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Identification, Nutrient Composition, and Evaluation of a Wild *Pleurotus citrinopileatus* Strain (X21156) from Tibet for Antioxidant and Cytotoxic Activities

Xiaoshan Xiao [1], Yun Li [1], Xiaomin Li [1], Xin Hu [1], Junli Zhang [2], Xiaoping Wu [3] and Junsheng Fu [3,*]

[1] College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China; fjxxs010412@163.com (X.X.); ly20240327@163.com (Y.L.); lxm2651277345@163.com (X.L.); hx1962024@163.com (X.H.)
[2] Tibet Academy of Agricultural and Animal Husbandry Sciences, Lasa 850000, China; xzyyxh888@163.com
[3] Mycological Research Center, College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China; fjwxp@126.com
* Correspondence: fujunsheng81@163.com

Abstract: A fungal strain (X21156) collected in Tibet was used as the material, identified based on its morphological characteristics and internal transcribed spacer (ITS) sequence; its optimal culture conditions were analyzed by single-factor experiments; artificial domestication and cultivation were carried out; its nutrient composition was determined; and the bioactivities of its polysaccharides were detected using chemical antioxidant assays and MTT assays. The results showed that the strain was *Pleurotus citrinopileatus* Sing. Its optimal culture conditions were a pH of 7, a temperature of 25 °C, glucose (20 g·L^{-1}) as the carbon source, and yeast powder (20 g·L^{-1}) as the nitrogen source. The fresh weight of a single domesticated fruiting body was 41.16 g. The strain had high protein (28.5%), high fiber (34%), and low fat (1.4%) contents, with high proportions of fresh and sweet amino acids. Polysaccharides had good scavenging ability on ABTS+, DPPH, and OH free radicals (EC_{50} 0.06 mg/mL, 1.21 mg/mL, and 3.62 mg/mL, respectively), and the cytotoxicity of polysaccharides to hepatocellular carcinoma cells (HepG2) (IC_{50}: 1.69 mg/mL) was higher than that of triple-negative breast cancer cells (MDA-MB-468) (IC_{50}: 1.76 mg/mL). In conclusion, the study provides a reference on the optimal culture conditions, domestication and cultivation, and dietary and medicinal values of wild *P. citrinopileatus* Sing.

Keywords: *Pleurotus citrinopileatus*; fungi identification; biological properties; nutritional composition; antioxidant activities; cytotoxic effect

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1. Introduction

Pleurotus citrinopileatus Sing., also known as golden oyster mushroom or elm mushroom, belongs to the division Basidiomycota, class Agaricomycetes, order Agaricales, family Pleurotaceae, and genus *Pleurotus* [1]. *P. citrinopileatus* is rich in bioactive substances such as polysaccharides, ergothioneine, sesquiterpenes, and glycoproteins [2,3], which have anti-cancer, anti-inflammatory, immunity-regulating, and blood sugar-lowering effects [4–7]. However, in recent years, due to excessive harvesting and habitat destruction, wild resources have plummeted. Although *P. citrinopileatus* can be cultivated artificially, its mycelial growth rate is slow and the yield is low. Identifying many wild *P. citrinopileatus* strains and selecting and breeding high-quality strains will greatly promote the artificial industrial cultivation of *P. citrinopileatus*.

The mean altitude of Tibet is above 4000 m, and it has a special geographical location and a complex and varied climate, which has nurtured a great variety of mycorrhizal resources. Wang et al. [8] collected *Pleurotus placentodes* in Rikaze City, Tibet, and the species was first reported in China in 2016. Liu et al. [9] collected *Ganoderma lucidum* from the

Tibetan Plateau and completed the first whole-genome assembly of *Ganoderma lucidum* from this location, based on high-quality DNA extracted from a monococcal strain (DH-8) on the Illumina and Nanopore platforms. Li [10] collected two wild strains of *Agaricus bisporus* (2091 and 2094) from the Tibetan Plateau and compared their domestication and cultivation in order to provide new germplasm resources.

Although several *P. citrinopileatus* strains have been reported in recent years, no studies have been carried out on *P. citrinopileatus* of Tibetan origin. The aims of this study were to isolate, identify, and characterize a fungal strain (X21156) collected in Tibet, including assessing its antioxidant and cytotoxic effects.In this study, we isolated and identified a wild fungal strain from Tibet. We then optimized the culture conditions, carried out artificial domestication and cultivation, and analyzed the nutrient composition. Finally, we measured the scavenging rate of *P. citrinopileatus* polysaccharides against $ABTS^+$, DPPH, and OH radicals, explored the ferric ion reducing antioxidant power (FRAP), and investigated the cytotoxic effects of triple-negative breast cancer cells (MDA-MB-468) and hepatocellular carcinoma cells (HepG2). The results provide a scientific basis for the full development and utilization of *P. citrinopileatus.*

2. Materials and Methods

2.1. Strain Collection

Wild mycorrhizal fungal substrate (strain no. X21156) was harvested in 2021–2022 by our research team in Yigong Township, Bomi, Tibet Autonomous Region (93°00′00″–97°40′00″ E, 29°21′00″–30°30′00″ N).

2.2. Isolation and Culture

A tissue isolation method was used to isolate the strain. A 0.5 cm^3 piece of tissue at the junction of the cap and stipe was taken and inoculated onto enriched potato dextrose agar (PDA) medium (200 g peeled potato, 20 g glucose, 20 g peptone, 1.5 g $MgSO_4$, 2 g K_2HP0_4, 10 mg vitamin B_1, 20 g agar, and 1 L distilled water; pH natural) to obtain the pure strain X21156, which was preserved at 4 °C until it was used for the inoculation process described directly below.

