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Unlocking the biorefining potential of *Miscanthus lutarioriparius* with a high performance mutant of the non-model fungus *Talaromyces* sp.

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KEYWORDS

Bioeconomy, biomass degradation, bioproducts, cellulase activity, *Miscanthus*, mutagenesis technique

HIGHLIGHTS

- Cellulose-degrading fungi inhabit the native environment *Miscanthus lutarioriparius*.
- The non-model *Talaromyces* sp. efficiently degraded *M. lutarioriparius* biomass.
- Ultraviolet and chemical mutagenesis effectively improved strain degradability.

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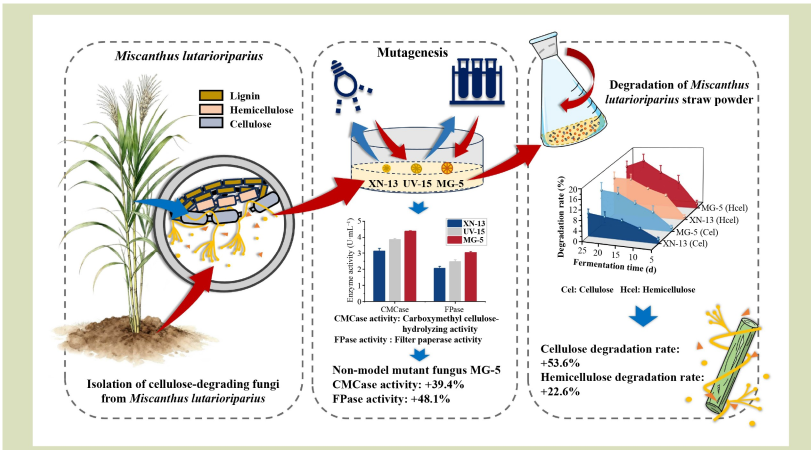
GRAPHICAL ABSTRACT



ABSTRACT

This study aimed to address two key bottlenecks in the microbial degradation of the endemic plant *Miscanthus lutarioriparius*: the scarcity of specialized degradative strains and the low cellulase activity of wild-type fungi. To isolate mutant strains with enhanced cellulolytic activity, this study applied a combined strategy of sequential screening (initially using Congo red followed by *M. lutarioriparius* straw powder) coupled with dual mutagenesis (UV and chemical). Seven cellulolytic fungal strains were isolated from soil samples from the native range of *M. lutarioriparius*. The wild-type strain XN-13 (*Talaromyces* sp.) had the highest initial activity for both carboxymethyl cellulase (CMCase, 3.2 $U\cdot mL^{-1}$) and filter paper cellulase (FPase, 2.1 $U\cdot mL^{-1}$). Following mutagenesis, the resulting mutant MG-5 had significantly enhanced CMCase (4.4 $U\cdot mL^{-1}$) and FPase (3.1 $U\cdot mL^{-1}$) activities, corresponding to increases of 39.4% and 48.1%, respectively. In degradation assays, MG-5 outperformed XN-13, achieving a 13.3% apparent cellulose degradation rate by day 25 (vs XN-13's 8.7%, an absolute difference of 4.6 percentage points) and an apparent hemicellulose degradation peak of 16.9% by day 20 (vs XN-13's 13.8%, an absolute difference of 3.1 percentage points). These findings demonstrate that the combined screening and mutagenesis strategy used successfully generated an efficient non-model mutant strain. Consequently, MG-5 thus represents a promising microbial resource for enhancing lignocellulose bioconversion of *Miscanthus* species.

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1 Introduction

The global transition toward a sustainable, green economy has spurred intense research interest in bioproducts as alternatives to petroleum-derived materials. These bioproducts, derived wholly or partially from renewable biomass resources (e.g., plants, animal manure and algae), are broadly categorized into bioenergy (e.g., bioethanol and biodiesel), and biobased materials (e.g., bioplastics and textiles)[1,2]. Driven by technological advances and rising market demand, the bioproducts industry has undergone rapid expansion, demonstrating considerable application potential[3]. However, its large-scale commercialization remains constrained by two persistent challenges: the sustainable supply of raw materials and the efficiency of conversion technologies.

Regarding feedstock supply, although agricultural residues such as crop straw are abundant[4,5], their seasonal availability and scattered distribution hinder the consistent supply required for continuous industrial production[6,7]. There is thus an urgent need to identify alternative biomass feedstocks that can support the sustainable growth of the bioproducts industry while guaranteeing a stable annual supply. Cultivating high-yielding perennial grasses on marginal lands is recognized as a highly promising solution for ensuring a sustainable biomass supply[8,9]. *Miscanthus* spp. represent a premier candidate for this strategy due to their low input requirement, high biomass yields (18.1–44.2 $t \cdot ha^{-1} \cdot yr^{-1}$ under China's climatic conditions)[10], strong environmental stress tolerance and superior material quality[11,12]. For example, in China, marginal lands suitable for *Miscanthus* cultivation have the potential to produce about 135 Mt of biomass annually[10]. This biomass has proven effective for producing biobased products such as cellulosic ethanol[13], xylooligosaccharides[14] and nanocellulose[15,16], offering higher production efficiency and superior product quality compared to agricultural straw.

