Degradation characteristics of lignocellulose by endophytic fungi of *Taxillus chinensis*

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Research Article

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Abstract

Background

There are several endophytic fungal strains from *Taxillus chinensis* that can degrade lignocellulose from different substrates. We systematically explored their enzyme-producing characteristics and degradation efficiency on these substrates with the aim of providing theoretical support for the screening and application of high-efficiency degrading strains.

Results

Among the 4 tested strains, the highest total organic matter mass loss rate (55.50%) was observed when wheat bran was used as the substrate for 44 days of fermentation with the average degradation rate reaching a peak at 9 d (4.28%/d). All 4 strains showed consistent temporal dynamic characteristics with the 4 substrates: the lignin peroxidase activity peaked at 9 d of fermentation, with the highest enzyme activity of 23.52 U/mL. For strain P6, when wheat bran was used as the substrate, the laccase activity peaked at 16 days of fermentation (108.39 U/mL), which was significantly higher than those in other substrates and at different fermentation times. For strains 4 and N6, with mulberry leaves and wheat bran as substrates, the manganese peroxidase activity reached the highest at 16 and 30 days of fermentation (118.32 and 111.69 U/mL), respectively. For strain N6, when mulberry xylem was used as the substrate, the cellulase activity peaked at 2 days (0.67 U/mL), which was higher than that in other substrates and at different fermentation times.

Conclusions

The degradation efficiency of the 4 screened endophytic fungal strains from *T. chinensis* on different lignocellulosic substrates, as well as the activities of key degrading enzymes such as lignin peroxidase and laccase, all showed significant strain specificity, substrate specificity, and temporal dynamic characteristics. The results of this study can provide a theoretical basis and practical reference for the excavation of high-efficiency lignin-degrading strain resources and the optimization of their fermentation conditions.

Background

Lignocellulose, the most abundant natural polymer in nature, is composed mainly of cellulose, hemicellulose, and lignin, which interweave to form a naturally compact structural complex [1]. The annual global production of lignocellulose is approximately 7.5 billion tons, which is equivalent to 10 times the annual total energy consumption. However, its high structural stability and resistance to degradation results in a low utilization efficiency for this process. At present, traditional disposal methods of agricultural and forestry wastes, (such as *in-situ* returns to fields, industrial raw material

processing, feed preparations, and direct combustion, have several limitations. Among them, incineration tends to cause regional haze and increases the difficulty of air pollution control [2–3]. Therefore, the potential development of efficient and environment-friendly lignocellulose degradation technologies is of great practical significance.

During microbial degradation of lignin, various changes occur in its chemical structure, including side-chain oxidation and demethylation. Lignin-degrading enzymes depolymerize the linkages within this macromolecule to ultimately degrade it. The key enzymes involved in degradation by white-rot fungi mainly include lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac) [4–6]. The synergistic action of these enzymes can effectively decompose the refractory lignin components [7–8]. In addition to these three major enzymes, other auxiliary ones also participate in the process. LiP (EC 1.11.1.14), also known as diarylpropane oxygenase, contains Fe^{3+} and was first discovered in the extracellular medium of fungi under nitrogen-limiting conditions [9]. It is a globular helical glycoprotein with 343 amino acid residues and a molecular weight of 38–46 kDa. It is initially oxidized by H_2O_2 to form compound I, which then reacts with the substrate to generate compound II. In comparison to conventional peroxidases, LiP can oxidize aromatic hydrocarbons with only 2–3 ether substituents, thereby reducing the stability of the lignin molecular bonds by promoting radical formation, and leads to lignin depolymerization [10].

MnP (EC 1.11.1.13) is a heme-containing, glycosylated peroxidase with a molecular weight of about 45 kDa. Its amino acid sequence shares high similarity with LiP, and its catalytic process is similar to those of LiP, horseradish peroxidase (HRP), and other peroxidases [8, 11]. The unique feature of this enzyme is that it uses Mn as an electron donor. Due to the lack of a corresponding tryptophan residue, it cannot directly oxidize non-phenolic lignin but generates highly oxidative Mn^{3+} through its manganese-binding site [12], which exerts a secondary oxidative effect via diffusion. In addition, it cooperates with other lignin-degrading enzymes to break macromolecular bonds, threby playing an essential role in lignin degradation [11].

Lac (EC 1.10.3.2), also known as hydroquinone: oxidoreductase) belongs to the multicopper oxidase family. As a copper-containing polyphenol oxidase, it plays a central role in lignocellulose degradation due to its broad substrate specificity and high catalytic efficiency [13–14]. Lac was first found in lacquer trees and later reported in many plants, microorganisms, and animals. Laccases from different sources differ significantly in content, structure, and properties [15–18]. Among them, microbial laccases, including those from fungus and bacteria, which can possess high redox potentials, are dominant enzyme sources for lignocellulose degradation [2]. Lac can catalyze the conversion of various aromatic compounds such as monophenols, diphenols, and polyphenols, while reducing molecular oxygen to water. It is widely used in industrial production, environmental protection, food processing, as well as other fields [19–22]. However, insufficient catalytic efficiency under harsh conditions limits its practical applications, so the screening of novel strains with extreme environmental tolerances and high enzyme production is crucial [23–24].

White-rot fungi account for approximately 80% of all wood-rotting fungi and they are widely distributed in forest ecosystems and are natural high-yield lac producers and efficient lignocellulose degraders [25–29]. Their unique degradation system consists of Lac, MnP, and LiP, which can efficiently degrade lignin by catalyzing the cleavage of lignin chemical bonds while maintaining the structural integrity of cellulose, without producing pigments during the process [30–31]. For example, Xu et al. [32] screened *Auricularia polytricha* 5.584 from 9 white-rot fungi strains, and the lignin degradation rate reached 36.24% after 15 days of submerged fermentation. Li [33] found that culture and fermentation conditions directly affected lac production and the synergistic effect of lignocellulolytic enzymes in *Trametes versicolor*, highlighting the importance of optimizing fungal culture conditions.

Fermentation protocols are the key factors that can affect lac yield. Submerged fermentation has long been the mainstream method for enzyme production due to uniform nutrient distribution and sufficient microbial absorption. In contrast, solid-state fermentation (SSF) has gradually become a research hotspot because it is closer to the natural living environment of microorganisms, with simplified technology, low energy consumption, less pollution, and high product recovery rates [34–35]. Lignocellulosic materials, including corn stover, wheat bran, and wood sawdust, as SSF substrates not only provide carbon and nitrogen but also mineral elements, such as K and Mg for microbial growth and enzyme synthesis. In addition, they contain natural inducers such as flavonoids and phenols, which can significantly improve fungal lac yield, making them low-cost and high-quality nutrient sources [36–38].

