

Response Mechanism of Extracellular Laccase Activity of *Lyophyllum decastes* to Cultivation Substrates and Subculture

Xue Xiong ^a, Peng Li ^{a,*}, Wei Li,^b and Gui-he Zhang^c

Different fermentation substrates were employed to investigate the variation patterns of lignocellulolytic enzymes in *Lyophyllum decastes* and the changes in laccase activity in subculture. The results showed that the activities of Lac, CMCase, and Xyl produced by the *L. decastes* F1 strain in liquid fermentation were significantly affected by different cultivating substrates. The optimal primary substrate for inducing Lac secretion in *L. decastes* F1 strain was corncob, followed by cottonseed hulls. The best supplementary substrate for Lac induction was soybean meal. The addition of corn cob and wheat bran was found to significantly stimulate the secretion of CMCase and Xyl in *L. decastes* F1 strain. The addition of different cultivation substrates enhanced Lac production in *L. decastes* subcultured strains (F1, F5, F10), but strains subjected to serial subculturing exhibited progressively diminished laccase production. The highest laccase activity detected in the fastest-growing subcultured strains within identical solid cultivation substrates demonstrated a phase-specific positive correlation between mycelial growth and extracellular laccase secretion.

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Keywords: *Lyophyllum decastes*; Cultivation substrates; Laccase activity; Subcultured strain; Growth rate

Contact information: a: Guizhou Provincial Institute of Biology, Guiyang, Guizhou 550009 China; b: Guizhou Guifu Mushroom Industry Development Co., Ltd., Yuping, Guizhou 554000; c: Guizhou Vocational College of Agriculture, Guiyang, Guizhou 551400;

* Corresponding author: LiPeng19870716@163.com

INTRODUCTION

Along with technological advancements, social development, and improved living standards, people not only seek an adequate food supply but also demand highly nutritious, high-quality, safe, and healthy grains, oils, fruits, and vegetables (Song *et al.* 2025). Edible mushrooms, with their unique taste and rich nutrition, have become the fifth largest pillar industry in China's agricultural products, following grains, vegetables, fruit trees, and oilseeds (Xu *et al.* 2023). Agricultural and forestry residues contain abundant lignocellulose, which can be efficiently degraded into bioavailable low-molecular-weight compounds by fungal-derived lignocellulolytic enzymes and subsequently assimilated to support mycelial growth and development (Munir *et al.* 2024). This establishes the edible mushroom industry as a highly profitable, green, and sustainable circular economy sector with exceptional resource efficiency. Scholars have identified significant correlations among edible mushroom yield, ligninolytic enzyme activity, and lignin degradation (Colla *et al.* 2023; Feng *et al.* 2025). Moreover, researchers posit that profiling extracellular enzyme activities under varied cultivation

conditions can provide critical insights into fungal decomposition capacity and metabolic vigor, enabling the creation of optimized environments for accelerated mycelial growth and enhanced biomass production (Huang *et al.* 2019).

Lyophyllum decastes (Fr.) Singer, belonging to the Basidiomycota phylum, is a typical and famous macrofungus with both high edible and medicinal value (Zhang *et al.* 2022; Li *et al.* 2024). The fruiting body is rich in polysaccharides, which have various effects, such as enhancing immunity, reducing blood lipids and blood sugar, protecting the liver, and exhibiting antioxidant and anti-inflammatory properties (Zhang *et al.* 2022; Li *et al.* 2024b; Sun *et al.* 2024). According to statistics from the China Edible Fungi Association, the annual production of *L. decastes* in China was 108,000 tons in 2022, and the production increased by 79.49% in 2023, ranking first in the world in terms of this mushroom production (China Edible Fungi Association 2024a,b). Therefore, the correct selection of lignocellulose-rich cultivation substrates is of great importance for the large-scale artificial cultivation of *L. decastes*. Xiong *et al.* (2024) proposed that laccase activity screening can serve as an effective method for selecting optimal cultivation substrates for *Volvariella brumalis* strains. The findings demonstrated that substrates inducing higher laccase production significantly enhanced mycelial growth of this species (Xiong *et al.* 2024). Li *et al.* (2024) confirmed that the Y1 substrate yielded the highest *Pleurotus eryngii* production among tested cultivation media, with concomitantly elevated extracellular laccase activity observed under Y1 growth conditions. Consequently, laccase-high-yielding strains typically correlate with accelerated growth cycles and elevated productivity (Sun *et al.* 2011; Fang *et al.* 2018; Xiong *et al.* 2024; Li *et al.* 2024). Whereas periodic subculturing serves as a standard method for strain preservation in the edible mushroom industry, excessive subculturing can induce strain mutations, diminished physiological vigor, and loss of superior traits, and ultimately compromising fruiting body yield (Castro-Ríos and Bermeo-Escobar 2021; Zhao *et al.* 2022). This raises a critical question: Do subcultured strains with higher laccase production exhibit greater mycelial vigor and accelerated growth? Multiple studies have confirmed that serial subculturing significantly modulates laccase activity in edible fungal strains. Within normal subculturing limits (typically 4 to 5 passages), laccase activity and mycelial vigor generally exhibit a positive correlation. However, both parameters may undergo co-degeneration with increasing passage numbers, demonstrating significant species-specific variations (Lin *et al.* 2018; Zhao *et al.* 2022; Zhou *et al.* 2023; Huang *et al.* 2025). Laccase activity assay can be regarded as an effective method for evaluating strain degeneration in edible fungi under specific conditions (Zhao *et al.* 2022). This conclusion has been validated in subculturing studies of edible fungal strains including *Volvariella volvacea*, *Ganoderma lucidum*, *Hypsizygus marmoreus*, and *Pleurotus ostreatus* (Zhao *et al.* 2022; Lin *et al.* 2018; Zhou *et al.* 2023). However, no comparable research has yet been reported to *L. decastes*.