Although biomass offers abundant potential for bioproduct conversion the related commercialization is still constrained by current techniques characterized by high production costs[17], environmental concerns[18] or low process efficiency[19]. For example, physical pretreatment methods, such as steam explosion and ball milling, have been applied in cellulosic ethanol production; however, their high energy consumption elevates operational costs, thereby undermining the economic competitiveness of the resulting bioethanol[20]. In contrast, chemical methods using strong acids or alkalis, while efficient and conceptually simple, raise environmental risks that limit their large-scale industrial adoption. Bioconversion methods, relying on microbial or enzymatic action, are regarded as a more sustainable technological pathway due to their mild reaction conditions and high environmental compatibility[21]. However, this strategy still faces two core challenges in practice: first, microbial degradation has significant substrate specificity, as the cross-linked structures of lignocellulose vary greatly among different biomass feedstocks. Strains capable of efficiently degrading loosely structured crop straws often have markedly reduced degradation efficiency when applied to highly crystalline, highly polymerized perennial grass biomass (e.g., *Miscanthus* species)[22,23]. Second, wild strains directly isolated from natural environments commonly have inherent limitations such as low enzyme activity and slow degradation rates[24,25], rendering them insufficient to directly meet the stringent performance requirements for industrial-scale production.

Overcoming these bottlenecks requires a dual approach: screening highly efficient strains from the native habitats of the target biomass, combined with mutagenesis techniques to enhance strain performance. Mutagenesis techniques typically involve treating wild-type strains with physical agents, such as ultraviolet (UV) radiation[26], or chemical agents, such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)[27]. These treatments induce genetic mutations, allowing for the selection of mutants with significantly improved enzymatic performance[28,29]. The objective extends beyond simply increasing total cellulase activity. More importantly, it aims to develop specialized strains that are highly adapted to specific target substrates. Fungi often present advantages over bacteria in strain selection programs. This is primarily because they secrete more complete and functionally synergistic extracellular cellulase systems[30–32]. As a result, they serve as superior candidates for the mutagenesis and improvement strategies described above.

Perennial grass biomass, particularly *Miscanthus*, holds enormous resource potential and promising industrial prospects in China. However, specialized research on its bioconversion remains limited. Therefore, developing efficient microbial strains tailored for biorefining this type of feedstock is of significant practical importance. This study aimed to isolate and screen fungi from the native habitat of *Miscanthus* that are capable of efficiently degrading its cellulose. Through mutagenesis strategies, we aimed further enhance their enzymatic activity and overall degradation efficiency. Their degradation capabilities and application potential will be systematically evaluated. The ultimate goal is to provide robust microbial strains that support the high-value conversion of *Miscanthus* resources.

2 Materials and methods

This study focused on *Miscanthus lutarioriparius* biomass as a feedstock. *M. lutarioriparius* is a *Miscanthus* species endemic to China, characterized by a large, concentrated growing area (88 kha) and a high annual yield potential (about 1 Mt)[33,34]. Also, its biomass has superior properties for biorefining, including high cellulose and hemicellulose contents, low ash content and a high degree of cellulose polymerization. These advantages establish *M. lutarioriparius* as a promising feedstock for bioproduct development in China[35].

Building on the rationale outlined above, this study aimed to obtain fungal strains with enhanced cellulolytic performance on *M. lutarioriparius* straw through a sequential screening and mutagenesis approach. This required first identifying a suitable wild-type strain with inherent cellulolytic activity, followed by mutagenesis to further improve its performance. The experimental design was as follows: (1) isolation of cellulose-degrading fungi from soil collected at *M. lutarioriparius* cultivation sites; (2) screening for the optimal wild-type strain through quantitative determination of carboxymethyl cellulase (CMCase) and filter paper cellulase (FPase) activities; (3) enhancement of cellulase activity in the selected strain via combined UV and MNNG mutagenesis; and (4) final validation of the degradation efficiency of the wild-type and mutant strains on *M. lutarioriparius* straw powder.

2.1 Isolation, screening, and identification of cellulolytic fungi special for *M. lutarioriparius*

Guided by the principle that microbial enzymatic systems have substrate specificity adapted to the composition of plant biomass in their native habitats[36,37], we focused our isolation efforts on the rhizosphere and cultivation sites of *M. lutarioriparius* to obtain strains proficient in degrading its distinct cellulose composite. Soil samples were collected from three representative locations: (1) the rhizosphere of wild *M. lutarioriparius* plants in Junshan District (29°50′ N, 112°35′ E), Yueyang City (a concentrated growing area); (2) a long-term (>10-year) *M. lutarioriparius* cultivated field in Lukou District (27°39′ N, 113°02′ E), Zhuzhou City, and (3) the *Miscanthus Germplasm Resource Garden* at Hunan Agricultural University (28°19′ N, 113°08′ E, Changsha City). Target microorganisms were then isolated via a standard serial dilution method[38]. Following isolation and purification, primary screening was conducted on a solid carboxymethyl cellulose sodium (CMC-Na) medium containing (L^{-1}): 15.0 g CMC-Na, 2.0 g peptone, 1.0 g yeast extract, 1.0 g NaCl, 1.0 g K_2HPO_4, 0.2 g $MgSO_4{\cdot}7H_2O$ and 20.0 g agar. This and all subsequent media were adjusted to pH 6.0 with NaOH or HCl unless otherwise stated. The plates were stained with a 1.0 $g{\cdot}L^{-1}$ Congo red solution for 30 min and destained with 1.0 $mol{\cdot}L^{-1}$ NaCl for 15 min. Isolates forming clear hydrolysis zones were selected as primary candidates and subsequently inoculated onto a secondary screening medium containing *M. lutarioriparius* straw powder as the sole carbon source[39]. The composition of this medium (L^{-1}) included 10.0 g *M. lutarioriparius* straw powder (250 μm sieved), 5.0 g K_2HPO_4, 5.0 g $MgCl_2{\cdot}6H_2O$, 0.25 g $MgSO_4{\cdot}7H_2O$, 0.2 g NaCl, 0.5 g $(NH_4)_2SO_4$ and 20.0 g agar. Strains having robust growth on this substrate-specific medium were designated as secondary candidates.