In an ultraclean workbench, the strain was inoculated in sterilized enriched liquid medium (200 g peeled potato, 20 g glucose, 5 g peptone, 1.5 g $MgSO_4$, 2 g K_2HPO_4, 10 mg vitamin B_1, and 1 L distilled water; pH natural) in a flask, which was sealed and incubated on a shaker (160 rpm) at 25 °C and protected from light. The strain was subsequently used in later experiments on the artificial domestication of cultivated mushrooms.

2.3. Morphological and Molecular Identification

For morphological identification, observations of the strain were compared to those in the *Chinese Illustrated Catalogue of Macrofungi in Primary Colours* (1998) by Huang Nian Lai [11] and the *Fungal Identification Manual* (1979) by Wei Jing Chao [12].

The internal transcribed spacer (ITS) molecular identification process involved the following: (1) DNA extraction. An OMEGA Fungal DNA Extraction Kit was used to extract DNA from the mycelium. (2) PCR amplification and sequencing. The primers for ITS amplification were ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTG ATATATGC-3′). The 25-μL PCR amplification system involved 12.5 μL 2×Fine Taq PCR SuperMix (+dye), 1 μL primer ITS1, 1 μL primer ITS4, 1 μL template DNA, and 9.5 μL ddH_2O. The PCR amplification procedure involved pre-denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 90 s; and a final extension at 72 °C for 7 min. Thereafter, 3 μL PCR products were separated by 1% agarose gel electrophoresis. PCR products were also purified and sent to Fuzhou Moby Dick Biotechnology Co., Fuzhou, China, for sequencing. (3) ITS sequence analysis. The obtained ITS sequences were entered ino the NCBI Nucleotide Database (http://www.ncbi.nlm.nih.gov) for BLAST comparison. ITS sequences with high similarity compared to the isolated strain were downloaded, and a phylogenetic tree was constructed

using the maximum likelihood method in MEGA (version 7.0, Mega Ltd., Auckland, New Zealand).

2.4. Optomization of Culture Conditions

Based on the method of Lai et al. [13], with slight adjustments. Carbon source single-factor experiment: PDA medium (pH natural, pH 5.7) was used as the basal medium, the carbon source was either glucose, sucrose, fructose, maltose, mannose, or starch, the nitrogen source was peptone, and the temperature was 25 °C.

Nitrogen source single-factor experiment: PDA medium (pH natural, pH 5.7) was used as the basal medium, the carbon source was glucose, the nitrogen source was either urea, yeast powder, ammonium sulfate, beef meal, peptone, or ammonium nitrate, and the temperature was 25 °C.

pH single-factor experiment: PDA medium was used as the base medium, the pH was adjusted with 1.0 mol/L NaOH and 1.0 mol/L HCl solutions to 5.0, 6.0, 7.0, 8.0, 9.0, or 10.0, and the temperature was 25 °C.

Temperature single-factor experiment: PDA medium was used as the basal medium (pH natural, pH 5.7), and the constant-temperature incubator was set at 15, 20, 25, 30, 35, or 40 °C.

2.5. Mycelial Growth Rate

Mycelial blocks were uniformly extracted using a 5-mm-diameter perforator and inoculated onto the center of the medium in each plate. The plates were inverted, placed in a constant-temperature incubator at 25 °C (except for the one-way temperature experiment), and protected from light. The incubation was stopped after the mycelium covered 2/3 of the plate, and the mycelial diameter was measured using the crossover method. Each single-factor experiment was repeated five times.

$$\text{Mycelial growth rate (mm/d)} = \text{colony diameter (mm)}/\text{number of days of growth (d)} \quad (1)$$

2.6. Mushroom Domestication

Cultivation material (60% wood chips, 20% cottonseed hulls, 18% bran, 1% lime, 1% sugar; according to mass fractions) was mixed well with water (moisture content: 60%), divided into cultivation bags, sterilized at 121 °C for 3 h, and then inoculated with *P. citrinopileatus* (X21156) in an ultraclean workbench after cooling.

The inoculated cultivation bags were moved to a culture room at 22–25 °C and cultivated in the dark with observation of the mycelium, including regarding whether there was any contamination. When each bag became full of mycelium, it was moved to a mushroom room, and the environment was adjusted according to the environmental factors for the optimal growth of different mycorrhizal fungi, including appropriate light, temperature regulation, and humidity regulation. The durations regarding the growth and development of the original base and substrate were observed and recorded.

2.7. Nutrient Analysis

The nutritional value of the studied mushrooms was determined according to the methods of the Association of Official Analytical Chemists [14]. Fresh fruiting bodies of *P. citrinopileatus*, which had been successfully domesticated, producing many fruiting bodies, were dried to a constant weight in a 70 °C electrically heated blast drying oven and kept sealed until use. The moisture content was determined by the direct drying method, protein content by the Kjeldahl method, ash content by the high-temperature burning method, fat content by the Soxhlet extraction method, total sugar content by an evaporative light scattering detector, dietary fiber content by the enzyme weight method, sodium content by flame atomic emission spectrometry, and amino acid content by an automatic amino acid analyzer. Three parallel experiments were conducted to calculate the mean of each index.

2.8. Antioxidant Activity of P. citrinopileatus Polysaccharides

Based on the method of Cui et al. [15], polysaccharides were extracted from *P. citrinopileatus* fruiting bodies. The polysaccharide solution was poured into a dialysis bag for water dialysis for 48 h. It was then freeze-dried to a constant weight to create a pure polysaccharide freeze-dried powder. Pure polysaccharide solutions at concentrations of 0.025, 0.05, 0.25, 0.5, 1, 2, and 5 mg/mL and the same concentrations of vitamin C (VC) solutions (positive control) were prepared. In all antioxidant assays, the optical density (OD) values were detected using a microplate reader. Five parallel experiments were conducted for each concentration, and the mean values were calculated.