Taxillus chinensis (DC.) Danser, a traditional Chinese medicinal herb, was first recorded in Shennong's Classic of Materia Medica as a high-grade medicine. Its dried leafy stems and branches have the effects of expelling wind-dampness, tonifying the liver and kidneys, strengthening tendons and bones, and the capacity to prevent miscarriages. It is officially listed in the Pharmacopoeia of the People's Republic of China (2025 Edition) [39]. Due to low natural parasitism rates and heavy reliance on wild resources, the contradiction between increasing market demand and insufficient supply of *T. chinensis* has become a prominent concern. It is urgent to alleviate this problem by improving the parasitism rates, analyzing reproductive mechanisms, and promoting artificial cultivations [40–42]. With respect to its parasitic mechanism, *T. chinensi*s penetrates the epidermal cell wall, which is composed mainly of lignin and cellulose, of host plants through specialized haustoria to establish a “physiological bridge” for obtaining water and mineral nutrients while maintaining its own photosynthesis [40–42]. Studies have shown that white-rot fungi can invade host plants by degrading their cell walls [43], and the role of endophytic fungi in lignocellulose decomposition has been preliminarily explored. However, most of the relevant studies have tended to focus on the enzymatic characteristics of single strains [44–45].

Previous studies in our laboratory have screened 4 endophytic fungal strains with high lignocellulolytic enzyme production from *T. chinensis* parasitizing different hosts [45–46]. Based on this, the present study selected 4 different substrates of mulberry bark, xylem, and leaves, as well as wheat bran. We investigated their effects on the kinetic characteristics of their ligninolytic enzymes (lac, MnP, and LiP) and cellulase production in a systematic way. In addition, we assessed the substrate degradation rates of the 4 endophytic fungi. The optimal substrate suitable for the growth of *T. chinensis* was determined

at the enzymatic level, with the aim of providing high-quality microbial resources and theoretical support for efficient lignin degradation as well as utilization of different substrate resources.

Materials and methods

The test strains

Four endophytic fungal strains isolated from *Taxillus chinensis* (DC.) Danser were used. The plant material identified as *T. chinensis* was collected from Nanning city, Gugnaxi. Taxonomic identification was performed by Yude Peng based on morphological characteristics. A voucher specimen (accession no.SJS-NN-101) was deposited at the Guangxi Key Laboratory of High-Quality Formation and Utilization of Dao-di Herbs, which is publicly accessible. These were strains 4 (*Colletotrichum* sp.), 15 (*Nigrospora sphaerica*), N6 (*Diaporthe phaseolorum*), and P6 (*Pestalotiopsis* sp.) [45]. The NCBI accession numbers (ITS and beta-tubulin sequences) of each strain were MZ823601/MZ964759, MZ823600/MZ934421, MZ823599/MZ934420, and MZ823598/MZ934419, respectively. All strains were preserved in a 4 ℃ refrigerator in the plant pathology laboratory of Guangxi Botanical Garden of Medicinal Plants for subsequent use.

Solid-state fermentation of the strains

Wheat bran used in the experiment was purchased from Zhonghe Modern Agricultural Development Group Co., Ltd., and its pretreatment was by the method of Tao et al [47] with slight modifications. Mulberry leaves and branch bark epidermis and xylem were obtained from the Research Base of Guangxi Medicinal Botanical Garden. The samples rinsed with clean water and then distilled water, and dried in a 50 ℃ oven for 24 hours. They were then crushed, passed through a 100-mesh sieve to prepare the culture substrates, which were sealed and stored for later use. 2.0 g of each prepared substrate was added to 250 mL Erlenmeyer flasks with 15 mL of 0.5% malt extract and the mixture was autoclaved at 121 ℃ for 30 minutes. After cooling, the test strains were activated and cultured at a constant temperature of 28℃ for 5 ~ 7 days, and 6 mm diameter mycelial discs were taken with a sterile puncher and inoculated into the sterilized flasks. The humidity was maintained at 90%, and the flasks were cultured in the dark at 28℃ in an incubator. During the fermentation and culture period, sampling was carried out on days 2, 9, 16, 23, 30 and 44, respectively. Each treatment carried out in triplicate.

Enzyme solution preparation and calculation of the average degradation rate

Total organic matter (TOM) mass loss and average (MLR) degradation rate were used as evaluation indicators to assess the degradation efficiency of the 4 strains on 4 substrates (wheat bran as well as mulberry leaves, bark and xylem) and these were determined based on Hao et al. [48] with slight modifications. After each sampling, 10 mL of sodium acetate buffer solution (pH 6.0, 50 mmol/L) was

pre-cooled to 4℃ in an Erlenmeyer flask, and extracted at 28℃ and 120 r·min⁻¹ for 20 minutes. Dried Whatman No.1 filter paper was weighed, and the mixture was filtered with this filter paper. The filtrate was collected and centrifuged at 4℃ and 10 000 × g for 10 minutes. The supernatant was used as the crude enzyme extract for the subsequent determination of various enzyme activities. The precipitate from the centrifuge tube was combined with the filter paper filtrate, placed on the original filter paper, and dried at 72 ℃ and weighed to calculate MLR.

Sample MLR (%) = (Initial dry mass of sample - Dry mass of sample after decomposition) / Initial dry mass of sample × 100%.

Average degradation rate (%) = TOM MLR / Culture days.

Determination of enzyme activities

The activity of lac was determined by the method of Buswell et al. [49]. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was the reaction substrate and it was carried out at 30 ℃. The amount of enzyme required to catalyze the oxidation of 1μmol ABTS per minute was defined as 1 enzyme activity unit (U) and this was measured by the absorbance change at 420 nm. The molar extinction coefficient of ABTS at 420 nm was 36000 L/(mol·cm). LiP activity was determined by Tien et al. [50] method with veratryl alcohol as the substrate and this was performed at 30℃. The amount of enzyme required to change the absorbance at 310nm change in 1mL of the reaction mixture to reach 0.1 per minute was defined as 1 enzyme activity unit (U), resulting in the production of veratraldehyde. The activity of MnP was determined by the method of Orth et al. [51] and used the changes in absorbance of the phenol red at 610 nm. The amount of enzyme required for the absorbance of 1mL of the reaction solution to increase by 0.1 unit per minute was defined as 1 enzyme activity unit (U·mL⁻¹).

The activity of cellulase was determined by the 3,5-dinitrosalicylic acid method. The enzyme activity was calculated by measuring the content of reducing sugars produced after cellulase degraded cellulose. A standard curve was constructed with 10 mg of anhydrous glucose standard (loss on drying < 0.2%) from the kit. This was dissolved in 1 mL of ultrapure water to prepare a glucose stock solution, which was then diluted to 1.0, 0.8, 0.6, 0.4, 0.2, 0.1, and 0 mg·mL⁻¹, respectively. 1 mL of fermentation broth was ultrasonicated ice bath conditions to lyse the cells and centrifuged at 4 ℃ and 8000×g for 10 minutes. The supernatants were stored on ice and 50μL samples were used for determinations for measurements by addition of 150μL of the 3,5-dinitrosalicylic acid reagent. The absorbance at 540 nm [52–53] was measured and the cellulase activity was calculated by using the standard curve.