All secondary candidates were subsequently sent for identification. The selected strains were first characterized based on colony and cellular morphology. Molecular identification was then performed by amplifying and sequencing the internal transcribed spacer (ITS) rDNA region (Sangon Biotech, Shanghai, China). The resulting sequences were compared against the NCBI database using BLAST. Final species identification was achieved by integrating phylogenetic analysis with morphological observations.

2.2 Screening and evaluation of elite wild-type strains based on CMCase and FPase activities

The secondary candidate strains were individually inoculated into a liquid fermentation medium for enzyme production[40] with the following composition (L^{-1}): 10.0 g CMC-Na, 2.5 g yeast extract, 5.0 g peptone, 1.0 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 2.0 g $(NH_4)_2SO_4$ and 2.5 g NaCl (pH 6.0). During a 5-day continuous fermentation, culture broth samples of equal volume were collected every 24 h to prepare crude enzyme extracts, with all samples collected in triplicate. CMCase and FPase activities in these extracts were determined using the 3,5-dinitrosalicylic acid method in accordance with the *National Standard of the People's Republic of China* (GB/T 35808-2018)[41]. One unit (U)of cellulase activity (X) was defined as the amount of enzyme required to liberate glucose at 1 $\mu mol{\cdot}min^{-1}{\cdot}mL^{-1}$ in the original enzyme solution ($U{\cdot}mL^{-1}$) under the specified assay conditions (37 °C, pH 5.5, incubation for 60 min, followed by a boiling water bath for 5 min). Based on the cellulase activity profile, the strain having the highest activity was selected as the starting strain for subsequent UV mutagenesis. Cellulase activity (X) was calculated as:

$$X = [m/(M \times t \times v)] \times 1000 \times n \tag{1}$$

where X is the cellulase activity ($U{\cdot}mL^{-1}$), m is the mass of glucose (mg) corresponding to the (A – A_0) value calculated from the standard curve, M is the molar mass of glucose (180.2 $g{\cdot}mol^{-1}$), t is the enzymatic reaction time (min), v is the volume of the crude enzyme solution used (0.5 mL); 1000 is the unit conversion factor and n is the dilution factor, which was defined as 25 in this study.

2.3 Sequential combined mutagenesis of wild strains enhanced cellulase activity

2.3.1 Screening of mutant with enhanced cellulase activity by UV mutagenesis

A spore suspension was prepared from the starting strain after 7 days on PDA medium at 28 °C. For UV mutagenesis, aliquots of the spore suspension were exposed to a Philips TUV 36 W UV lamp at a distance of 30 cm for various durations (0, 30, 60, 90, 120, 150, 180, and 210 s). The lethality rate, calculated as in Eq. 2, was used to determine the optimal mutagenic dosage. An appropriate lethality rate (typically 70%–90%)[42,43] was targeted because it maximizes the probability of generating beneficial mutations while maintaining a sufficient number of surviving cells for screening. The optimal mutagenic dosage was determined based on the resulting lethality curve. Primary screening of mutants was performed on a CMC-Na solid medium supplemented with Congo red to identify those with a significantly increased hydrolysis zone ratio. Promising candidates were then subjected to secondary screening via liquid fermentation and selected based on enhanced CMCase and FPase activities. The high-yielding mutant strain obtained was confirmed to be genetically stable over five consecutive subcultures and was designated as the starting strain for subsequent chemical mutagenesis. Its percentage lethality rate (L) was calculated as:

$$L = (1 - N_m/N_0) \times 100\% \tag{2}$$

where N_m is the number of colonies in the mutagenized group and N_0 is the number of colonies in the control group.

2.3.2 Screening of mutants with enhanced cellulase activity by chemical mutagenesis

The superior mutants obtained from the primary UV screening were then advanced to chemical mutagenesis. MNNG was selected as the mutagenic agent for this second round. To determine the optimal treatment conditions, a concentration gradient (0, 0.2, 0.4, 0.6, 0.8 and 1.0 $mg{\cdot}mL^{-1}$) was first tested with a fixed 20-min exposure. The mutagenic reaction was terminated with a 10% sodium thiosulfate solution, and the optimal MNNG concentration was determined based on the lethality curve. A time gradient experiment, testing exposures of 0, 5, 10, 15, 20, 25 and 30 min, was then conducted at this optimal concentration to identify the corresponding optimal exposure duration. The resulting dual-mutagenized strain was treated under the optimized MNNG conditions. Mutants were primarily screened on a CMC-Na solid medium via Congo red staining, and subsequently in liquid fermentation based on CMCase and FPase activities. The high-yielding mutant was selected, and its genetic stability was confirmed over five consecutive subcultures before being designated as the final target strain for subsequent studies.

2.4 Degradation assay with *M. lutarioriparius* straw powder

The wild-type strain and the mutant were inoculated at a 1% (v/v) ratio into a liquid medium containing *M. lutarioriparius* straw powder as the sole carbon source. The medium composition (L^{-1}) was as follows: 20.0 g straw powder, 1.0 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.5 g $NaNO_3$, 0.1 g $CaCl_2$, 0.01 g $FeSO_4{\cdot}7H_2O$, and 0.01 g $ZnSO_4{\cdot}7H_2O$. Cultures were incubated at 28 °C with shaking at 180 $r{\cdot}min^{-1}$ for up to 25 days, and samples were harvested at 5-day intervals. An uninoculated medium, subjected to the same sterilization and incubation procedures, served as the control to account for non-biological degradation (e.g., from autoclaving and abiotic hydrolysis). After fermentation, the culture broth was centrifuged and filtered to remove microbial cells. The solid residue was collected and dried to a constant weight. The monosaccharide composition of the residue was analyzed by acid hydrolysis followed by high-performance liquid chromatography, according to the National Energy Administration standard of (NB/T 34057.5-2017)[44]. The residual cellulose and hemicellulose contents were calculated based on the monosaccharide analysis, allowing for the determination of the respective degradation rates. The degradation rate was calculated as in Eq. 3. Owing to the difficulty in completely separating fungal mycelia from the *M. lutarioriparius* straw powder, the percentage degradation rates (D) in this study are presented as apparent values, serving as conservative estimates of the actual efficiency and were calculated as:

$$D = [(C_0 - C_e)/C_0] \times 100\% \quad (3)$$

where C_0 is the residual cellulose/hemicellulose content in the sterilized and incubated uninoculated control (g), representing the baseline content after accounting for non-biological losses, and C_e is the residual cellulose/hemicellulose content in the experimental group (g).