2.8.1. ABTS Free Radical Scavenging Capacity Assay

Based on the method of Miller [16] with slight adjustments, $ABTS^+$ mother liquor was prepared by mixing 5 mL of 7.0 mmol/L ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) solution and 88 µL of 2.45 mmol/L aqueous potassium persulfate solution and allowing the solution to stand for 12–16 h at room temperature, protected from light. To create an $ABTS^+$ working solution, the mother liquor was diluted with distilled water to achieve an OD_{734} of 0.70 ± 0.023. Next, 100 µL polysaccharide/VC solution and $ABTS^+$ working solution were added to a 96-well plate, mixed well, and left at 25 °C for 20 min, protected from light. OD_{734} was recorded as OD_y. For the blank group, distilled water was used to replace the polysaccharide, and OD_{734} was recorded as OD_o. For the control group, distilled water was used to replace ABTS, and OD734 was recorded as OD_p.

$$\text{ABTS free radical scavenging rate (\%)} = [1 - (OD_y - OD_p)/OD_o] \times 100 \quad (2)$$

2.8.2. DPPH Free Radical Scavenging Capacity Assay

Based on the method of Saiga [17], with slight adjustments, 100 µL polysaccharide/VC solution and 0.2 mmol/L DPPH (2,2-diphenyl-1-picrylhydrazyl) solution were added to a 96-well plate, gently stirred for 30 min at room temperature, and protected from light. OD_{517} was recorded as OD_y. For the blank group, anhydrous ethanol was used to replace the polysaccharide, and OD_{517} was recorded as OD_o. For the control group, anhydrous ethanol was used to replace DPPH, and OD_{517} was recorded as OD_p.

$$\text{DPPH free radical scavenging rate (\%)} = [1 - (OD_y - OD_p)/OD_o] \times 100 \quad (3)$$

2.8.3. Hydroxyl Free Radical Scavenging Capacity Assay

Based on the method of Winterbourn and Satton [18] with slight adjustments, 75 µL polysaccharide/VC solution and 15 µL of a mixture of $FeSO_4$ solution (9 mmol/L), salicylic acid-ethanol solution (9 mmol/L), and H_2O_2 solution (8.8 mmol/L) were added to a 96-well plate, gently shaken, and then 100 µL distilled water was added to the mixture, which was incubated in a water bath at 37 °C for 30 min. OD_{510} was recorded as OD_y. For the blank group, OD_{510} of distilled water was assessed and recorded as OD_o. For the control group, OD_{510} of the reaction system was assessed and recorded as OD_p.

$$\text{Hydroxyl free radical scavenging rate (\%)} = [1 - (OD_y - OD_p)/OD_o] \times 100 \quad (4)$$

2.8.4. Ferric Ion Reducing Antioxidant Power (FRAP) Assay

Based on the method of Benzie et al. [19] with slight adjustments, a FRAP working solution was prepared using 0.3 mol/L acetic acid–sodium acetate buffer solution (pH 3.6), 0.02 mol/L $FeCl_3$ solution, and 0.01 mol/L TPTZ (2,4,6-tris(2-pyridyl)-1,3,5-triazine) solution in a volume ratio of 10:1:1. To create a standard curve, 0.5 mL of 0.025, 0.1, 0.15, 0.2, 0.4, 0.5, 0.8, 1.0, and 1.5 mmol/L $FeSO_4$ solution was mixed with 3.0 mL FRAP working solution, reacted in a 37 °C water bath for 15 min, and subjected to OD_{593} measurement to construct the standard curve. 3.0 mL FRAP working solution was also mixed with 1.5 µL polysaccharide/VC solution and reacted in a 37 °C water bath for 15 min, and

OD_{593} was recorded as OD_y. For the blank group, distilled water was used to replace the polysaccharide, and OD_{593} was recorded as OD_o. For the control group, distilled water was used to replace the FRAP working solution, and OD_{593} was recorded as OD_p. FRAP was determined based on the difference between OD_y, OD_o, and OD_p and the corresponding $FeSO_4$ concentration on the standard curve.

2.9. Cytotoxic Effect of P. citrinopileatus Polysaccharides

HepG2 and MDA-MB-468 cells were cultured in PRMI-1640 medium containing 10% fetal bovine serum and 1% penicillin-streptomycin in a 5% CO_2 incubator at 37 °C.

The effect of *P. citrinopileatus* polysaccharides on the proliferation of HepG2 and MDA-MB-468 cells was detected using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assays based on the method of Latronico et al. [20]. Logarithmic cells were digested with trypsin, centrifuged, placed in a 96-well plate, and mixed with 200 μL cell culture medium containing polysaccharides (0.025, 0.05, 0.25, 0.5, 1, 2, or 5 mg/mL). The control group involved culture medium without polysaccharides, and the blank group involved culture medium without cells, with five parallel wells per group. After 24 h of culture in an incubator, the cell culture medium was removed, and 100 μL cell culture medium containing 10% MTT was added to each well. After another 4 h of culture in the incubator, the cell culture medium was removed, 150 μL dimethyl sulfoxide (DMSO) was added to each well, and the wells were gently shaken until the purple-brown precipitate was completely dissolved. OD_{490} was measured using a microplate reader. The effect of different concentrations of polysaccharides on the half-maximal inhibitory concentration (IC_{50}) was determined based on the cell survival rate, assessed using the following formula:

$$\text{Cell survival rate (\%)} = (OD_{\text{polysaccharide}} - OD_{\text{blank}})/(OD_{\text{control}} - OD_{\text{blank}}) \times 100 \quad (5)$$

2.10. Statistical Analysis

The data were processed and analyzed using SPSS 26.0 (IBM Crop., Armonk, NY, USA) and Prism 5.0 (GraphPad software, San Diego, CA, USA). Data for all experiments from at least three biological replicates are presented as means ± SD. The significance of differences between groups was determined by one-way and repeated-measures analysis of variance (ANOVA). $p < 0.05$ indicates significant differences, and $p < 0.01$ indicates extremely significant differences.

