture Media

Subculture medium: 5 g corn straw powder (washed with boiling water until colorless, passed through 200-mesh sieve), 1.65 g ammonium sulfate, 0.5 g dipotassium hydrogen phosphate (K HPO), 0.25 g magnesium sulfate heptahydrate (MgSO ·7H O), 18–20 g agar, natural pH, diluted to 1 L with distilled water.

Microcrystalline cellulose Congo red medium: 5 g microcrystalline cellulose, 1.65 g ammonium sulfate, 0.3 g Congo red, 0.5 g K HPO , 0.2% sodium deoxycholate, 0.25 g MgSO ·7H O, 18–20 g agar, natural pH, diluted to 1 L with distilled water.

Solid fermentation basal medium: 7.0 g corn straw (passed through 2 mm sieve), 3.0 g wheat bran, 2% ammonium sulfate, solid-to-water ratio 1.0:2.2, natural pH.

1.3 Spore Suspension Preparation

Spore suspension was prepared by inoculating 2.5 mL of *Trichoderma* spore suspension (10 spores/mL) into 50 mL of limited culture solution [0.1% glucose, 0.05% (NH) SO] and culturing at 30 °C with 150 r/min shaking. The spore germination process was recorded, with 20%–30% germination rate considered optimal.

1.4 UV, Chemical, and Compound Mutagenesis of *T. koningii*

For UV mutagenesis, *T. koningii* spore solution was diluted to 40 spores/mL. Ten milliliters were placed in a 6 cm sterile petri dish in a UV mutagenesis apparatus (preheated for 30 min in darkroom) and irradiated at 365 nm wavelength with continuous stirring for 8, 11, 14, 17, 20, or 23 min. All post-UV operations were performed under red light to prevent reversion.

For chemical mutagenesis, 3.5 mL spore suspension was mixed with 1 mL diethyl sulfate and 0.5 mL anhydrous ethanol for 10, 20, 30, 40, 50, or 60 min, then terminated with 0.5 mL 25% Na S O . Sterile water treatment served as blank

control. Mutagenized solutions were spread on microcrystalline cellulose Congo red medium and incubated at 30 °C for 36 h. Colony counts were performed to calculate lethality rates before and after mutagenesis.

For compound mutagenesis, first-round mutants were subjected to sequential UV and chemical mutagenesis using the same procedures.

1.5 Screening of Optimal Mutants

Using the transparent halo size produced by the original *T. koningii* 13006 on microcrystalline cellulose Congo red medium as control, colonies with fast growth, short hyphae, late sporulation, and large transparent halos were selected. These were washed with 0.9% saline solution, spread on subculture medium, and after 4–6 days of single colony growth, were collected and preserved. Strains were inoculated into solid fermentation medium, and cellulase (NY/T 912-2004), xylanase (GB/T 23874-2009), and filter paper enzyme (GB/T 23881-2009) activities were measured to identify high-yield fiber-degrading strains.

1.6 Fermentation Condition Optimization

1.6.1 Single-Factor Experiments Using basal medium at 30 °C, single-factor experiments were conducted for fermentation time (48, 60, 66, 72, 78, 84, 90, 96, 102, 108, 114, 120 h), ammonium sulfate concentration (0, 1%, 2%, 3%, 4%, 5%, 6%), potassium dihydrogen phosphate concentration (0, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%), and spore inoculum (10 spores/mL) size (0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4 mL), with three replicates per treatment. Cellulase, xylanase, and filter paper enzyme activities of mutant *T. koningii* UH-1 were measured under different conditions to determine optimal enzyme production parameters.

1.6.2 Orthogonal Experiments Based on single-factor results, L (3) orthogonal experiments were conducted using four major influencing factors at three levels each to determine optimal solid-state fermentation conditions for xylanase, cellulase, and filter paper enzyme production by *T. koningii* UH-1. The test strain was inoculated in optimal fermentation medium under optimal conditions, and enzyme activities were compared with the original *T. koningii* 13006.

1.7 Statistical Analysis

SPSS 19.0 software was used for single-factor ANOVA, with results expressed as mean ± standard deviation.