Statistical analysis

All experimental data were analyzed by one-way analysis of variance (ANOVA) using SPSS 19.0 software, and the results were expressed as means±standard deviations. The homogeneity of variance test was first performed on each group of data and Origin 2018 software was used for plotting the data.

Results

The mass loss of different substrates of four endophytic fungal strains

The mass loss rates (MLRs) of the 4 strains varied significantly across the 4 substrates, mulberry bark, xylem, and leaves, as well as wheat bran, used (Fig. 1). The MLR of each strain on mulberry bark displayed an overall trend of first increasing and then decreasing with prolonged incubation times (Fig. 1A). Strain 15 showed the best degradation performance, with its MLR reaching 50.50% at 30 d of fermentation, which was notably higher than those of the control and the other three strains. The MLRs of the 4 strains on mulberry xylem increased gradually with increased incubation times. At 44 d, strains P6 and 4 achieved significantly higher MLR rates than Strain N6 and the control, at 34.50 and 32.50%, respectively (Fig. 1B). The MLRs of the strains on mulberry leaves showed a fluctuating pattern of increase to decrease over time (Fig. 1C). At 44 days, Strains P6 and 15 both showed the highest degradation efficiencies, with a MLR of 62.50%, exceeding the other two strains and the control. The MLRs of the strains using wheat bran as substrate increased continuously with incubation times (Fig. 1D). Except for the control and Strain N6, Strains 15, P6, and 4 exhibited higher MLRs at 44 d, and reached 55.50, 48.50, and 43.00%, respectively. Overall, Strain 15 showed the highest MLR on mulberry bark and leaves, as well as wheat bran. Strain P6 presented the optimal degradation performance on mulberry xylem and leaves, and wheat bran. Strain 4 achieved the highest MLRs on mulberry xylem and wheat bran. These results suggested that Strains 15, P6, and 4 possess high degradation capacity on diverse substrates.

Daily average degradation rates of the four strains with different substrates

The average daily degradation rates of the 4 strains on the 4 substrates used all showed a decreasing trend with increased incubation times (Fig. 2). However, there were significant differences in the peak times and rates of degradation of the different strains on each substrate used (Fig. 2, $P < 0.05$). Using mulberry bark substrate, the average daily degradation rates of strains P6 and 4 decreased gradually with time (Fig. 2A) but were at relatively high levels from 9 ~ 16 days, ranging from 2.03 to 2.67%, and these were significantly different from other time periods ($P < 0.05$). The average daily degradation rate of strain N6 reached its peak of 2.56% at 16 days, which was significantly higher than that in other time periods ($P < 0.05$). That of Strain 15 was 23 d, with the maximum of 1.91%. With mulberry xylem as substrate, except for Strain N6, the average daily degradation rates of the other three strains all decreased with the extension of time and Strain 4 reached the highest average daily degradation rate (3.17%) at 9 days. Strain P6 maintained a relatively high degradation level during 9 ~ 16 days, with rates ranging from 1.56 to 1.61%, which was significantly different from other time periods (Fig. 2B; $P < 0.05$). The peak average daily degradation rate of Strain 15 was also at 9 days, with a maximum of 2.00%. The average daily degradation rates of the 4 strains on mulberry leaf substrate all showed a decreasing trend

with time, but there were differences observed in the peaks (Fig. 2C). Strain 4 reached the highest average daily degradation rate (4.39%) at 9 days and Strains 15 and P6 maintained relatively high degradation levels during 9 ~ 16 days, with rate ranging from 3.28 ~ 3.38% and 2.28 ~ 2.39%, respectively ($P < 0.05$). Strain N6 reached a relatively high degradation level during 16 ~ 23 days, with rates of 2.07–2.19% ($P < 0.05$). With wheat bran substrate, the average daily degradation rates of the 4 strains all decreased continuously with time, and the highest average daily rate of all the strains was at 9 days. These were 3.56, 3.78, 4.28 and 3.00%, respectively ($P < 0.05$).

Therefore, all the strains reached relatively high average daily degradation rates on wheat bran substrate at 9 d, which were significantly different from other time periods ($P < 0.05$). The average daily degradation rates of Strain 4 on mulberry xylem and leaves as substrates both reached their peaks at day 9, and these were 3.17 and 4.39%, respectively, ($P < 0.05$), reflecting their strong short-term degradation advantage.

Changes in LiP production by the four strains

When mulberry bark was used as the substrate, the LiP activities of the P6, 4, 15, and N6 strains all showed a trend of first increasing and then decreasing as the time of fermentation increased (Fig. 3A). The activities reached their peaks on the 9th day, and were 6.77, 2.00, 6.94, and 4.17 U/mL, respectively, all of which were higher than that of the control group. When mulberry xylem was used as the substrate, the trend of LiP activity of the 4 strains was similar to that of when mulberry bark was the substrate, and the activities also reached the peak at the 9 days, and were 6.49, 5.14, 5.96, and 3.89 U/mL, respectively (Fig. 3B). Except for Strain N6, the enzyme activity peaks of the other three strains were all higher than the control group, and none was detected on the second day of culture. When mulberry leaves were used as the substrate, the LiP activities of the 4 strains still showed a trend of first increasing and then decreasing, reaching the peak on day 9, and were 3.33, 21.15, 2.45, and 1.51 U/mL, respectively (Fig. 3C). Among them, Strain 4 had the highest enzyme activity peak, and except for strain N6, the peaks of the other strains were all higher than that of the control group. With wheat bran as the substrate, the LiP activities of the 4 strains was also consistent and reached the highest on day 9, and were 7.01, 23.52, 4.5, and 2.19 U/mL, respectively (Fig. 3D). With this substrate, Strain 4 had the highest enzyme activity peak, and the peaks of all 4 strains were also higher than that of the control group.

To summarize, under the 4 different substrates and culture times used the LiP activities of the 4 endophytic fungal strains all showed a consistent trend of "first increasing and then decreasing", and peaked on day 9 of culture. Among them, when cultured with mulberry leaves and wheat bran the enzyme activity peaks of Strain 4 were significantly higher than those of the other two substrates and other strains while using the same substrate.