3. Results

3.1. Identification of Wild Mushroom Species from Tibet

3.1.1. Morphological Identification

The wild strain (strain X21156) (Figure 1a) collected in the field was isolated. Its morphological characteristics included clustered or clustered substrates with golden-yellow, flat hemispherical to funnel-shaped or flat fan-shaped caps, and a smooth surface. The mushroom flesh was white, thin, and brittle, with a distinct flavor. The hyphae were white or slightly yellowish, lamellate, crowded, elongated, and unequal. The stipe was partial, white to slightly yellow, solid inside, 2–12 cm long, and 0.5–2 cm in diameter. These characteristics were consistent with the description of *P. citrinopileatus* Sing. In the *Chinese Illustrated Catalogue of Macrofungi in Primary Colours* (1998) [11] and the *Fungal Identification Manual* (1979) [12].

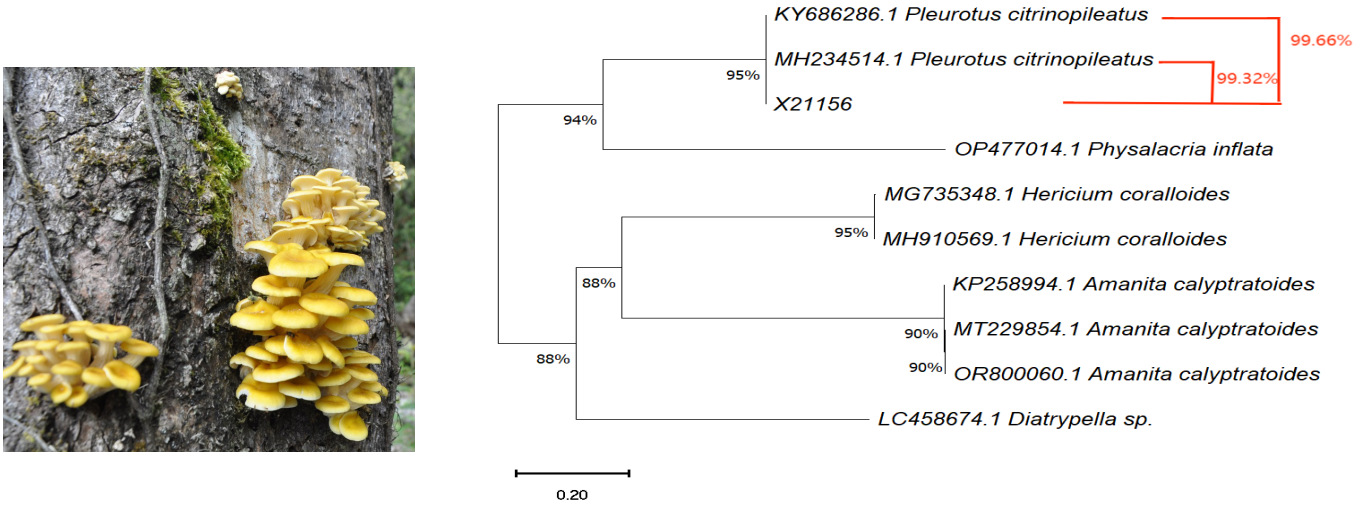

Figure 1. Molecular identification of *P. citrinopileatus* (X21156). (**a**) Wild *P. citrinopileatus* fruiting body. (**b**) Maximum likelihood phylogenetic tree based on ITS sequences.

3.1.2. Molecular Identification

The ITS sequences of strain X21156 had 99.66% and 99.32% similarity with the reported sequences of two *P. citrinopileatus* strains (NCBI accession numbers KY686286.1 and MH234514.1, respectively). The maximum likelihood method was used to construct a phylogenetic tree of ITS sequences of *Pleurotus* spp., using *Hohenbuehelia grisea* AY265835.1 as an outgroup. Strain X21156 clustered with *P. citrinopileatus* and was genetically distant from other *Pleurotus* spp. (Figure 1b). Based on morphological and molecular identification, strain X21156 is *P. citrinopileatus* Sing.

3.2. Optimization of Culture Conditions

The pH single-factor experiment showed that the strain grew well at all tested pH values. At pH 7, the aerial mycelial growth was vigorous, and the mycelial growth rate was optimal, with a daily mycelial growth rate of 8.71 mm/d. The temperature single-factor experiment showed that, at 25 °C, the aerial mycelial growth was the most vigorous and the mycelial growth rate was optimal, with a daily mycelial growth rate of 10.00 mm/d, which was clearly different from the results at 40 °C. The carbon source single-factor experiment showed that when the carbon source was fructose, the mycelial density was the highest, but the mycelial growth rate was slow. When the carbon source was maltose, the mycelial growth rate was the fastest, but the mycelial density was low. Based on both the mycelial density and growth rate, the most suitable carbon source was glucose (9.00 mm/d). The nitrogen source single-factor experiment showed that when the nitrogen source was yeast powder, mycelial growth was the most vigorous and the daily mycelial growth rate was 8.29 mm/d, which was significantly different from that related to urea. Combining the results of the above experiments, it was concluded that the optimal culture conditions for X21156 were a pH of 7, a temperature of 25 °C, glucose as the carbon source, and yeast powder as the nitrogen source (Figure 2).