Therefore, the present work was carried out based on the conventional substrate formulations and supplements typically employed in *L. decastes* cultivation (Luo and Chen 2010). The experimental design incorporated three lignocellulosic substrates (*Quercus acutissima* sawdust, cottonseed hulls, corncobs) and three nitrogen-rich supplements (soybean meal, wheat bran, rice bran) to profile lignocellulase production in *L. decastes* F1. Through serial subculturing coupled with liquid and solid-state fermentation, substrate-specific laccase production profiles were established. Generation-dependent enzyme activity changes were quantified, and mycelial growth patterns were correlated with strain vitality. This systematic approach is expected to advance

understanding of both substrate optimization and degeneration prevention in commercial *L. decastes* cultivation.

EXPERIMENTAL

Materials

Tested strain

The tested strain, *L. decastes* F0, was isolated and purified from fruiting bodies collected from Guizhou Guifu Mushroom Industry Development Co., Ltd. The strain was first subjected to DNA identification (GenBank accession number: PQ804433) and fruiting trials. After the F0 strain achieved full plate colonization, it was transferred to fresh subculture medium and designated as F1, followed by incubation at 25 °C in a constant-temperature incubator. Upon complete colonization of F1, the culture was subcultured into new medium, designated as F2. The strains were preserved at the Guizhou Institute of Biology.

The culture medium formula for subculturing was provided as follows: potato extract powder 4.0 g/L, sucrose 20 g/L, soybean protein peptone 2.0 g/L, MgSO₄·7H₂O 0.5 g/L, KH₂PO₄ 1.0 g/L, and agar powder 20 g/L. The potato extract powder was purchased from Beijing Hongrun Baoshun Technology Co., Ltd.

Cultivation substrates

Quercus acutissima sawdust, corncob, cottonseed hull, soybean meal, wheat bran, and rice bran, used as cultivation substrates for edible mushrooms, were all purchased from the agricultural and sideline products market in Guiyang, Guizhou Province, China. These materials were dried at 60 °C, crushed by an electric grinder, and sieved through a 40-mesh screen. All materials were dried to a constant weight prior to being used.

Methods

Liquid cultivation and crude enzyme extraction methods

The F1, F5, and F10 strain plates of *L. decastes* were selected. Five agar blocks (5 mm in diameter) containing the mycelial tips of the same strain were inoculated into 250 mL Erlenmeyer flasks containing 100 mL of liquid fermentation medium, with five replicates for each strain. The liquid fermentation media consisted of the following components: potato infusion powder 4.0 g/L, sucrose 20 g/L, soybean protein peptone 2.0 g/L, MgSO₄·7H₂O 1.0 g/L, and KH₂PO₄ 0.5 g/L. The inoculated Erlenmeyer flasks were incubated in a shaking incubator (Boxun, BXYC-LX2400, Shanghai, China) at 25 °C and 120 r/min for 7 days. The cultivated liquid mycelia were homogenized using a homogenizer (HFJ-10, Tianjin, China) and used later as the seed liquid.