Results

2.1 UV and Chemical Mutagenesis and Mutant Screening of *T. koningii*

T. koningii spores germinated for 20 h (20% germination rate) were subjected to UV and chemical mutagenesis. The optimal UV exposure time was 10 min (80% lethality rate), and optimal chemical mutagenesis time was 30 min (80% lethality rate). After multiple rounds of UV and chemical mutagenesis, primary screening selected mutants with larger transparent halo diameter to colony diameter ratios on microcrystalline cellulose Congo red medium (cultured for 7 days). Rescreening through solid-state fermentation revealed the enzyme production capacity of *T. koningii* 13006 mutants . The results showed two mutants were obtained through colony size comparison. Rescreening identified that mutant *T. koningii* U11-2 exhibited increased filter paper, cellulase, and xylanase activities compared with the original *T. koningii* 13006 ($P>0.05$), indicating that single-method mutagenesis could not obtain superior mutants. Given the improved enzyme production trend of *T. koningii* U11-2, it was selected as the starting strain for subsequent cross-compound mutagenesis.

Table 1 Enzyme production ability of *Trichoderma koningii* 13006 mutant strains (U/g)

Items	*T. koningii* 13006	*T. koningii* H1-6	*T. koningii* U11-2
Transparent halo diameter/Colony diameter	0.65±0.11	0.65±0.10	0.83±0.10
Filter paper enzyme	575.56±29.38	589.03±36.32	638.11±50.16
Xylanase	68.38±1.34	71.24±8.50	72.67±2.34

In the same row, values with different small letter superscripts mean significant difference ($P<0.05$), while with the same or no letter superscripts mean no significant difference ($P>0.05$). The same as below.
U: UV mutagenesis; H: chemical mutagenesis. The same as below.

2.2 Cross-Mutagenesis of *T. koningii* U11-2 and Mutant Screening

Under optimal mutagenesis conditions (UV 20 min, chemical 30 min), *T. koningii* U11-2 underwent multiple rounds of cross UV-chemical mutagenesis. Transparent halo production on microcrystalline cellulose Congo red medium

(7 days) and fermentation enzyme production were evaluated . All mutants (*T. koningii* U11-2, UH30-2, UH-1) showed significantly increased xylanase and cellulase activities compared with the original *T. koningii* 13006 ($P<0.05$), with improved filter paper enzyme activity ($P>0.05$).

Table 2 Enzyme production ability of *Trichoderma koningii* U11-2 mutant strains

Items	*T. koningii* 13006	*T. koningii* U11-2	*T. koningii* UH30-2	*T. koningii* UH-1
Transparent halo diameter/Colony diameter	0.47±0.03	0.56±0.09	0.53±0.12	0.60±0.12
Filter paper enzyme	478.26±47.47	631.88±56.81	716.76±28.79	682.62±16.86
Xylanase	45.84±2.94	77.90±1.03	81.02±2.64	81.81±2.45

As shown in [Figure 1: see original paper], the fermented sample of mutant *T. koningii* UH-1 was significantly darker than that of the original *T. koningii* 13006, indicating successful mutation and demonstrating that cross-compound mutagenesis is a suitable method for *T. koningii*. Given its strong comprehensive enzyme production capacity, mutant *T. koningii* UH-1 was selected for subsequent studies.

Figure 1 Color change of fermented samples of *Trichoderma koningii* original and mutant strains

2.3 Optimization of Fermentation Conditions for *T. koningii* UH-1

2.3.1 Effect of Fermentation Time on Enzyme Production Fermentation time significantly affected enzyme production . With increasing fermentation time, filter paper, cellulase, and xylanase activities showed a trend of increase-decrease-increase, peaking at 84 h. After 84 h, filter paper enzyme activity decreased, while xylanase and cellulase activities showed irregular changes. Since shorter fermentation times reduce production costs, 84 h was selected as the optimal fermentation time for *T. koningii* UH-1.