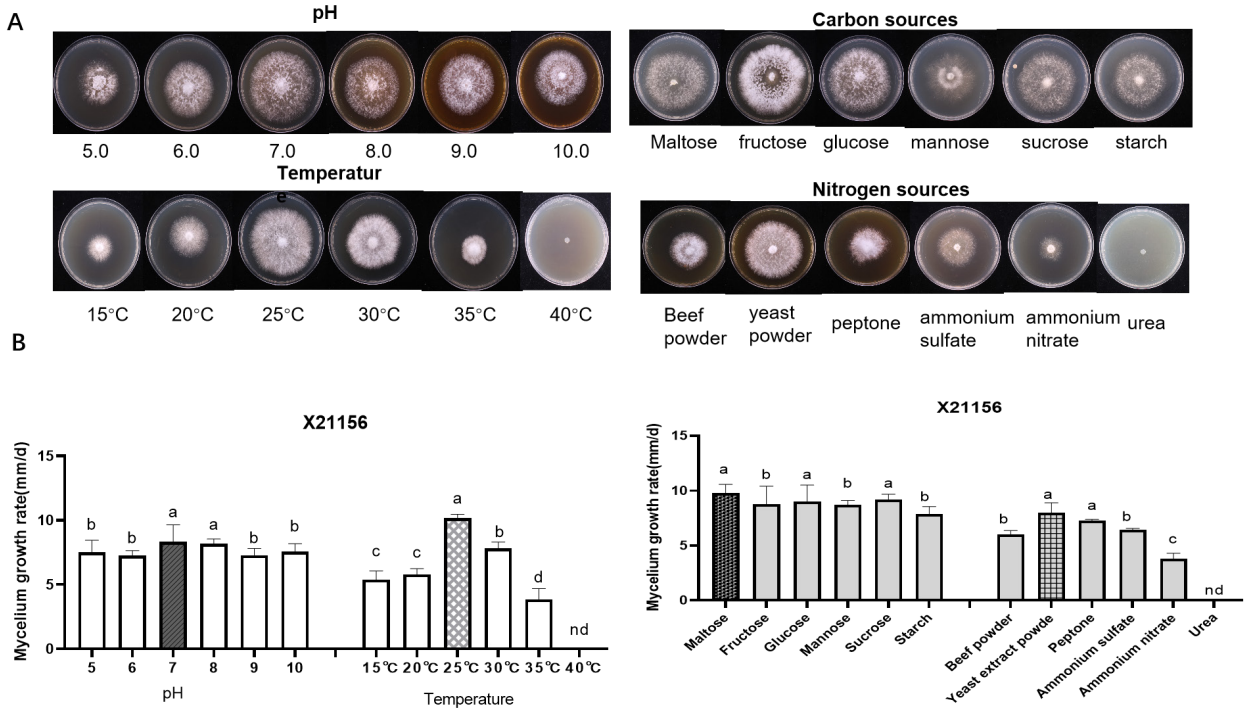

Figure 2. Biological properties of *P. citrinopileatus* (X21156). (**A**) Growth trend diagram. (**B**) Mycelial growth rate chart. On the bar chart, the same letters indicate no significant difference, while different letters indicate a significant difference ($p < 0.05$).

3.3. Domestication and Cultivation of Mushrooms

The optimal culture conditions for the mycelium of *P. citrinopileatus* (X21156) were exploited to domesticate the wild strain. It took 25 d for the mycelium to fill the bag. In the early stage of mushroom production (at about 7 d), yellow and white primordia the size of rice grains appeared on the surface of the culture material. After differentiation in the middle stage, the structures unfolded, were yellowish, and had a slightly curled edge. After maturity, they were medium-sized. The caps were light-yellow to golden-yellow. After harvesting, the mean fresh weight of a single mature P. citrinopileatus fruiting body was 41.16 g (Figure 3). The results indicated that strain X21156 has a short growth cycle and a high yield.

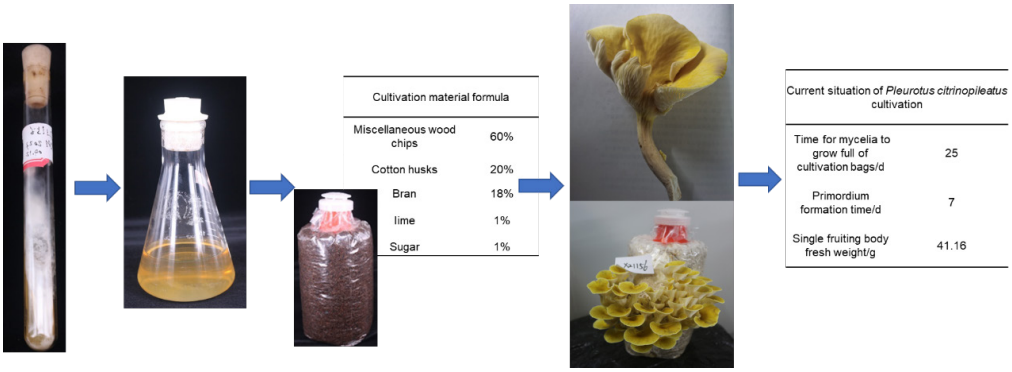

Figure 3. Domestication and cultivation process.