Changes in laccase production by the four strains used

When mulberry bark was used as the substrate, the lac activities of the P6, 4, 15, and N6 strains all showed a trend of first increasing and then decreasing (Fig. 4A). Except for strain 15 and the control

group, the other strains all reached their peak enzyme activities on the 9th day of culture, with the peak values being 21.88, 3.19, 1.10, and 11.73 U/mL respectively. The peak laccase activity of Strain 15 appeared on the 23rd day, with a value of 62.80 U/mL. When mulberry xylem was used as the substrate, the lac activities of the 4 strains used also showed a trend of first increasing and then decreasing (Fig. 4B). Except for strains P6 and 4, the other ones all reached their peak activities on the 9th day, with values of 7.35, 9.09, 31.30, and 30.86 U/mL respectively. With mulberry leaves as the substrate, there were differences in the peak lac activities among the 4 strains (Fig. 4C). Strain P6 reached its peak on day 9, with an activity of 28.94 U/mL. The peak activities of Sstrains 15 and N6 both appeared on the 16th day, and were 5.08 and 3.91 U/mL respectively. and that of Strain 4 was on the 23rd day, at 6.45 U/mL. In addition, the lac activities of all 4 strains were higher than the control group. When wheat bran was used as the substrate, the lac activities of the 4 strains all showed a trend of first increasing and then decreasing, and the peaks occurred on day 16, with peak values of 108.39, 73.20, 11.99, and 68.64 U/mL, respectively (Fig. 4D). In summary, there were differences in the lac activities of the 4 strains when using different substrates and culture times, and the peak enzyme activities were different.

Changes in MnP production by the four strains

With mulberry bark, xylem, and leaves and wheat bran as substrates, the MnP activities of P6, 4, 15, and N6 all showed trends of first increasing and then decreasing (Figs. 5A-5D). For mulberry bark, the 4 strains reached their peak activities on day 30, with the peak values being 11.76, 45.64, 45.21, and 78.45 U/mL, respectively, and Strain N6 had the highest activity (Fig. 5A). For mulberry xylem, except for Strains N6 and 15, the others (P6 and 4) both reached their peak enzyme activities on the 30th day, with peak values of 51.75 and 38.08 U/mL, respectively (Fig. 5B). When mulberry leaves were used, the peak enzyme activity of strain P6 appeared on day 30, while that of Strain 4 reached the peak on the 16th day, with values being 36.26 and 118.32 U/mL, respectively (Fig. 5C). With wheat bran as the substrate, the MnP activities reached their peak activities on day 30, with values of 62.28, 55.05, 84.33, and 111.69 U/mL respectively, and strain N6 had the highest activity (Fig. 5D). In summary, when using different substrates and culture times, the MnP activities of the 4 strains all showed an increasing and then a decreasing trend. Among them, only when Strain 4 used mulberry leaves as the substrate, the peak MnP activity appeared on the 16th day, and that occurred on day 30 for the other strains.

Changes in cellulase production by the four strains

When mulberry bark, xylem, and leaves were used as substrates, the cellulase activities of Strains P6, 4, 15, and N6 all showed trends of first increasing and then decreasing (Figs. 6A-6C). For mulberry bark, with the exceptions of P6 and the control group, the other three strains all reached their peak cellulase activities on the 2nd day of culture, with peak values being 0.19, 0.37, and 0.21 U/mL respectively (Fig. 6A). The peak of Strain P6 appeared on day 23, with a value of 0.39 U/mL. With mulberry xylem and the exception of N6, the other three strains all reached their peak enzyme activities on the 2nd day, with the peak values being 0.49, 0.35, and 0.67 U/mL respectively (Fig. 6B). The peak activity of strain N6 was 0.16 U/mL and appeared on day 9. When mulberry leaves were used as the substrate, the cellulase activities of the 4 strains reached their peaks on the 2nd day, with values of 0.14, 0.15, 0.23, and 0.20

U/mL respectively (Fig. 6C). With wheat bran as the substrate, except for Strain 15, the cellulase activities of P6, 4, and N6 all showed a trend of first increasing and then decreasing, and all reached their peaks on the 2nd day, with values of 0.27, 0.20, and 0.26 U/mL respectively. That of Strain 15 was on day 9, with a value of 0.28 U/mL (Fig. 6D). In summary, there were significant differences in the changes of cellulase-producing activities of the 4 endophytic fungi when using different substrates and culture times.

Discussion

Previous studies have shown that the composition of the fermentation medium is one of the key factors affecting fermentation efficiency [53], and the differences in enzyme types and their production times also led to differences in the lignin degradation ability of strains on different substrates [54]. In this study, four endophytic fungal strains isolated from *T. chinensis*. These were Strains 4 (*Colletotrichum* sp.), 15 (*N. sphaerica*), N6 (*D. phaseolorum)*, and P6 (*Pestalotiopsis* sp.) and were inoculated separately onto the 4 substrates of mulberry, leaves, and xylem, as well as wheat bran to undergo the process of solid-state fermentation. The changes in TOM mass loss, average degradation rates, and lignocellulose-degrading enzyme activities caused by the strains during the degradation of different culture substrates were determined.

Our studies showed that all 4 fungi exhibited high lignin degradation levels when wheat bran was used as the substrate, and the highest rate reaching 55.50% under optimal culture conditions. Guo et al. [55] found that a strain of *Pseudomonas guanguanensis* isolated from lignin-degrading bacteria the could secrete LiP, MnP, and lac, with a lignin degradation rate of about 38% after 3 days of fermentation. Zhou et al. [56] found that *Alternaria eichhhorniae* Strain G6 and *Trichoderma shangrilaense* Strain G59 could both produce LiP and lac, and the enzyme-producing capacity of the composite strain was significantly improved, with a degradation rate of 54.53% for corn stover. Current studies have shown that the degradation efficiency of composite microbial communities was significantly higher than that of individual strains. In the natural ecosystems, lignin degradation does not primarily result from the independent action of specific strains, but rather it stems from the inhibition and synergism of communities of microorganisms in the same habitat. Therefore, future studies should attempt to construct composite microbial communities, explore their degradation effects on lignocellulose of different substrates, and analyze the optimal conditions required by different lignocellulose-degrading bacteria, including temperature, pH, light, and types and concentrations of carbon and nitrogen sources.

Lignin degradation relies on the synergistic effects of lac, MnP, and LiP. These enzymes can break the chemical bonds in the lignin structure to achieve degradation, and lac plays a leading role in the decomposition process. Its activity can be used as an important indicator reflecting the overall activity of the lignin-degrading enzyme system. During the 44-day full cultivation period, when Strains 4 (*Colletotrichum* sp.), 15 (*N. sphaerica*), P6 (*Pestalotiopsis* sp.), and N6 (*D. phaseolorum*) were fermented with wheat bran as the lignocellulosic biomass substrate, the activities of lac and cellulase were significantly higher than those of the control group. Zhao et al. [57] showed that increased lac

concentrations were needed for mycelial degradation of lignin. They showed that the laccase activity of the white-rot fungus, *Pleurotus eryngii*, was the highest in the culture substrate with the highest lignin content, which is consistent with our results. When wheat bran was used as the substrate, cellulase activity increased significantly on the second day of cultivation, while lac activity reached a high level on day 9. Previous studies [58–60] have reported that this phenomenon is closely related to the non-selective lignin degradation mode of white-rot fungi, which consumes large amounts of cellulose. In addition, the reducing sugars and other substances released by cellulase degradation of cellulose can provide the necessary substrates and a suitable reaction environment for lac to act, thereby indirectly regulating its activity. Several others have also reported [61–63] significantly improved the lignin degradation rates by adding wheat bran to the medium, confirming that this substrate can induce the enhancement of enzyme-producing capacity by a strain, which is consistent our findings.