2.5 Data analysis

Data were managed in Microsoft Excel (Version 2023), and statistical analyses were performed using IBM SPSS Statistics (Version 27.0). Prior to analysis, the normality of the data distribution was verified using the Shapiro-Wilk test, and the homogeneity of variances was confirmed using Levene’s test. A two-way repeated-measures ANOVA followed by Duncan’s post hoc test was applied to assess: (1) the main and interactive effects of strain type and fermentation time on cellulase activities (CMCase and FPase), (2) differences in cellulase activities between the wild-type and mutant strains at individual time points, (3) the genetic stability of the mutants over successive subcultures, and (4) the comparative cellulose degradation efficiency of the wild-type and mutant strains throughout the fermentation period. The significance threshold was set at $p < 0.05$ for all statistical tests. Figures were generated using a combination of Origin (Version 2022) and GraphPad Prism (Version 10.1.2), depending on the specific data visualization requirements. Data are presented as the mean ± standard error.

3 Results and analysis

3.1 Identification and enzymatic profile of the screened wild cellulolytic fungi specifically for degrading the *M. lutarioriparius* biomass

Seven fungal strains were isolated from soil humus by primary

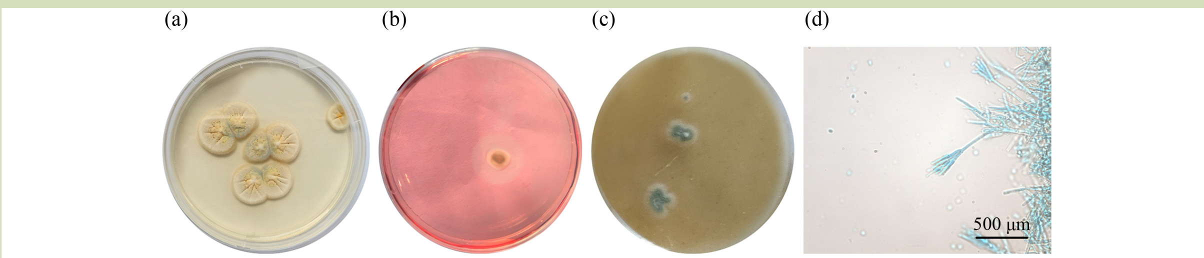

Fig. 1 Morphological and functional characterization of the wild-type strain XN-13 for degrading *M. lutarioriparius* biomass. (a) Colony morphology on PDA medium; (b) hydrolytic halo formation on CMC-Na medium; (c) growth profiling on *M. lutarioriparius* straw powder medium; and (d) microscopic morphology of spores and hyphae (lactophenol cotton blue staining at 400× magnification).

screening on a CMC-Na solid medium and designated as XN-13, XN-15, XN-121, XN-221, MZ-710, MY-2, and MY-3. Their colonial morphologies were examined after cultivation on PDA medium (Fig. 1(a)). Cellulose- degrading ability was evaluated via Congo red staining, with the formation of clear hydrolysis zones indicating cellulolytic activity (Fig. 1(b)). Following a 5-day cultivation period on a CMC solid medium, the hydrolysis zone ratios (D/d) of the seven isolated and purified fungal strains was found to ranged from 1.4 to 2.4 (Table 1). Generally, a higher D/d ratio indicates a stronger cellulose degradation capacity. Results showed that strain XN-15 (D/d = 2.4) had significantly higher D/d values than all other strains ($p < 0.05$) except for strains XN-13 (D/d = 2.1) and strain MZ-710 (D/d = 2.1). These three strains were therefore considered the most promising candidates for degrading the *M. lutarioriparius* biomass.

All seven strains had robust growth on a solid secondary screening medium containing *M. lutarioriparius* straw powder as the sole carbon source (Fig. 1(c)), confirming their ability to utilize this specific substrate. Preliminary taxonomic identification integrated morphological characterization (Fig. 1d) with sequence alignment based on Sanger sequencing data, as summarized in Table 1. Results revealed a distinct taxonomic clustering: strains MY-2, MY-3 and XN-221 were assigned to the genus *Penicillium*, while XN-13 and XN-15 were identified as members of *Talaromyces*. Sequence comparison between XN-13 and XN-15 had high similarity, with divergence limited to a few nucleotide positions, indicating that they represent closely related but genetically distinct strains within the same genus. Morphologically, all *Penicillium* and *Talaromyces* isolates had broom-shaped conidiophores with abundant conidia and relatively rapid

Table 1 Morphological characteristics and molecular identification of the seven isolated fungal strains for degrading *M. lutarioriparius* biomass