Different cultivation substrates (0.9 g each) were added to 500 mL Erlenmeyer flasks containing 300 mL of the basal medium. An equal amount of the basal medium without any additional substrate was used as the experimental control (CK). The basal medium consisted of the following main components: sucrose 20 g/L, soybean protein peptone 2 g/L, MgSO₄·7H₂O 0.5 g/L, and KH₂PO₄ 1.0 g/L. All Erlenmeyer flasks were sterilized in an autoclave at 121 °C for 30 min. After cooling, 10 mL of seed liquid was inoculated into each Erlenmeyer flask, with three replicates per treatment. The inoculated Erlenmeyer flasks were incubated in a shaking incubator at 25 °C and 120 r/min for 11 days. For each treatment, a 3 mL sample was collected once daily, starting from the 2nd

day of cultivation. Samples were centrifuged at 12,000 r/min for 20 min at 4 °C. The supernatant, designated as the crude enzyme extract, was stored at 4 °C for subsequent analysis.

Methods for mycelial growth rate determination and crude enzyme extraction in solid-state cultivation

Solid-state cultivation substrate formulation: Cottonseed hulls (58%), corncobs (25%), wheat bran (12%), and soybean meal powder (5%), were mixed each other with moisture content adjusted to 65%. *L. decastes* strains F1, F5, and F10 were cultivated using 18 mm × 180 mm stoppered test tubes as culture vessels with a solid medium. The substrate weight was 28.0 g per tube. All medium-loaded test tubes were sterilized in an autoclave at 121 °C for 120 min. After cooling to room temperature, each test tube was inoculated with one 5-mm diameter inoculum plug. Five replicates were prepared per treatment. After mycelial colonization of the substrate, the mycelial growth length was recorded every 5 days. When mycelia fully colonized the test tubes, a 10 g mixture sample containing mycelia and growth substrate was aseptically collected from the bottom of each tube. This sample was transferred to a 250-mL Erlenmeyer flask containing 90 mL of sterile water, followed by oscillatory extraction at 25 °C and 120 r/min for 3 h. The liquid suspension was separated through filtration, followed by centrifugation at 4 °C and 4000 rpm for 10 minutes. The resulting supernatant was collected as the crude enzyme extract and stored at 4 °C for subsequent use.

Assay of lignocellulolytic enzyme activity

The method for measuring laccase, carboxymethyl cellulase, and xylanase described by Han *et al.*(2020) was referenced in this study, with specific procedures as follows: Laccase (Lac) activity was determined in a 3-mL reaction system containing: 1 mL of 1 mmol/L 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) substrate. 1.9 mL of 50 mmol/L acetate buffer (pH 4.2) 100 µL of appropriately diluted enzyme extract absorbance changes at 420 nm were monitored for 5 minutes. One unit of enzyme activity (U) was defined as the amount of enzyme required to oxidize 1 µmol of ABTS per minute. The molar extinction coefficient of oxidized ABTS at 415 nm is 3.6×10^4 L/(mol·cm). Carboxymethyl cellulase (CMCase) activity was determined as follows: 1.5 mL of 1% (w/v) sodium carboxymethyl cellulose solution (prepared in 50 mmol/L citrate-sodium citrate buffer, pH 5.0) and 0.5 mL of appropriately diluted crude enzyme extract were added into a test tube. The tube were incubated at 40 °C for 30 minutes in a water bath. Consequently 3 mL of 3,5-dinitrosalicylic acid (DNS) reagent was added to terminate the reaction. The mixture was boiled for 10 minutes. The boiled mixture was cooled to room temperature under running water. 25 mL distilled water was mixed with the mixture thoroughly. Absorbance measurements were taken at 540 nm using a UV-4802 dual-beam UV-Vis spectrophotometer. Enzyme blanks were prepared using heat-denatured enzyme extract (boiled for 10 minutes). One unit of CMCase activity (U) is defined as the amount of enzyme that liberates 1 µmol of glucose equivalent from carboxymethyl cellulose per minute under standard assay conditions. Xylanase (Xyl) activity was determined as follows: 0.9 mL of 1% (w/v) beechwood xylan solution (prepared in 50 mmol/L citrate-sodium citrate buffer, pH 5.0) and 0.1 mL of appropriately diluted crude enzyme extract were added into a test tube. After incubation at 40 °C for 30 minutes in a water bath, subsequent steps followed the CMCase activity assay protocol.

One unit of Xyl activity (U) is defined as the amount of enzyme that liberates 1 μ mol of xylose equivalent per minute from beechwood xylan under standard assay conditions.