3.4. Nutrient Analysis

3.4.1. Conventional Nutrient Analysis Results

Conventional nutrient analysis of the domesticated and cultivated *P. citrinopileatus* (X21156) fruiting bodies showed that the protein content (28.5%) was higher than the

nutritional standard for edible mushrooms (24.00%) [21]. The total sugar content was 4.5%, which was nearly six times higher than that of eggs (0.7%). The dietary fiber content was 34%. The fat content was 1.4%, which was much lower than that of eggs (8.6%) (Table 1). All in all, *P. citrinopileatus* is a tasty, high-protein, high-fiber, and low-fat food that meets the requirements for a healthy diet for humans.

Table 1. Nutrient content of *P. citrinopileatus* fruiting bodies.

Nutrient Composition	Content (g/100 g)	
	Pleurotus citrinopileatus	Egg [22]
Moisture (g) (fresh)	91.7 ↑	75.2
Moisture (g) (dry)	11.4 ↑	-
Crude protein (g)	28.5 ↑	12.04
Ash (g)	10.2 ↑	0.9
Fat(g)	1.4	8.6
Total sugar (g)	4.5 ↑	0.7
Dietary fiber (g)	34.0 ↑	0
Na (mg)	13.0	131.5

↑ higher than in eggs.

3.4.2. Amino Acid Content

The *P. citrinopileatus* (X21156) fruiting bodies contained 15 amino acids, including valine, lysine, and phenylalanine. The total and essential amino acid contents were 16.46% and 5.22%, respectively. The essential/total amino acid ratio was 0.32, and the essential/nonessential amino acid ratio was 0.46, which were close to the ideal values (0.40 and 0.60, respectively) proposed by the Food and Agriculture Organization/World Health Organization. In addition, the contents of glutamic acid (an umami-flavored amino acid) (3.81%) and glycine (a sweet amino acid) (0.99) were nearly twice as high as those of eggs (1.59 and 0.39, respectively), indicating potentially better taste and freshness compared to those of eggs. Taken together, this suggests that *P. citrinopileatus* can be used as a high-quality, palatable source of protein (Table 2).

Table 2. Amino acid content of *P. citrinopileatus* fruiting bodies.

Amino Acid Composition		Content (g/100 g)	
		Pleurotus citrinopileatus	Egg [23]
EAA	IIe	0.49	0.65
	Val	0.93 ↑	0.64
	Lys	1.16 ↑	0.85
	Met	-	0.33
	Leu	1.02	1.05
	Phe	0.69 ↑	0.65
	Thr	0.93 ↑	0.59
	Trp	-	0.19
NEAA	Arg	0.89 ↑	0.74
	His	0.41 ↑	0.27
	Try	0.40	0.50
	Ala	1.14 ↑	0.66
	Pro	0.78 ↑	0.34
	Ser	1.08 ↑	0.91
	Glu	3.81 ↑	1.59
	Gly	0.99 ↑	0.39
	Asp	1.74 ↑	1.21
	Cys	-	0.50

Table 2. *Cont.*

Amino Acid Composition	Content (g/100 g)	
EAA	5.22 ↑	4.93
NEAA	11.18 ↑	7.11
TAA	16.40 ↑	12.04
E/T	0.32	0.41
E/N	0.44	0.69

↑ higher than in eggs.

3.5. Antioxidant Activity of P. citrinopileatus Polysaccharides

The in vitro antioxidant activity of *P. citrinopileatus* polysaccharides from fruiting bodies was analyzed. In the $ABTS^+$ free radical scavenging experiment, the scavenging ability of the $ABTS^+$ free radical was enhanced with an increase in polysaccharide concentration. At 0.5 mg/mL, the rate was 97.22%, and the half maximal effective concentration (EC_{50}) was 0.06 mg/mL (Figure 4A). The scavenging ability of the DPPH free radical was analyzed, and the scavenging ability was positively correlated with the concentration of polysaccharide. At 5 mg/mL, the reaction rate was 89.88%, and the EC_{50} was 1.21 mg/mL (Figure 4B). In the hydroxyl radical scavenging experiment, the clearance rate was also positively correlated with the concentration of polysaccharide. At 5 mg/mL, the reaction rate was 58.01%, and the EC_{50} was 3.62 mg/mL (Figure 4C). In the experimental concentration range, the polysaccharide showed good reducing ability. At 5 mg/mL, FRAP peaked at 4.24 mmol/L (Figure 4D). In summary, the results showed that the polysaccharide from the *P. citrinopileatus* fruiting bodies had good chemical antioxidant activity.