When using lac activity as the evaluation index, strain P6 had the highest activity (108.39 U/mL) on the wheat bran substrate. Existing literature reports show that *Pestalotiopsis* sp. of fungi possess high efficiencies for the production of lac and cellulase, which can effectively degrade forest litter [48]. This further confirms the excellent enzyme-producing and degradation characteristics of strain P6 in this study. During the early stages of fermentation, the enzyme activities of Strains N6, 4, 15, and P6 on different substrates all showed a gradual upward trend, but as the cultivation times increase, they tend to decrease. This is mainly because the nutrients and oxygen in the substrates were gradually consumed, leading to restricted microbial growth and even death, thereby reducing enzyme production efficiency. The buildup of inhibitor metabolites is another possible explanation for this phenomenon [64].

In summary, the 4 endophytic fungal strains isolated from *T. chinensis* screened in this study are all able to degrade lignin efficiently, providing new high-quality strain resources for biological lignin degradation. This can have important theoretical and practical significance for the development of bioenergy and the sustainable utilization technology of lignocellulosic resources.

Conclusion

The degradation efficiency of the 4 screened endophytic fungal strains from T. chinensis on different lignocellulosic substrates, as well as the activities of key degrading enzymes such as lignin peroxidase and laccase, all showed significant strain specificity, substrate specificity, and temporal dynamic characteristics. The results of this study can provide a theoretical basis and practical reference for the excavation of high-efficiency lignin-degrading strain resources and the optimization of their fermentation conditions.

Abbreviations

LiP
lignin peroxidase
MnP

manganese peroxidase
Lac
laccase
MLRs
the mass loss rates
TOM
total organic matter
ABTS − 2,2'
azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
kDa
kilodalton
HRP
horseradish peroxidase
SSF
solid-state fermentation

Declarations

Ethics approval and consent to participate

Strains 4 (*Colletotrichum* sp.), 15 (*Nigrospora sphaerica*), N6 (*Diaporthe phaseolorum*), and P6 (*Pestalotiopsis* sp.) were kept in the Plant Pathology Department of the Guangxi Botanical Garden of Medicinal Plants and we obtained written consent from them to use these fungal strains in our studies. The experimental methods conducted in this study complied with all the current Chinese laws and regulations.

Consent for publication

Not applicable.

Conflict of interest

The authors declare no competing interest.

Authors' information

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Author Contribution

Lisha Song implemented the experiments, analyzed and interpreted the data and completed the article writing. Lingjian Gui and Wenjing Liang participated in the experiments and collected the samples. Ni Jiang, Ru Chen and Cuihong Yang integrated information into the tables. Shugen Wei and Jine Fu reviewed and finalized manuscript. Yuqiong Li and Ya Qin generated the figures. Lingyun Wang and Huijie Zhang edited the manuscript. The final manuscript was approved by all authors who agreed to be accountable for the content of this work.

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Availability of data and materials

ITS and beta-tubulin sequences of 4 strains, 4,15, N6, and P6, were submitted to the GenBank database and the NCBI accession numbers were MZ823601/MZ964759, MZ823600/MZ934421, MZ823599/MZ934420, and MZ823598/MZ934419, respectively. Other data generated and analyzed during this study are included in the article and its supplementary information files: (https://www.ncbi.nlm.nih.gov/search/all/?term=MZ823600;https://www.ncbi.nlm.nih.gov/pmc/?term=MZ823598;https://www.ncbi.nlm.nih.gov/search/all/?term=MZ823601;https://www.ncbi.nlm.nih.gov/search/all/?term=MZ823599)

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Tables

Tables 1 to 6 are available in the Supplementary Files section.