Data Statistics

Analyses of variance (ANOVA) among the test groups were performed with the PASW Statistics 18.0 (International Business Machines Corporation, version 18.0, New York, NY, USA). The figures were created by WPS Office (Kingsoft, V12.1.0.16388, Beijing, China).

RESULTS AND DISCUSSION

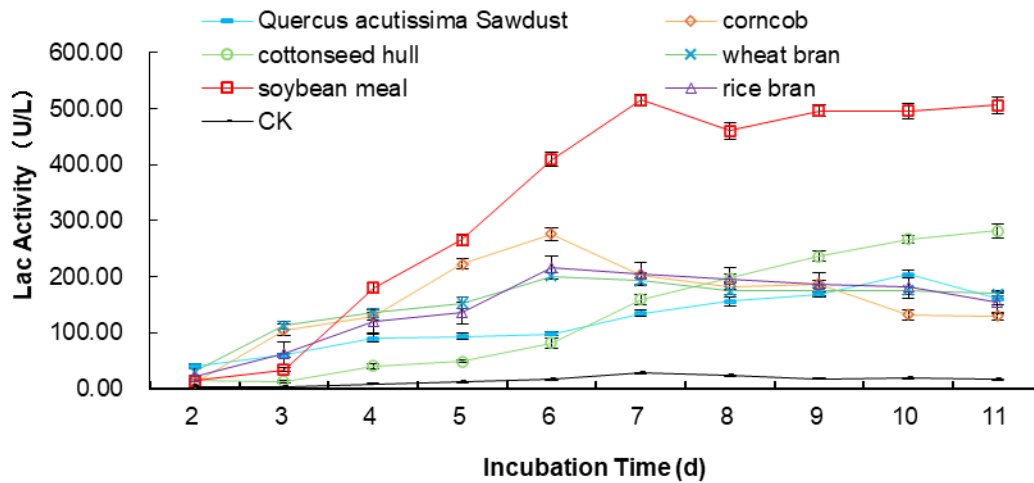
Effects of Different Cultivation Substrates on the Lignocellulolytic Enzyme Activity of *L. decastes* F1 Strain

Six cultivation substrates were selected for liquid fermentation of the *L. decastes* F1 strain, including primary substrates (*Q. acutissima* sawdust, corncob, cottonseed hull) and supplementary substrates (soybean meal, wheat bran, rice bran). Figure 1-A shows that the *L. decastes* F1 strain had a strong Lac secretion capability. This may be related to the differential expression of 19 Lac genes identified in its transcriptomic analysis (Niu *et al.* 2021; Wang *et al.* 2025). Compared to the control (CK), all tested culture substrates effectively promoted the secretion of Lac by the *L. decastes* F1 strain (Fig. 1-A). Soybean meal exhibited the strongest ability to stimulate Lac secretion in the *L. decastes* F1 strain, with the highest Lac activity reaching 515 U/L on the 7th day of cultivation (Fig. 1-A). This may be because the addition of soybean meal allows soybean protein to be adsorbed through hydrophobic interactions between the alkyl carbon chains of amino acid residues and the phenyl or biphenyl groups of lignin (Huang *et al.* 2022; Fan *et al.* 2024). This process alleviated the inefficient adsorption of Lac protein onto lignin, thereby enhancing the catalytic efficiency of Lac (Huang *et al.* 2022; Fan *et al.* 2024). The primary cultivation materials, corn cob and cottonseed hull, exhibited significantly stronger Lac-inducing ability of *L. decastes* F1 compared to *Q. acutissima* sawdust (Fig. 1-A). Compared to cottonseed hulls, corn cob could stimulate *L. decastes* F1 to secrete more Lac in a shorter period (Fig. 1-A). However, cottonseed hulls could sustain Lac secretion for a longer time, demonstrating greater potential (Fig. 1-A). Similarly, cottonseed hulls exhibited greater potential in stimulating Lac secretion of *Pleurotus ostreatus* and *Flammulina filiformis* compared to corn cob or poplar wood (Han *et al.* 2021). Cottonseed hulls had a greater advantage over corn cobs and *Q. acutissima* sawdust in stimulating Lac secretion from *Auricularia cornea* strain (Li *et al.* 2021). Xiong *et al.* (2025) confirmed that cottonseed hulls had a greater advantage in stimulating Lac secretion in *Auricularia cornea* ‘Yumuer’ strain compared to corn cobs and *Q. acutissima* sawdust. Moreover, Li *et al.* (2021) also revealed a consistent trend in their liquid induction experiments conducted on three distinct *Auricularia cornea* strains.