Strain number	Colony morphology	Hydrolysis zone ratio	Microscopic morphology	Reference strain	Homology (%)	Genus	GenBank_accession
MY-2	Flat and circular in form, with a rough surface; white initially, becoming dark green at maturity	1.6 ± 0.1 bc	Broom-shaped conidiophores with abundant conidia	*Eupenicillium javanicum* isolate AFTOL-ID 429	99.6	*Penicillium*	PX485168
MY-3	Circular with radial folds; white initially, maturing to dark green	1.7 ± 0.2 bc	Broom-shaped conidiophores bearing secondary branches and abundant conidia	*Penicillium* sp. strain R57	99.8	*Penicillium*	PX485170
XN-13	Circular with radial folds; pale yellow initially, turning dark green at maturity	2.1 ± 0.3 ab	Broom-shaped conidiophores with abundant conidia	*Talaromyces wortmannii* culture CBS:316.63	99.5	*Talaromyces*	PX485144
XN-15	Circular with radial folds; initially off-white, becoming dark green at maturity	2.4 ± 0.6 a	Broom-shaped conidiophores with abundant conidia	*Talaromyces variabilis* strain ZHKUCC 23-1029	100.0	*Talaromyces*	PX485146
XN-121	Raised and circular, with a dark greyish-black, cotton-wool-like surface	1.8 ± 0.2 bc	Elongated, dendritic hyphae with sparse conidia	*Cladosporium* sp. strain P6	99.2	*Cladosporium*	PX485148
XN-221	Flat and circular, with a rough texture; the colony had a white periphery and a pinkish-brown center initially, transitioning uniformly to dark green at maturity	1.4 ± 0.1 c	Broom-shaped conidiophores bearing secondary branches and abundant conidia	*Penicillium* sp. isolate LSZ-1	99.8	*Penicillium*	PX485171
MZ-710	Raised and circular, with a light greyish-white, cotton-wool-like surface	2.1 ± 0.4 ab	Elongated, dendritic hyphae with sparse conidia	*Nigrograna magnoliae* culture UESTCC:23.0082	99.4	*Nigrosabulum*	PX485160

Note: Means with the same letters are not significantly different ($p > 0.05$).

growth. In contrast, isolate XN-121 was classified as *Cladosporium* and MZ-710 as *Nigrosabulum*, both of which lacked broom-shaped conidiophores and instead developed slender, dendritic hyphae with sparse conidiation.

The cellulolytic profiles of the screened strains were assessed based on the activities of CMCase and FPase (Fig. 2). All strains had higher CMCase activity than FPase activity. For example, the average CMCase activity across all strains was 38.8% higher than the average FPase activity (1.3 vs 1.0 U·mL^{-1}). Significant differences (p < 0.05) were detected among strains for both enzymes, particularly over the course of fermentation. On day 4 of fermentation, most strains reached peak activity for both CMCase and FPase; even when not the highest, enzyme activities on day 5 did not differ significantly from those on day 4. Therefore, activities measured on day 4 were used for inter-strain comparisons. Among all strains, XN-13 had the highest CMCase activity (3.2 U·mL^{-1}), significantly outperforming all other strains except XN-15 (p < 0.001). The difference between XN-13 and XN-15 (2.8 U·mL^{-1}) was not statistically significant (p = 0.130). A similar trend was observed for the FPase activity: although XN-13 (2.1 U·mL^{-1}) had higher activity than XN-15 (1.8 U·mL^{-1}), the difference was not significant. Collectively, this comparative evaluation identified the wild-type strain XN-13 as the top performer. Classified as *Talaromyces* sp. (Ascomycota), XN-13 was consequently chosen as the parental strain for subsequent dual mutagenesis studies.

3.2 UV mutagenesis and screening of mutant strains

The UV mutagenesis lethality curve of strain XN-13 is shown in Fig. 3(a). Lethality rates increased proportionally with extended UV exposure time, reaching 89.2% at 60 s. Given that the probability of positive mutations is generally higher within the 70% to 90% lethality range, 60 s was selected as the optimal UV exposure time. Based on the 5-day enzyme activity profiles presented above, strain XN-13 reached peak CMCase and FPase activities on the fourth day of fermentation. Therefore, all subsequent experiments used 4 days of fermentation as the fixed time point for enzyme activity measurement.

Five mutant strains (UV-3, UV-5, UV-8, UV-9 and UV-15) were selected via Congo red staining, and their enzyme activities were determined (Fig. 3(b)). On average, the mutants had 61.9% higher CMCase activity than FPase activity (3.5 vs 2.2 U·mL^{-1}). Of these, mutant UV-15 showed the highest CMCase activity (3.9 U·mL^{-1}), which was 22.5% greater than that of the best-performing wild-type strain XN-13 (3.2 U·mL^{-1}) and significantly exceeded that of all other mutants (p < 0.05). Its FPase activity (2.5 U·mL^{-1}) was also significantly higher than that of all other strains (p < 0.05) except UV-8 (2.3 U·mL^{-1}, p = 0.063), representing a 19.9% increase over strain XN-13 (2.1 U·mL^{-1}).

To evaluate genetic stability, the highest-activity mutant, UV-15, was subjected to five consecutive subculturing passages.