Figures

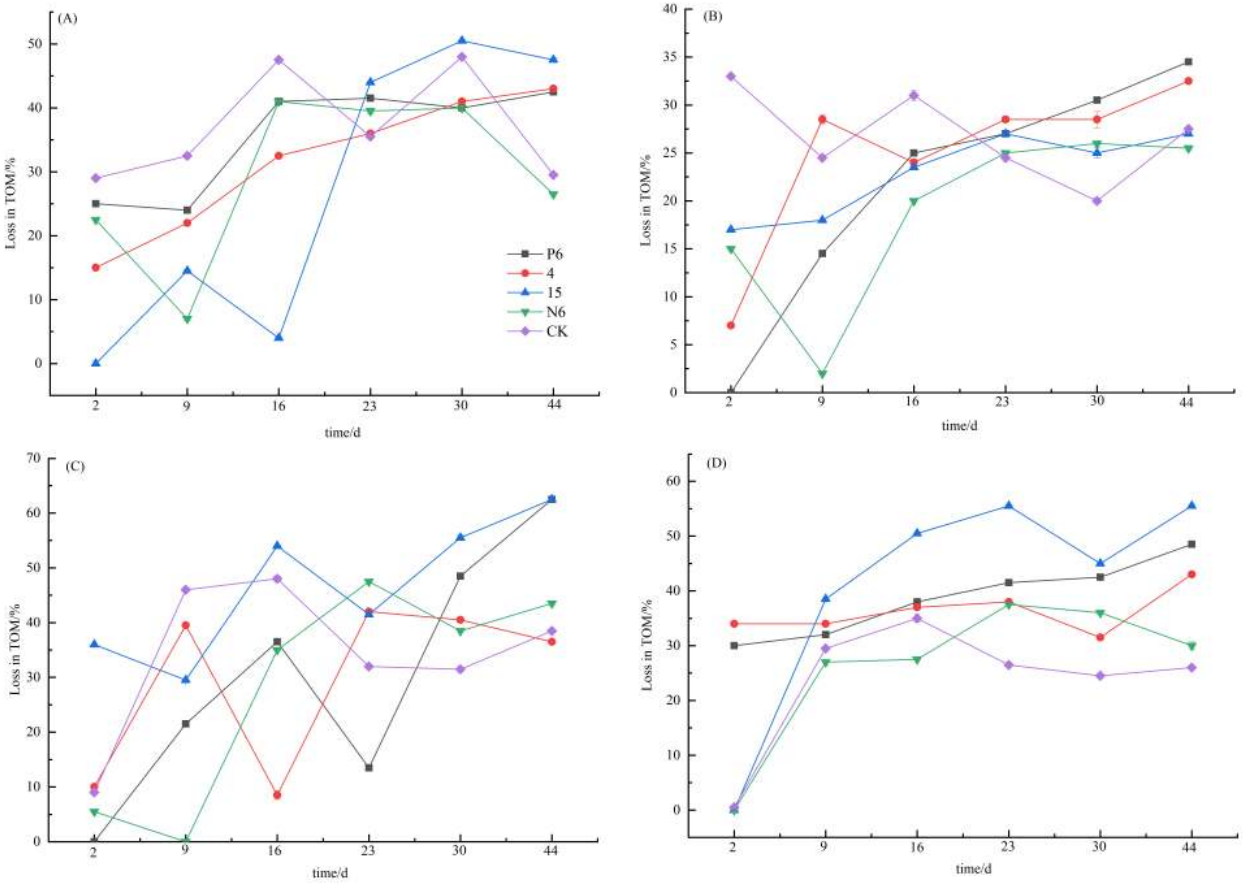

Figure 1

The changes observed in mass loss of four endophytic fungalstrains during the degradation of different substrates.

Note：A: mulberry bark B: mulberry xylem C: mulberry leaves D: wheat bran.

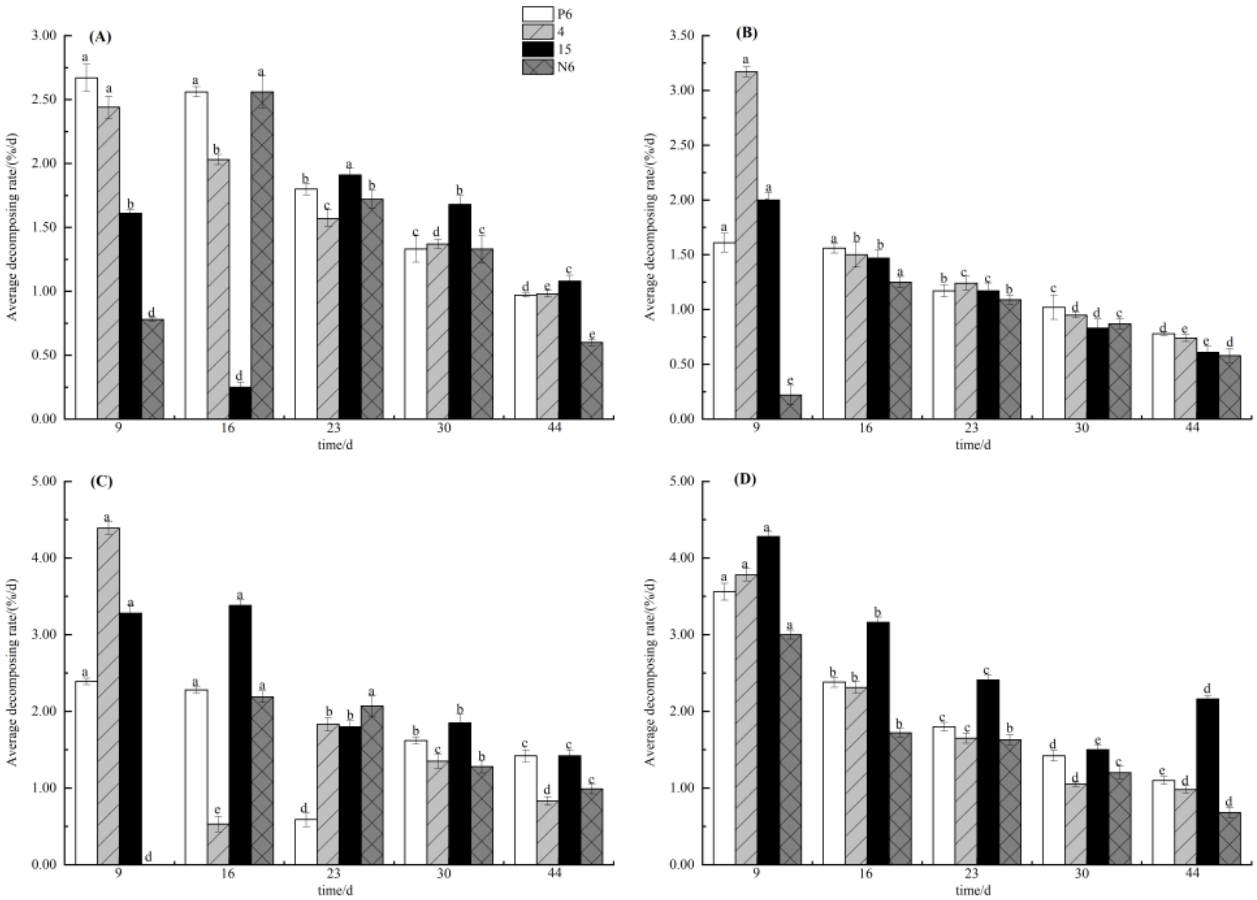

Figure 2

The changes observed in the daily average degradation rates of four endophytic fungal strains during the degradation of different substrates.

Note：A: mulberry bark B: mulberry xylem C: mulberry leaves D: wheat bran.