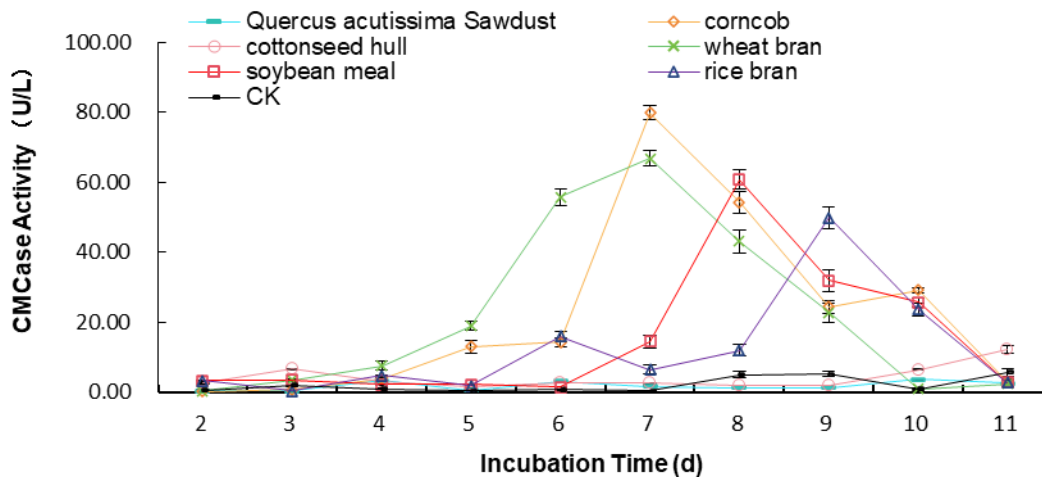
Compared to the control medium (CK), the addition of *Q. acutissima* sawdust and cottonseed hulls had little effect on stimulating the secretion of CMCase and Xyl in the *L. decastes* F1 strain (Fig. 1-B and C). However, the addition of wheat bran, rice bran, or corncob to the culture medium significantly enhanced the activities of CMCase and Xyl in *L. decastes* F1 strain (Fig. 1-B and C). Moreover, the drastic changes in CMCase and Xyl activities occurred after the peak of Lac activity in the same culture medium (Fig. 1). This is most likely related to the degradation of lignin, which disrupted the complex

cross-linked structure of lignocellulose, and released a large amount of cellulose and hemicellulose into the substrates (Wu *et al.* 2022; Elisashvili *et al.* 2023). In the soybean meal-induced medium, which exhibited the highest Lac activity, relatively high CMCase activity was also detected, second only to the corn cob- and wheat bran-induced media (Fig. 1). But Xyl activity was inconspicuous (Fig. 1-C).

A



B



C

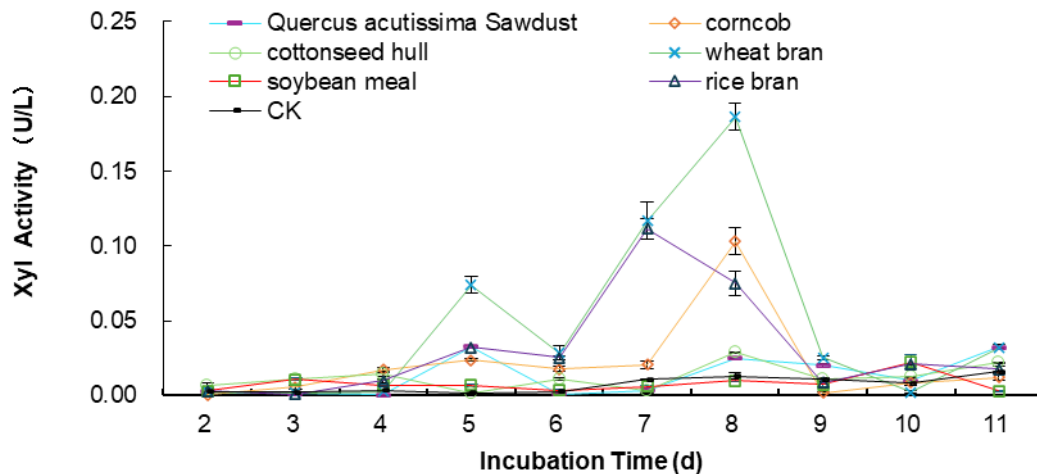


Fig. 1. Effect of different culture materials on the lignocellulolytic enzyme activity of *L. decastes* F1 strain in liquid fermentation: **A:** Lac activity; **B:** CMCase activity; **C:** Xyl activity