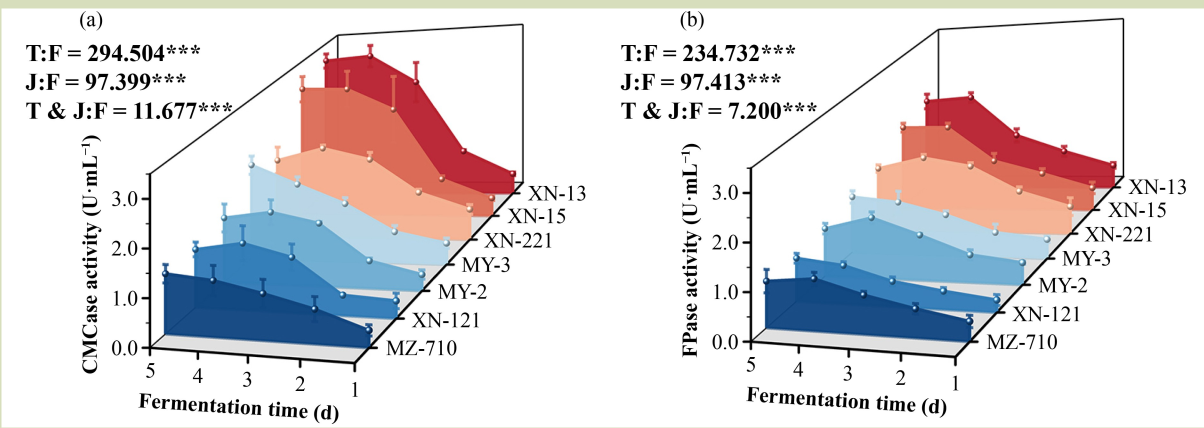

Fig. 2 Enzymatic profile of the seven screened cellulolytic fungi: comparative cellulose activities on *M. lutarioriparius* biomass. (a) Carboxymethyl cellulase activity (CMCase activity), and (b) filter paperase activity (FPase activity). T, fermentation time; J, strain type; T & J, interaction between fermentation time and strain type; ***, statistically highly significant (p < 0.001).

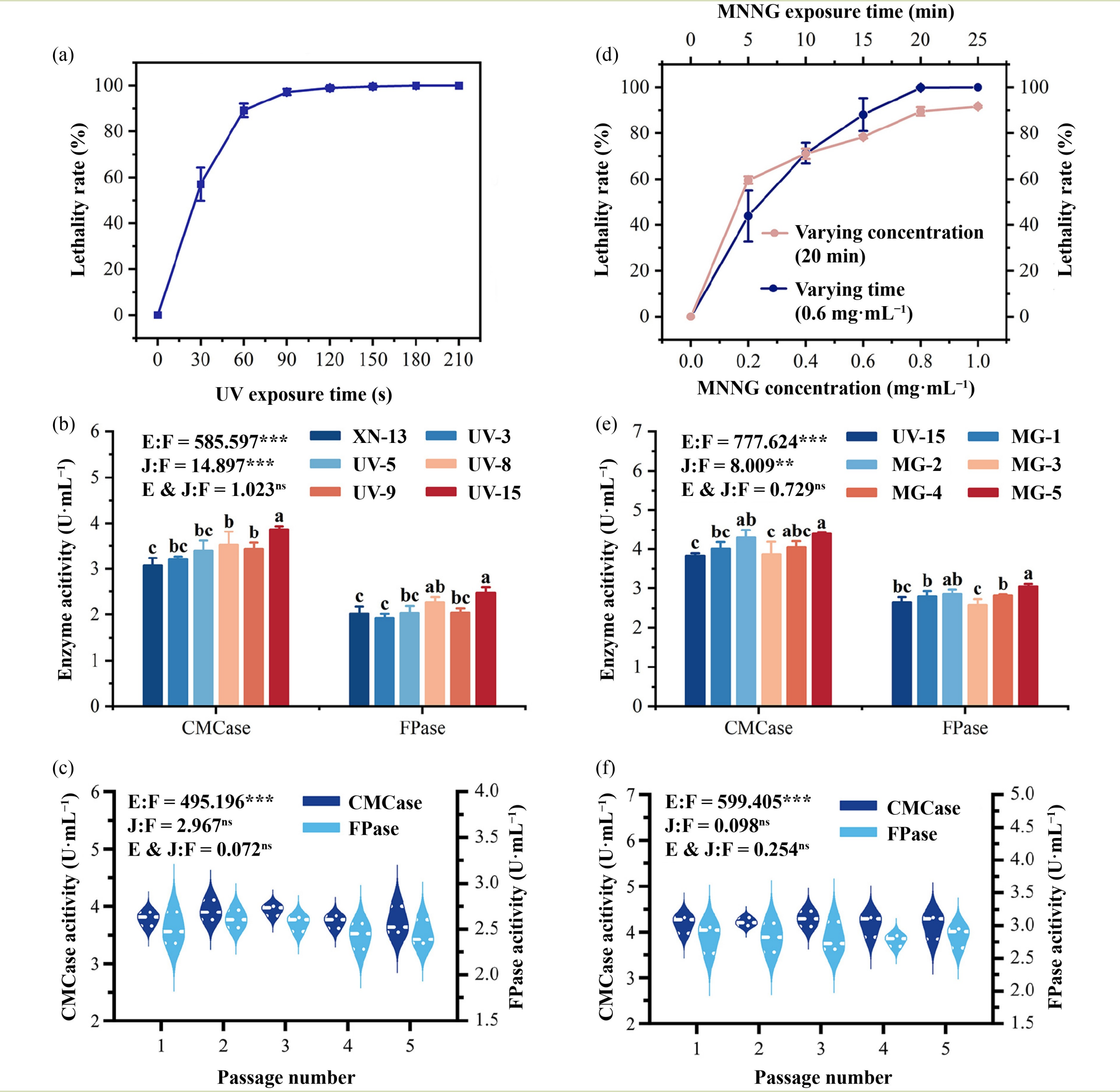

Fig. 3 (a) The UV-induced lethality curve of strain XN-13; (b) comparative analysis of carboxymethyl cellulase (CMCase) and filter paperase (FPase) activities in UV-induced mutant strains and the wild-type strain XN-13; (c) genetic stability of cellulase activities in mutant strain UV-15 over five serial subcultures; (d) dual-axis analysis of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) induced lethality. Lethality rates are plotted on two independent *y*-axes sharing an identical scale. Left *y*-axis, lethality versus MNNG concentration (lower *x*-axis), measured at a fixed exposure time of 20 min; right *y*-axis, lethality versus exposure time (upper *x*-axis), measured at a fixed MNNG concentration of 0.6 mg·mL^{-1}; (e) comparative analysis of CMCase and FPase activities in combinatorial mutant strains and the UV mutant strain UV-15; (f) genetic stability of cellulase activities in mutant strain MG-5 over five serial subcultures. E, cellulase activity (CMCase or FPase); J, strain type; E & J, interaction between cellulase activity and strain type; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant ($p > 0.05$); and F, F-statistic. Means with the same letters are not significantly different ($p > 0.05$).