Table 3 Effects of fermentation time on enzyme producing ability of *Trichoderma koningii* UH-1

Fermentation time (h)	Filter paper enzyme	Xylanase	Cellulase
48	0.62±0.03	560.46±9.97	75.46±2.14
60	0.72±0.12	615.47±13.17	77.73±0.88
66	0.40±0.06	916.83±34.89	80.12±1.90
72	0.48±0.04	861.16±20.32	81.31±0.59
78	0.67±0.10	819.87±45.87	84.02±1.60
84	0.85±0.04	962.92±9.16	90.62±2.81
90	0.78±0.02	868.32±36.06	88.20±3.87
96	0.80±0.14	964.79±3.04	85.60±9.54
102	0.80±0.12	911.26±2.60	94.41±3.56
108	0.68±0.13	805.83±60.28	96.58±6.75
114	0.51±0.07	814.11±12.46	90.02±5.67
120	0.57±0.02	989.96±54.03	91.94±3.03

2.3.2 Effect of Ammonium Sulfate Concentration on Enzyme Production Ammonium sulfate concentration significantly affected enzyme activities . Filter paper and cellulase activities were highest at 6% ammonium sulfate, significantly higher than at 0, 1%, and 2% ($P<0.05$), with 2% being significantly higher than 0 and 1% ($P<0.05$). Xylanase activity peaked at 2% ammonium sulfate, significantly higher than all other concentrations ($P<0.05$). Considering comprehensive effects on all three enzyme activities, 2% ammonium sulfate was selected as optimal.

Table 4 Effects of ammonium sulfate concentration on enzyme producing ability of *Trichoderma koningii* UH-1

Ammonium sulfate concentration (%)	Filter paper enzyme	Xylanase	Cellulase
0	0.61±0.08	1,045.02±96.47	74.95±1.75
1	0.60±0.09	682.20±93.95	81.80±4.32
2	0.88±0.06	1,160.64±35.20	92.02±7.16
3	1.04±0.07	800.63±51.38	89.65±2.63
4	1.05±0.14	787.70±29.88	89.16±1.05
5	1.09±0.06	852.75±19.29	90.79±1.69
6	1.10±0.06	928.81±25.07	91.37±4.35

2.3.3 Effect of Potassium Dihydrogen Phosphate Concentration on Enzyme Production Potassium dihydrogen phosphate concentration showed no significant effect on filter paper, cellulase, or xylanase activities ($P>0.05$) . However, enzyme activities were relatively high at 1% concentration. Considering cost-effectiveness, 1% potassium dihydrogen phosphate was selected as optimal.

Table 5 Effects of KH PO concentration on enzyme producing ability of *Trichoderma koningii* UH-1

KH PO concentration (%)	Filter paper enzyme	Xylanase	Cellulase
0	1.02±0.16	1,173.69±101.80	90.62±2.56
1.0	1.05±0.08	1,216.23±45.87	87.80±6.42
1.5	0.90±0.20	1,125.63±131.92	92.63±1.67
2.0	0.90±0.23	1,194.60±52.25	85.62±1.67
2.5	0.97±0.23	1,111.08±45.34	84.41±5.89
3.0	1.10±0.36	1,086.59±21.93	93.73±6.42
3.5	1.00±0.25	1,172.44±50.20	87.04±1.39
4.0	0.88±0.12	1,094.51±42.45	83.30±2.42

2.3.4 Effect of Inoculum Size on Enzyme Production Inoculum size significantly affected enzyme production . Filter paper enzyme activity peaked at 1.2 mL, significantly higher than at 0.8, 1.1, and 1.4 mL ($P<0.05$). Cellulase activity was highest at 1.0 mL, significantly higher than at 1.3 mL ($P<0.05$). Xylanase activity peaked at 1.3 mL, significantly higher than at 0.9 mL ($P<0.05$). Considering all factors comprehensively, 1.0 mL was selected as the optimal inoculum size.

Table 6 Effects of inoculation quantity on enzyme producing ability of *Trichoderma koningii* UH-1

Inoculum size (mL)	Filter paper enzyme	Xylanase	Cellulase
0.7	0.87±0.13	1,144.06±95.81	89.95±2.93
0.8	0.48±0.13	1,143.66±113.43	91.94±3.18
0.9	0.71±0.15	995.66±78.08	91.74±0.89
1.0	0.84±0.14	1,021.00±222.11	92.16±0.69
1.1	0.59±0.32	1,056.88±176.07	90.45±4.54
1.2	1.00±0.14	1,227.71±103.25	87.99±3.11
1.3	0.80±0.06	1,258.35±61.92	86.29±2.28
1.4	0.56±0.31	1,021.56±85.71	89.62±3.43

2.3.5 Effect of Culture Medium Moisture on Enzyme Production Culture medium moisture showed no significant effect on enzyme production ($P>0.05$) . Considering practical aspects, 67% moisture content was selected as optimal.