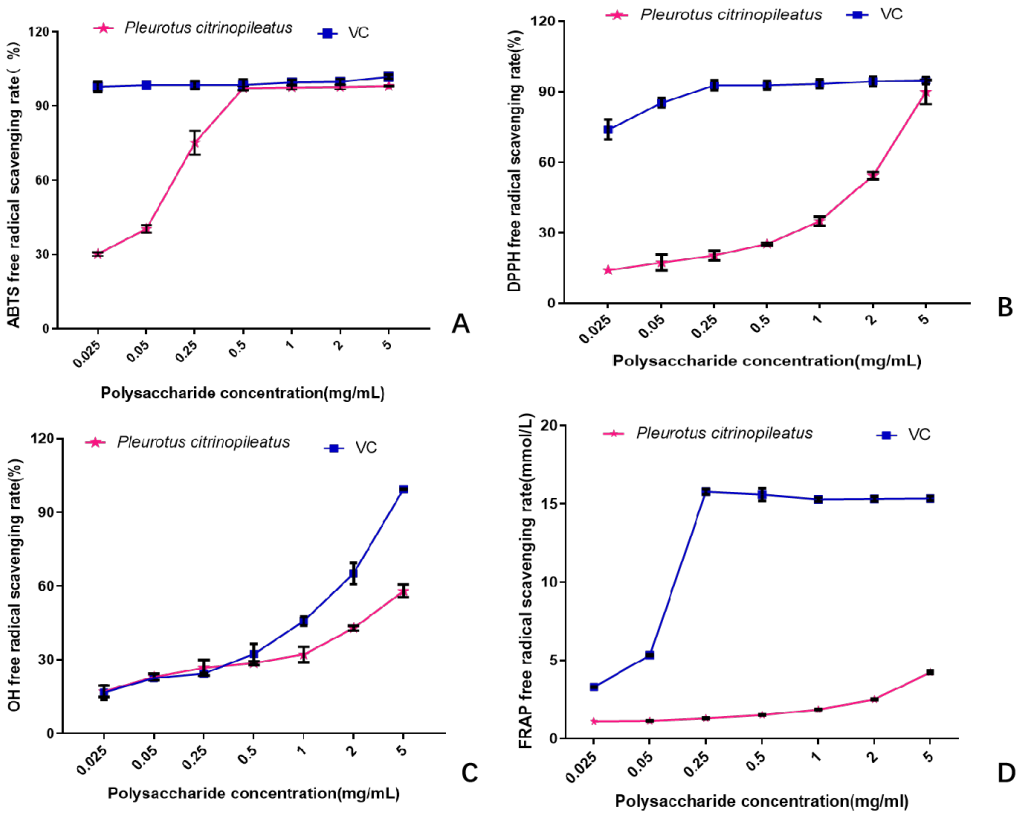

Figure 4. In vitro antioxidant activity of *P. citrinopileatus* polysaccharides. (**A**) ABTS free radical scavenging ability. (**B**) DPPH free radical scavenging ability. (**C**) Hydroxyl free radical scavenging ability. (**D**) Ferric ion reducing antioxidant power (FRAP). Vitamin C (VC) was the positive control. Quantitative results are plotted. All data represent five independent experiments. Results represent the means ± SD of one representative experiment.

3.6. Cytotoxic Effect of *P. citrinopileatus* Polysaccharides

Cell-based experiments are close to the complex environment inside an organism, being able to reflect the mechanisms of action of bioactive substances. These experiments are also quicker and have lower costs compared to animal experiments. Therefore, in vitro MTT assays were used to detect the anticancer effects of *P. citrinopileatus* polysaccharides against triple-negative breast cancer cells (MDA-MB-468) and hepatoma cells (HepG2). *P. citrinopileatus* polysaccharides concentration-dependently inhibited the proliferation of both cancer cells at 1–5 mg/mL ($p < 0.01$). The IC_{50} values showed that the effect was more pronounced in the HepG2 cells (1.69 mg/mL) than the MDA-MB-468 cells (1.76 mg/mL) (Figure 5).

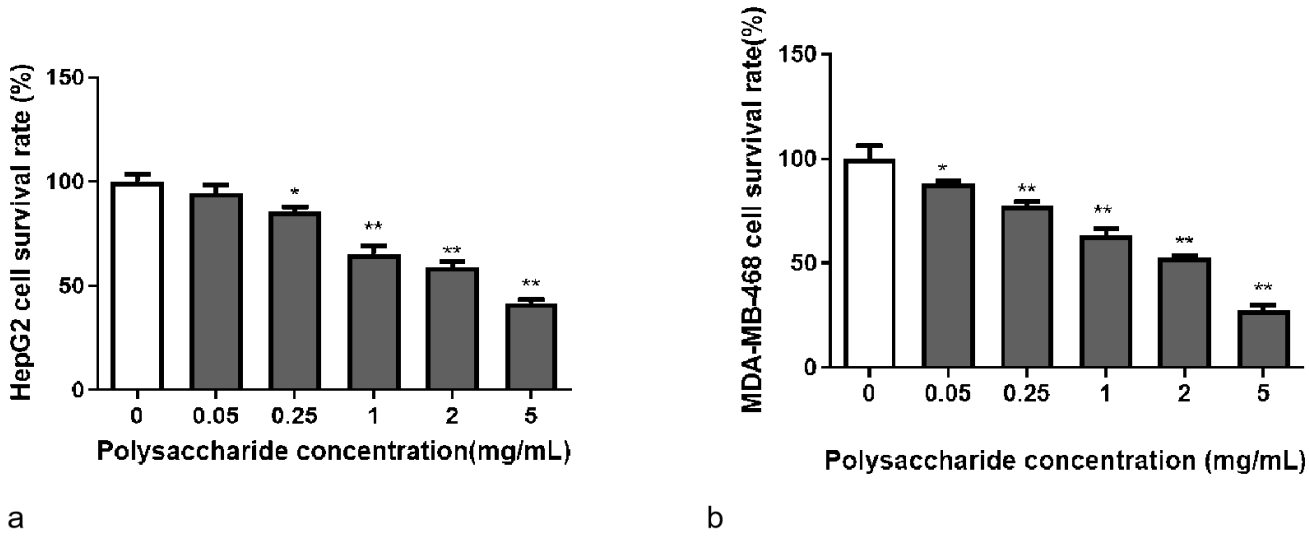

Figure 5. Effects of *P. citrinopileatus* polysaccharides on cancer cell proliferation. (**a**) HepG2 cell inhibition. (**b**) MDA-MB-468 cell inhibition. Quantitative results are plotted. All data represent five independent experiments. Results represent the means ± SD of one representative experiment. * $p < 0.05$, ** $p < 0.01$ using ANOVA.