The results (Fig. 3(c)) revealed that both CMCase and FPase activities remained stable across generations, with average values of 3.8 and 2.5 U·mL^{-1}, respectively. The absence of significant intergenerational differences in enzymatic activities were observed (CMCase: $p = 0.356$; FPase: $p = 0.431$), coupled with low coefficients of variation (4.1% and 5.1%, respectively), demonstrated the robust genetic stability of strain UV-15, supporting its selection as the parental strain for subsequent chemical mutagenesis.

3.3 Compound mutagenesis and screening of mutant strains

The lethality of the UV-15 mutant strain in response to MNNG concentration and exposure time is shown in Fig. 3(d). The results indicated that lethality increased with higher MNNG concentrations and longer exposure times. Under the conditions of 0.6 mg·mL^{-1} MNNG and a 20-min treatment, lethality fell within the targeted 70%–90% range (88.1% and 89.4%, respectively); these parameters were therefore selected for subsequent chemical mutagenesis.

Five chemical mutant strains (MG1 to MG5) were isolated via Congo red staining, and their enzyme activities were assessed (Fig. 3(e)). On average, the mutants had 32.5% higher CMCase activity than FPase activity (4.1 U·mL^{-1} vs 3.1 U·mL^{-1}). Of these, mutant MG5 showed significantly higher CMCase (4.4 U·mL^{-1}) and FPase (3.1 U·mL^{-1}) activities than all other strains ($p < 0.05$), except MG2 (CMCase 4.3 U·mL^{-1}, $p = 0.526$; and FPase 2.9 U·mL^{-1}, $p = 0.051$) ($p < 0.05$). Relative to the wild-type strain XN13 (CMCase 3.2 U·mL^{-1} and FPase 2.1 U·mL^{-1}), these values represent increases of 39.4% and 48.1%, respectively.

To evaluate genetic stability, mutant MG5 was subcultured for five consecutive passages (Fig. 3(f)). Both CMCase and FPase activities remained stable across generations, with average values of 4.2 and 2.8 U·mL^{-1}, respectively. No significant intergenerational differences were detected (CMCase $p = 0.907$ and FPase $p = 0.995$), and the coefficients of variation were low (4.0% and 5.5%, respectively). Based on these results, MG5 was established as a genetically stable and high-performing candidate strain for subsequent fermentation process optimization.

3.4 Degradation assay with *M. lutarioriparius* straw powder

The degradation effects of the wild-type strain XN-13 and the mutant strain MG-5 on *M. lutarioriparius* straw powder are shown in Fig. 4. The degradation rates of cellulose and hemicellulose increased significantly with fermentation time in both strains ($p < 0.001$). The average degradation rates achieved by MG-5 (cellulose 8.5% and hemicellulose 10.9%) were significantly higher than those of XN-13 (cellulose 5.9% and hemicellulose 9.5%) ($p < 0.05$).

The degradation curves of the two strains followed a similar trend. At the end of fermentation (25 days), cellulose degradation reached its maximum in both strains (MG-5 13.3% and XN-13 8.7%), corresponding to an absolute increase of 4.6 percentage points for MG-5 compared to XN-13. For hemicellulose degradation, MG-5 peaked earlier at day 20 (16.9%), which was 3.1 percentage points higher than that of XN-13 at the same time point (13.8%, $p < 0.05$). These results collectively demonstrate that the mutant strain MG-5 outperforms the wild-type strain XN-13 in degrading *M. lutarioriparius* biomass.

4 Discussion

4.1 *Talaromyces* as a promising non-model fungus for degrading *Miscanthus* biomass

In this study, a wild fungal strain with a strong capacity to degrade cellulose was isolated from the native habitat of *M. lutarioriparius*. It was identified as a non-model fungus belonging to the genus *Talaromyces*. Energy crops such as

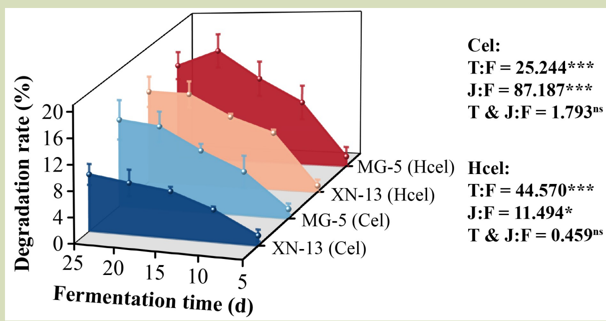

Fig. 4 Cellulose and hemicellulose degradation by the wild-type strain XN-13 and mutant MG-5. Cel, cellulose; Hcel, hemicellulose; T, fermentation time; J, strain type; T & J, interaction between fermentation time and strain type; ***, $p < 0.001$; *, $p < 0.05$; ns, not significant ($p > 0.05$); and F, F-statistic.

Miscanthus possess a more compact and robust lignocellulosic structure compared to common agricultural residues such as maize stover. Common model fungal strains, including *Trichoderma reesei*[45] and *Aspergillus* spp.[46], often show limited degradation efficiency on such specialized substrates. This observation leads to a key hypothesis: due to long-term coevolutionary relationships, microorganisms native to *Miscanthus* habitats are more likely to have developed efficient strategies for degrading the lignocellulose of their host plants. The discovery of this strain therefore provides valuable material for investigating such adaptive mechanisms and for developing novel enzyme resources.