Table 7 Effects of culture medium moisture on enzyme producing ability of *Trichoderma koningii* UH-1

Culture medium moisture (%)	Filter paper enzyme	Xylanase	Cellulase
60	0.80±0.33	976.93±29.78	96.79±4.72
63	0.66±0.07	1,179.81±74.91	91.53±1.26
65	0.73±0.09	1,197.10±84.23	93.63±12.16
67	0.58±0.27	1,060.36±51.74	74.75±4.45
70	0.50±0.02	1,204.66±64.31	85.93±2.05
73	0.84±0.14	1,191.61±92.92	92.36±4.37

2.3.6 Orthogonal Experiment Analysis Based on single-factor results, L (3) orthogonal experiments were conducted using four major factors (fermentation time, ammonium sulfate concentration, inoculum size, culture medium moisture) . For filter paper enzyme activity, the influencing factors ranked: ammonium sulfate concentration > culture medium moisture > fermentation time > inoculum size, with optimal conditions of 3% ammonium sulfate, 67% moisture, 78 h fermentation, and 1.2 mL inoculum. For cellulase activity, the ranking was: ammonium sulfate concentration > fermentation time > inoculum size > culture medium moisture, with optimal conditions of 4% ammonium sulfate, 90 h fermentation, 1.0 mL inoculum, and 68% moisture. For xylanase activity, the ranking was: ammonium sulfate concentration > inoculum size > culture medium moisture > fermentation time, with optimal conditions of 2% ammonium sulfate, 1.1 mL inoculum, 67% moisture, and 84 h fermentation.

Table 8 Results of orthogonal test

Factors	Ammonium sulfate concentration (%)	Fermentation time (h)	Inoculum size (mL)	Culture medium moisture (%)	Filter paper enzyme (1)	Cellulase (2)	Xylanase (3)
K1(1)							
K2(1)							
K3(1)							
K1(2)							
K2(2)							
K3(2)							
K1(3)							
K2(3)							
K3(3)							

K1, K2 and K3 represent enzyme production at factor levels 1, 2, and 3, respectively; R is the range; (1) is filter paper enzyme activity; (2) is cellulase activity; (3) is xylanase activity.

2.3.7 Orthogonal Experiment Verification Optimal fermentation conditions for the original *T. koningii* 13006 and mutant UH-1 were verified . For *T. koningii* UH-1, optimal filter paper and xylanase production conditions were 2% ammonium sulfate, 1.1 mL inoculum, 67% moisture, and 84 h fermentation, achieving activities of 0.86 and 1,150.32 U/g, respectively. Optimal cellulase production conditions were 4% ammonium sulfate, 90 h fermentation, 1.0 mL inoculum, and 68% moisture, reaching 103.99 U/g activity. The original *T. koningii* 13006 achieved maximum activities of 53.17, 812.78, and 0.30 U/g for cellulase, xylanase, and filter paper enzyme, respectively, all of which were lower than the mutant strain.

Table 9 Verification by orthogonal test

Items	Optimal fermentation conditions	Filter paper enzyme	Cellulase	Xylanase
T. koningii 13006 (Filter paper enzyme)		0.16±0.06	49.51±3.31	663.77±31.05
T. koningii UH-1 (Filter paper enzyme)		0.76±0.06	103.34±3.77	819.29±30.28
T. koningii 13006 (Cellulase)		0.30±0.08	53.17±4.31	812.78±53.32
T. koningii UH-1 (Cellulase)		0.86±0.15	99.81±1.61	1,150.32±53.31

Items	Optimal fermentation conditions	Filter paper enzyme	Cellulase	Xylanase
T. koningii 13006 (Xylanase)		0.23±0.08	46.38±2.41	677.86±62.02
T. koningii UH-1 (Xylanase)		0.76±0.12	103.99±5.76	1,006.28±81.8