Obviously, the differences in lignocellulose significantly affected the activities of Lac, carboxymethyl cellulase, and Xyl in the strain (Fig. 1). However, the relationship among the lignin, cellulose, and hemicellulose contents in lignocellulose and the synthesis of lignocellulolytic enzymes remains ambiguous (Elisashvili *et al.* 2023). The induction of CMCase and Xyl in white-rot fungi represents a highly coordinated regulatory process when specific plant polymers act as carbon and energy sources (Okal *et al.* 2020; Elisashvili *et al.* 2023). In absence of inducers, such as in the control medium, the basal activity of CMCase and Xyl is sustained through the synthesis of specific mRNA (Sukumaran *et al.* 2021; Elisashvili *et al.* 2023). In the lignin-rich *Q. acutissima* sawdust and cottonseed hull-induced media, only a small amount of CMCase and Xyl activity was detected, which was comparable to that in the CK medium. It is possible that this is linked to the inhibitory influence of lignin on enzymes, which has not been overcome yet. However, the specific mechanism requires further research.

In summary, the primary substrates, corncob and cottonseed hulls, or the supplementary substrate, soybean meal, can all stimulate the *L. decastes* F1 strain to secrete more extracellular Lac (Fig. 1). The primary substrate corncob and the supplementary substrate wheat bran can induce the *L. decastes* F1 strain to secrete CMCase and Xyl within a shorter period. Therefore, in the production and cultivation of *L. decastes*, the primary substrates corncob and cottonseed hull, as well as the supplementary materials soybean meal and wheat bran, should be prioritized.

The Response of Extracellular Lac from *L. decastes* to Different Culture Substrates and Subculturing

As shown in Table 1, except for day 4th of cultivation, both the subcultured *L. decastes* strains and different culture substrate-induced media had a significant impact on Lac activity under liquid fermentation conditions ($p < 0.05$). Both the subcultured strains and different induced media had the greatest impact on the Lac activity of *L. decastes* on day 7th of the cultivation period (Table 1). Under the interaction of subcultured strains and induced media, the most significant impact on *L. decastes* Lac activity occurred on day 5th of the cultivation period, followed by day 7th (Table 1).

Table 1. The Effects of Six Kinds of Culture Substrates on Lac Activity in Different Subcultured Strains of *L. decastes* (two-way ANOVA)

Incubation Period (d)	Substrated Strains	Induced Media	Substrated Strains × Induced media
2	38.195***	29.642***	5.094***
3	4.381*	135.270***	5.203***
4	2.150	143.890***	2.842**
5	12.298***	323.385***	11.875***
6	21.722***	524.860***	5.661***
7	40.805***	743.782***	10.006***
8	27.187***	454.385***	6.699***
9	35.416***	728.913***	7.200***
10	15.557***	552.275***	6.415***
11	6.210**	631.314***	1.863