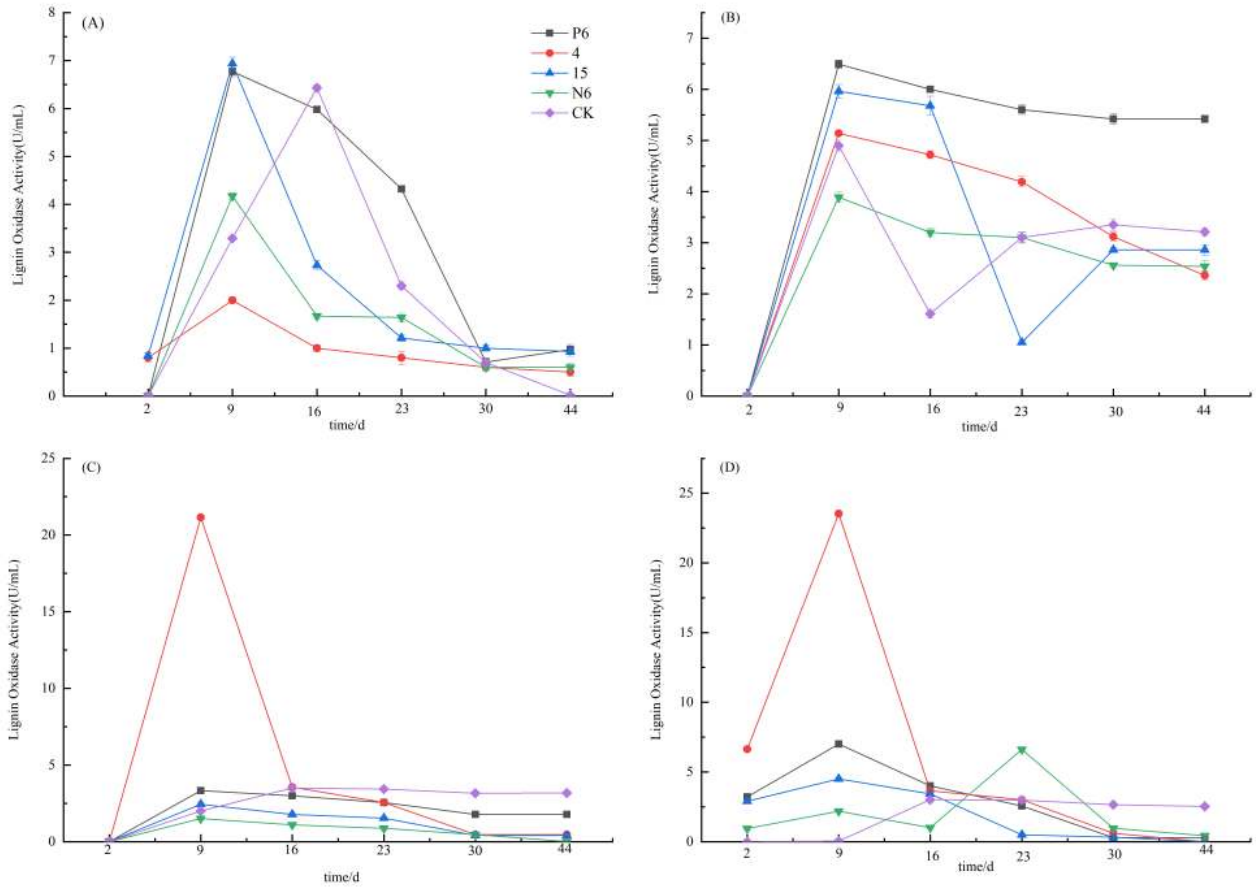

Figure 3

The changes observed in lignin peroxidase of four endophytic fungal strains.

Note：A: mulberry bark B: mulberry xylem C: mulberry leaves D: wheat bran.

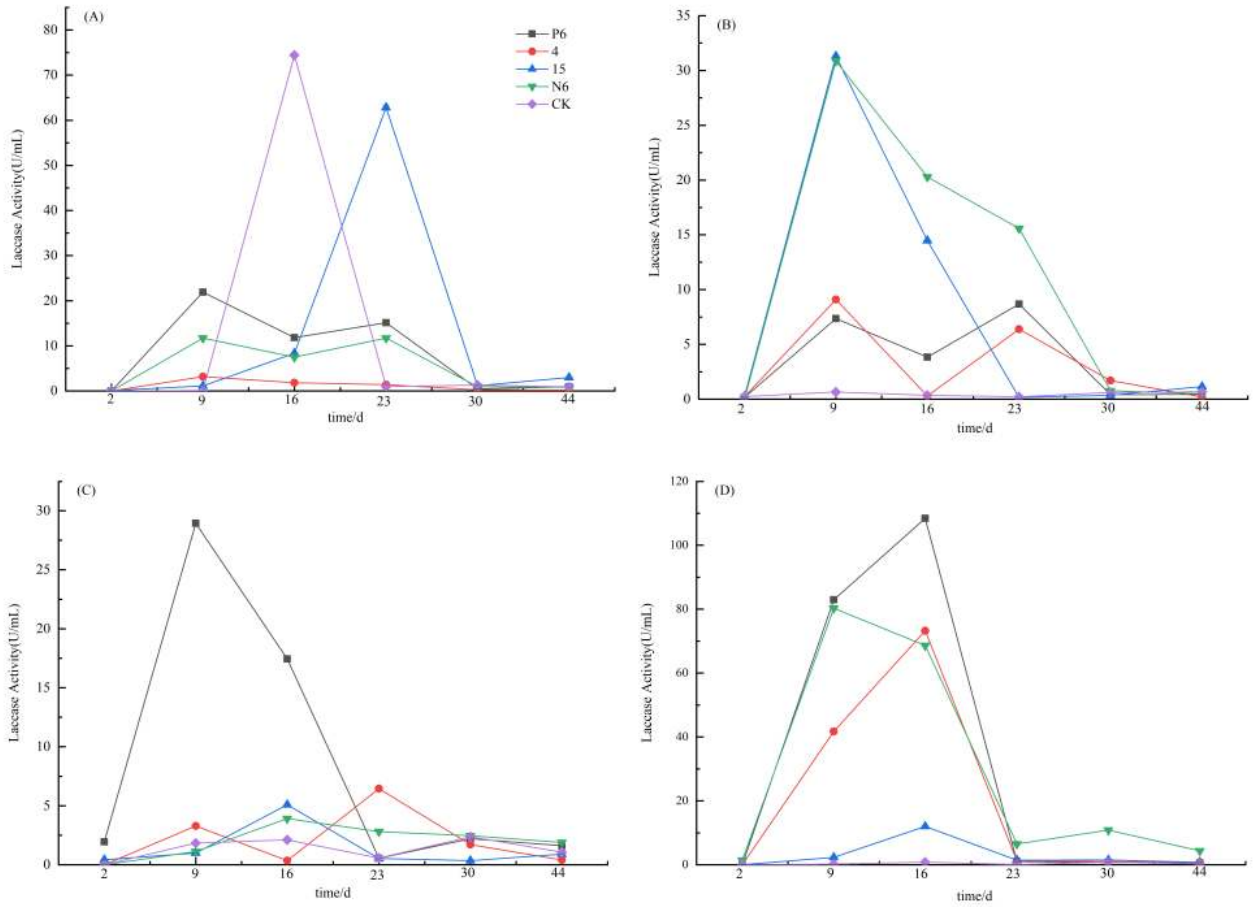

Figure 4

The changes observed in laccase activities of four endophytic fungal strains.

Note：A: mulberry bark B: mulberry xylem C: mulberry leaves D: wheat bran.