Discussion

The complex chemical structure of corn straw makes it difficult for animals to utilize efficiently. Converting corn straw into easily digestible feed resources using microbial fiber-degrading enzymes is a research hotspot in comprehensive straw utilization. Obtaining superior strains with high enzyme production capacity through mutagenesis and genetic modification is key to biological enzymatic straw processing. Since mature conidia of molds are dormant, germinating spores can improve mutagenesis effectiveness. Liu demonstrated that using germinated spores for mutagenesis increases positive mutation rates. Therefore, this study germinated *T. koningii* spores and performed mutagenesis at 20% germination rate, achieving high mutation efficiency. Through UV, diethyl sulfate, and UV-diethyl sulfate compound mutagenesis of *T. koningii* 13006, the high enzyme-producing mutant *T. koningii* UH-1 was obtained, showing 42.7% increased xylanase activity and 78.5% increased cellulase activity compared with the original strain. This improvement may be attributed to single mutagenesis methods having limited mutation points, low probability, instability, and potential for strain resistance to single mutagens, suggesting compound mutagenesis may be more effective for filamentous fungi.

Rapid and efficient screening of superior mutants is crucial in mutagenesis breeding. This study employed transparent halo and solid-state fermentation enzyme activity assays for primary and secondary screening. Although some correlation existed between enzyme production and transparent halo to colony diameter ratio, mutants with larger ratios did not always exhibit higher enzyme activities. Since filamentous fungi grow rapidly on natural crop straw plates with indistinct halos, this method is unsuitable for mutant screening. Microcrystalline cellulose plates used in this study are representative but only demonstrate microcrystalline cellulose decomposition capacity, not necessarily straw degradation ability. Additionally, *T. koningii* solid-state fermentation produces multiple enzyme systems including cellulase, xylanase, filter paper enzyme, exoglucanase, -xylosidase, and -glucosidase, which play different roles in straw

degradation. Therefore, enzyme activity measurement is essential for mutant screening, though time-consuming. Establishing rapid screening methods for efficient crop straw-degrading filamentous strains requires further research.

Nitrogen is an essential nutrient for microbial cells and key for enzyme protein synthesis. Microorganisms can utilize both organic and non-protein nitrogen. Inorganic nitrogen sources like ammonium sulfate are commonly used in microbial fermentation due to low cost. Sun reported 2% ammonium sulfate and 84 h fermentation as optimal for *T. koningii* cellulase production, consistent with our findings. Phosphorus is an essential mineral element for microbial growth. Acid production from carbon source metabolism decreases fermentation pH, affecting growth and enzyme production. Phosphate addition in solid-state fermentation provides buffering and promotes growth and metabolism. Xie and Wang reported 0.1% potassium dihydrogen phosphate as optimal for *T. koningii* cellulase production, differing from our results, possibly due to different carbon source compositions in fermentation media.

Inoculum size is critical in filamentous fungal solid-state fermentation without nutrient supplementation. Sun determined 10% as optimal for *Trichoderma pseudokoningii*, consistent with our results. Low moisture causes medium surface drying affecting growth and enzyme production, while excessive moisture blocks air pores, limiting oxygen supply and inhibiting growth. Sun reported 55%–60% as optimal moisture for *T. pseudokoningii*, differing from our 67% result. Our experiments showed moisture had minimal effect on enzyme production, possibly due to higher initial moisture content. Lin reported 67% moisture for rice straw and bran medium with *T. koningii* AS3.2774, consistent with our findings. Therefore, optimal moisture content should be determined based on specific medium composition.

Conclusion

1. Through multiple rounds of UV, chemical, and cross-compound mutagenesis of *T. koningii* 13006, mutant strain *T. koningii* UH-1 with strong fiber-degrading enzyme production capacity was obtained, showing 42.7% increased xylanase activity and 78.5% increased cellulase activity compared with the original strain.

2. Optimal solid-state fermentation conditions for *T. koningii* UH-1 were: corn straw to wheat bran ratio 7:3, ammonium sulfate concentration 2%, inoculum size 1.1 mL, moisture content 67%, fermentation time 84 h, and potassium dihydrogen phosphate concentration 1%. Under these conditions, filter paper enzyme activity reached 0.86 U/g, cellulase activity reached 99.81 U/g, and xylanase activity reached 1,150.32 U/g.

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Note: Figure translations are in progress. See original paper for figures.