df:2,6,12; *p<0.05, **p<0.01, ***p<0.001

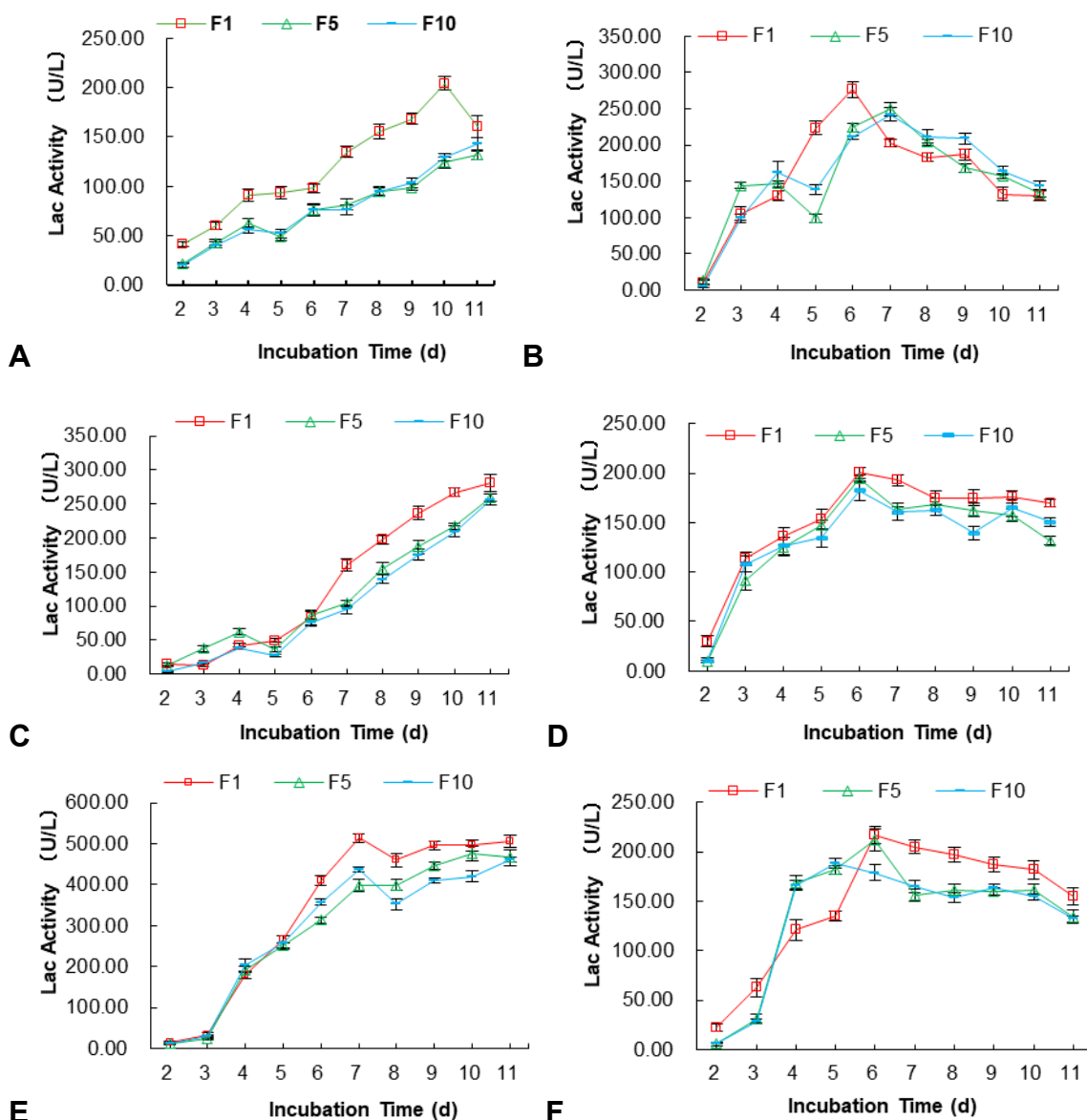
In the control medium (CK), strain F5 exhibited the highest Lac production (Fig. 2-G). Compared to the control, the addition of the tested culture materials stimulated the subcultured strains (*L. decastes* F1, F5, and F10) to secrete more Lac. Moreover, under almost all tested culture material inductions, the maximum Lac activity of *L. decastes* F1 was higher than that of F5 and F10 ((Fig. 1-A, Fig. 2). Except for the main material corncob, the Lac activity of *L. decastes* F1 in liquid fermentation was higher than that of *L. decastes* F5 and *L. decastes* F10 strains after 6th days under the induction of other culture materials (Fig. 2). Only in the media containing cottonseed hulls were a continuously increasing trend in Lac activity being detected among the different *L. decastes* subcultured strains (F1, F5, F10), without reaching a peak during the cultivation period (Fig. 2-C). This indicated that cottonseed hulls exhibited great potential in promoting Lac secretion in *L. decastes* strains. *Q. acutissima* sawdust-induced Lac activity in *L. decastes* F1 strain showed an initial increase followed by a decline, while *L. decastes* F5 and *L. decastes* F10 strains exhibited a continuous increasing trend (Fig. 2-A). However, the Lac secretion ability of the *L. decastes* F1 strain was significantly stronger than that of the *L. decastes* F5 and *L. decastes* F10 strains.