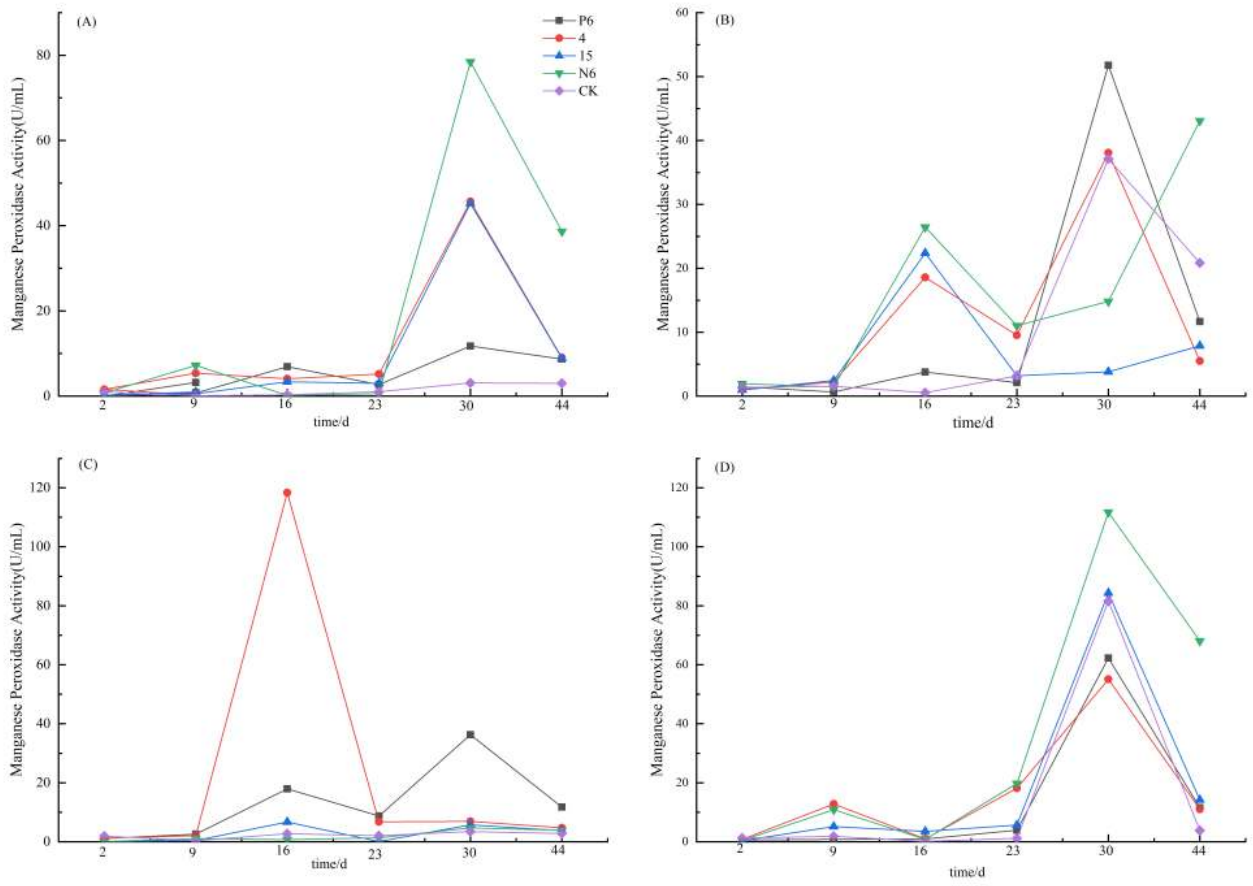

Figure 5

The changes observed in manganese peroxidase activities of four endophytic fungalstrains.

Note：A: mulberry bark B: mulberry xylem C: mulberry leaves D: wheat bran.

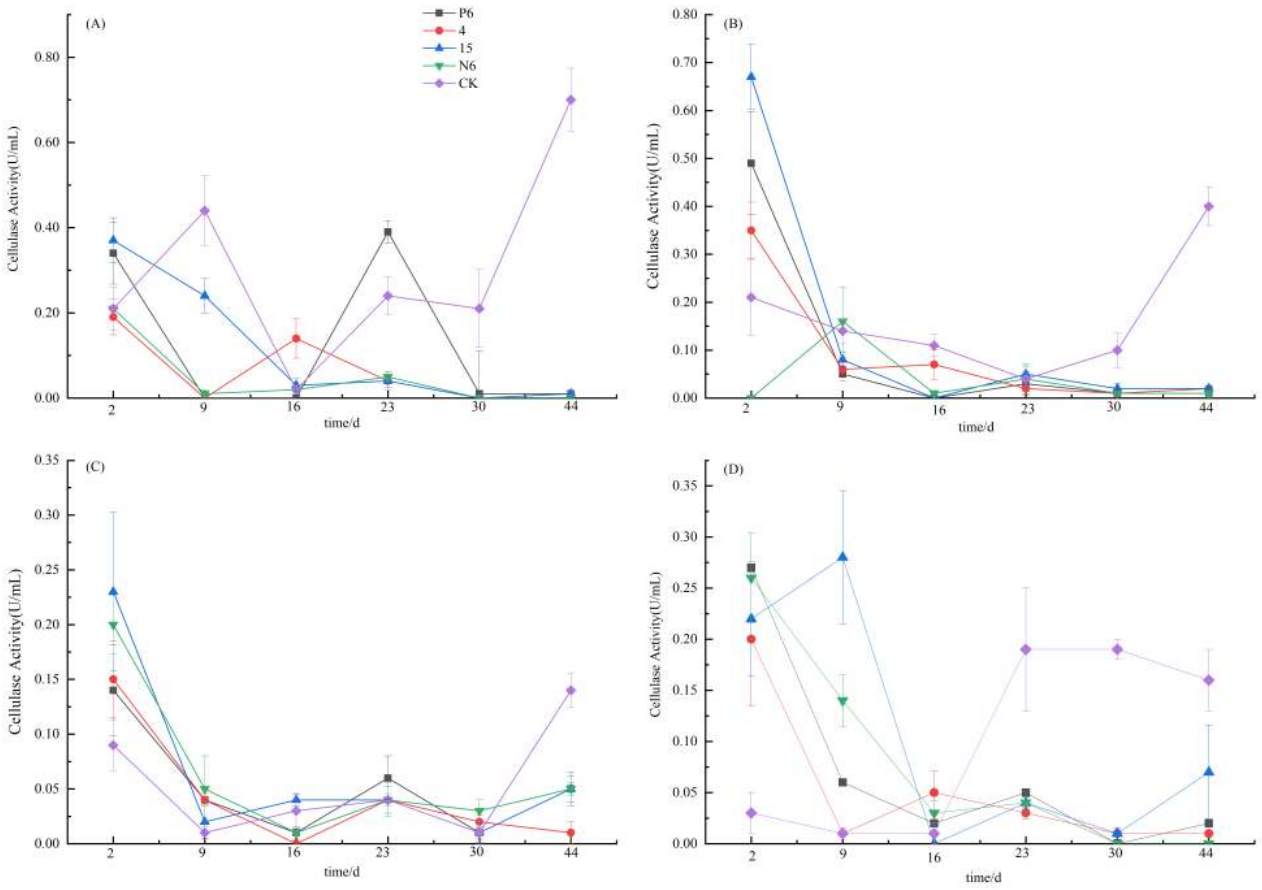

Figure 6

The changes observed in cellulase activities of four endophytic fungalstrains.

Note：A: mulberry bark B: mulberry xylem C: mulberry leaves D: wheat bran.

Supplementary Files

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