Enzymatic activity assays revealed that *Talaromyces* sp. XN-13 has a CMCase/FPase activity ratio exceeding 1. This specific enzyme profile carries both biological and technological significance. As a filamentous fungus[47], *Talaromyces* sp. generates mechanical forces through hyphal growth, which aid in physically disrupting plant cell walls[48]. Its high proportion of endoglucanase (CMCase) activity allows it to function as a pioneer enzyme during the initial stages of degradation, efficiently targeting the amorphous regions of cellulose to rapidly reduce substrate polymerization and loosen the cell wall framework. This creates more accessible binding sites for subsequent exoglucanase activity[49]. While the excellent lignocellulose degradation capabilities of certain fungal genera have been reported, existing research has largely focused on model substrates such as cellulose powder or agricultural residues such as maize stover[50]. For highly resistant energy crops with denser cell wall structures, such as *Miscanthus*, systematic investigations into fungal degradation mechanisms and enzyme adaptability remain limited. *Talaromyces* sp. XN-13, directly screened from the native habitat of *Miscanthus*, offers an ideal research model and germplasm resource for understanding how fungi adapt to and deconstruct such highly recalcitrant substrates.

4.2 Mutagenesis enhances rate-limiting enzyme activity in *Talaromyces* sp.

The *Talaromyces* sp. isolate is strongly substrate adapted but had lower overall cellulase activity than commercial strains. Its enzyme profile featured high CMCase activity but relatively low FPase activity, indicating a weak capacity for crystalline cellulose hydrolysis[51]. This limitation is common in wild isolates[52,53] and restricts their direct industrial application. We therefore employed a mutagenesis strategy[54] to specifically enhance FPase activity. The mutant strain MG-5 had simultaneous increases in both enzyme activities. This coordinated improvement suggests that the mutation may affect global regulatory pathways rather than individual enzyme genes[55]. Future transcriptomics or whole-genome resequencing analyses could help identify the underlying mechanisms. These results highlight an important principle: in lignocellulose degradation, the synergy between endo- and exoglucanases often dictates the overall hydrolysis efficiency[56]. When a wild-type strain already possesses good substrate adaptation, targeting rate-limiting enzymes through mutagenesis can effectively boost this synergy. This approach enabled a significant improvement in *Miscanthus* degradation by *Talaromyces* sp. and provides a valuable reference for modifying other non-model filamentous fungi.

4.3 Significance and prospects of mutant strain MG-5 in constructing the *Miscanthus* sugar platform

The development of a bioeconomy hinges on establishing economically viable conversion of agricultural biomass to fermentable sugars (a sugar platform). Two cornerstones support such platforms: a stable supply of high-quality feedstock and efficient, low-cost saccharification technology[57]. From a feedstock perspective, *Miscanthus* presents clear advantages, including high biomass yield, high cellulose content, and moderate lignin levels. Importantly, it does not compete with food crops for land, making it an ideal non-grain feedstock for sugar platforms[58]. Given these feedstock benefits, breakthroughs in conversion processes become increasingly critical. The degradation capability demonstrated by the mutant strain MG-5 toward *Miscanthus* cellulose provides initial evidence that a tailored sugar conversion system is achievable. Once *Miscanthus* resources are converted into fermentable sugars through such a system, they can feed into various biorefining pathways, including the production of fuel ethanol[59], lactic acid[60] and polylactic acid[61]. The global markets for these products continue to expand, which in turn extends the value chain of *Miscanthus* resources and enhances their economic potential[62].

Nevertheless, translating laboratory achievements into industrial applications presents numerous challenges. Based on the current degradation performance of MG-5, future research should proceed along two parallel tracks. At the strain improvement level, genome resequencing can help identify key mutations, providing targets for the precise modification of rate-limiting enzymes. At the process development level, efforts should focus on reducing costs and improving efficiency. Optimizing fermentation conditions could boost enzyme activity while shortening fermentation cycles. Additionally, introducing lignin-degrading bacteria for sequential co-cultivation may help overcome the lignin barrier, enabling the cascade saccharification of both cellulose and hemicellulose. Ultimately, measuring actual fermentable sugar yields and calculating unit production costs will determine whether this route is economically viable.

In summary, this study successfully obtained a mutant strain with enhanced degradation capability toward *Miscanthus* cellulose. Through a combination of in situ screening, enzymological characterization, and targeted mutagenesis, we isolated and developed the *Talaromyces* mutant strain MG-5. This strain not only provides a core microbial resource for *Miscanthus* biomass conversion but also offers methodological insights for discovering and modifying non-model microorganisms. With continued multidimensional research, MG-5 has the potential to be a key contributor to construction of a *Miscanthus* sugar platform, representing a viable technological pathway for non-grain biomass refining.

5 Conclusions

This study reports the isolation of a wild *Talaromyces* sp. XN-13 capable of efficiently degrading *M. lutarioriparius* cellulose. The strain had CMCase and FPase activities of 3.2 and 2.1 U·mL^{-1}, respectively, and achieved 8.7% cellulose degradation from *M. lutarioriparius* straw during a 25-day fermentation period.

To improve its enzymatic performance, a combined UV-MNNG mutagenesis strategy was used, yielding the high-activity mutant MG-5. The mutant showed increased CMCase and FPase activities (4.4 and 3.1 U·mL^{-1}), corresponding to improvements of 39.4% and 48.1% over the wild-type strain, and enhanced cellulose degradation by 4.6 percentage points (from 8.7% to 13.3%) under identical conditions.

These results demonstrate that the combined mutagenesis approach for *Talaromyces* sp. XN-13 can substantially improve its cellulolytic capacity on *M. lutarioriparius* biomass. The MG-5 mutant represents a promising candidate for further strain development toward applications in lignocellulose bioconversion.

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Compliance with ethics guidelines

Jun Li, Xiaoyue Wang, Meng Li, Tongcheng Fu, Zili Yi, and Shuai Xue declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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