In summary, under fermentation with the tested culture materials, subculturing *L. decastes* strains reduced the Lac activity (Fig. 2). This suggests that Lac activity could be used as a characteristic indicator to assess the degree of degeneration in *L. decastes* strains. In previous studies, Lac activity had also been considered a characteristic indicator for assessing the degree of degeneration in edible mushroom strains such as *Volvariella volvacea*, *Ganoderma lucidum*, *Hypsizygus marmoreus*, and *Pleurotus ostreatus* (Lin *et al.* 2018; Zhao *et al.* 2020; Zhou *et al.* 2023). Lac activity changed among different edible mushrooms due to differences of the subcultured strains. In the inducing media with cottonseed hulls, the Lac activity of *Volvariella volvacea* gradually decreased with an increasing number of subcultures (Zhao *et al.* 2020). When Feng *et al.* (2024) conducted transcriptome sequencing of *V. volvacea* subcultured strains, they found that the key expressing genes, such as Lac-4 and Lac-8, were downregulated, which may have led to the decline in Lac activity (Feng *et al.* 2024). Under solid-state fermentation conditions, as the number of subcultures increasing, the Lac activity of *G. lucidum* and *H. marmoreus* also showed a gradually decreasing trend (Lin *et al.* 2018; Zhou *et al.* 2023). Also, along with the number of subcultures increasing, the Lac activity of *Agrocybe*

aegerita exhibited a trend of first increasing and then decreasing, with a significant decline observed after the seventh generation (F7) (Huang *et al.* 2025).

Mycelial Growth Characteristics of Successive Subcultures of *L. decastes* in Solid Medium

In solid culture, the mycelial growth of *L. decastes* slowed with successive subculturing (Tab. 2, Fig. 3). After 50 days of cultivation, no Xyl activity was detected in the test tube substrate, whereas strain F5 showed comparatively higher CMCase production (Tab. 2). Lac activity was significantly higher in the F1 strain than in the F5 and F10 strains ($P < 0.05$) (Table 2). This result aligns with those observed in liquid fermentation culture. The highest Lac activity detected in the fastest-growing subcultured strains within identical solid cultivation substrates demonstrates a phase-specific positive correlation between mycelial growth and extracellular Lac secretion.



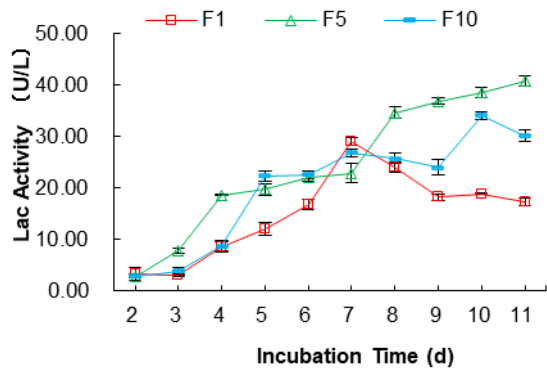
**G**

Fig. 2 The Lac activity of subcultured *L. decastes* strains induced by different culture substrates: **A:** *Quercus acutissima* sawdust; **B:** Corncob; **C:** Cottonseed hull; **D:** Wheat bran; **E:** Soybean meal; **F:** Rice bran; **G:** CK (Control)

Table 2. Lignocellulolytic Enzyme Activity and Average Growth Rate of Different Subculture Generations of *Lyophyllum decastes* in Solid-State Cultivation

Strains	CMCase Activity (U/L)	Lac Activity (U/L)	Xyl Activity (U/L)	Growth Rate (mm/d)
F1	0.41±0.1 ab	18.69±1.16 a	-	1.76±0.02 a
F5	0.63±0.18 a	12.83±1.12 b	-	1.71±0.01 ab
F10	0.16±0.03 b	11.54±0.51 b	-	1.68±0.01 b

Note: The data in the table are presented as mean ± standard error (n = 5). Different lowercase letters indicate significant differences between groups ($P < 0.05$), while the same letters indicate no significant difference ($P > 0.05$).



Fig. 3 Comparative mycelial growth of subcultured strains after 50 days of cultivation

CONCLUSIONS

1. Differences in cultivation substrates significantly affected the secretion of Lac, CMCase, and Xyl activity in *L. decastes* F1 strain. The optimal primary substrate for inducing Lac secretion in *L. decastes* F1 strain was corncob, followed by cottonseed hulls. The best supplementary substrate for Lac induction was soybean meal. The addition of corncob and wheat bran can significantly stimulate the secretion of

CMCase and Xyl in *L. decastes* F1 strain. The addition of corn cob and wheat bran can significantly stimulate the secretion of CMCase and Xyl in *L. decastes* F1 strain.

2. The addition of different cultivation substrates enhanced Lac production in *L. decastes* subcultured strains (F1, F5, F10), but strains subjected to serial subculturing exhibited progressively diminished Lac production.
3. The highest Lac activity was detected in the fastest-growing subcultured strains within identical solid cultivation substrates. This finding demonstrates a phase-specific positive correlation between mycelial growth and extracellular Lac secretion.

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