4. Discussion

The identification and classification of edible mushrooms provide a basis for research, development, and utilization. The isolation of strains of large wild fungi is easily influenced by the environment, the characteristics of the sample itself, and operator factors. The morphological characteristics are prone to differ, so the classification and identification of wild fungi based on morphological characteristics have limitations. However, the ITS fragments of eukaryotes are small, easy to analyze, and can substantially reflect the differences between genera, species, and strains. They have been widely used in phylogenetic research on different or similar species within fungal genera [24]. Based on the comprehensive morphological characteristics and ITS sequence results, the wild strain (X21156) collected in this study was identified as *P. citrinopileatus*. The optimal pH for mycelial growth was 7; the optimal temperature was 25 °C; the optimal carbon source was glucose; and the optimal nitrogen source was yeast powder. The mycelial growth rate was fast during artificial cultivation, and the appearance and odor of the mushroom were good. This study has significance for protecting large-scale fungal germplasm resources, enriching international edible mushroom resource banks, and promoting in-depth exploration of the economic value of fungal germplasm resources.

Edible mushrooms are rich in nutrients such as protein, amino acids, polysaccharides, vitamins, minerals, and purines, which play important roles in maintaining human biological functions, and edible mushrooms are known as “plant meat”. Due to factors such as climate, habitat, and growth cycle, wild edible mushrooms typically contain higher levels of various nutrients compared to common cultivated edible fungi [25]. This study

determined the nutritional components of *P. citrinopileatus* (X21156) and found that it contains abundant crude protein content (28.5 g/100 g), which is higher than that of *Pleurotus ostreatus* (12.8 g/100 g), *shiitake mushroom* (26.6 g/100 g), and *Agrocybe aegerila* (28.8 g/100 g) [26]. The sodium content (13.0 mg/100 g) is higher than that of *Hericium erinaceus* (9.52 mg/100 g) and *Black Ganoderma lucidum* (6.69 mg/100 g) [27]. The essential amino acid content (5.22 g/100 g) is higher than that of *Pleurotus ostreatus* (4.96 g/100 g) and *Flammulina velutipes* (Fr.) Sing. (4.79 g/100 g) [28]. Thus, *P. citrinopileatus* (X21156) is an ideal healthy ingredient. It is not only a high-protein edible mushroom, but it also has more mineral elements than other edible mushroom species, making it highly nutritious. Improving existing edible mushroom germplasm resources is of great value.

Some fungal polysaccharides are known as host immune enhancers and also as biological response modulators (BRMs). They have many bioactivities, such as lowering blood sugar, inhibiting cancer cell growth, regulating immunity, and having antiviral, antioxidant, and antibacterial effects. This study found that the ABTS free radical scavenging rate of the *P. citrinopileatus* polysaccharides (1 mg/mL) was 97.52%, higher than that of *Pleurotus djamor* polysaccharides (1 mg/mL) (59.64%) [29]. It can be used as a widely available, effective, natural, and low-toxicity free radical scavenger and antioxidant. According to the 2023 Cancer Statistics Report of the United States [30], cancer remains the second most common cause of death after heart disease. Various mushroom polysaccharides have been reported for the development of anticancer drugs, such as *shiitake mushroom* polysaccharides [31] (from China), *mushroom* polysaccharides [32] (from the US), and *Grifola frondosa* polysaccharides [33] (from Japan). This study also showed that *P. citrinopileatus* (X21156) polysaccharides have cytotoxic effects on MDA-MB-468 and HepG2 cancer cells, which provides a new direction and material basis for further drug development.

Polysaccharides are compounds consisting of more than 10 sugar groups bound by glycosidic bonds and are one of the four basic substances of life. Polysaccharides have antioxidative stress properties, and the antioxidant mechanisms include direct scavenging of ROS, enhancement of antioxidant enzyme activity, and binding of polysaccharide molecules to metal ions necessary for ROS inhibition of free radical production [34]. Li et al. [35] found that astragalus polysacharin alleviates H_2O_2-triggered oxidative injury in HUVECs by elevating the expression of KLF2 via the MEK/ERK pathway. Liu et al. [36] showed that *Lycium barbarum* polysaccharides could protect retinal ganglion cells from $CoCl_2$-induced apoptosis by reducing mitochondrial membrane potential and reactive oxygen species. Zhang et al. [37] demonstrated that AP attenuated cellular oxidative response damage in HaCaT cells by positively regulating miR-126. However, there are few reports about the specific molecular oxidation mechanism of edible fungi. MTT/MTS in vitro cell proliferation assays are colorimetric-based assays used to evaluate the initial anticancer activity of synthetic natural products and their extracts. This assay fully reveals the cytotoxicity of an unknown sample, while the kinase inhibition assay or enzyme inhibition screening assay is used to determine the exact molecular target [38]. Therefore, this research group will conduct a systematic study on the antioxidant and anticancer mechanisms of the polysaccharides of *P. citrinopileatus* (X21156), based on making up for the deficiency of the antioxidant and anticancer mechanism of edible fungi.

5. Conclusions

Pleurotus citrinopileatus Sing. is a precious homologous bacterium of medicine and food that has extensive medical value because it contains abundant active substances. The aims of this study were to isolate, identify, and characterize a fungal strain (X21156) collected in Tibet, including assessing its antioxidant and cytotoxic effects. The results show that the tested strains for *Pleurotus citrinopileatus* Sing's optimal culture conditions were a pH of 7, a temperature of 25 °C, glucose (20 $g{\cdot}L^{-1}$) as the carbon source, and yeast powder (20 $g{\cdot}L^{-1}$) as the nitrogen source. The fresh weight of the mushroom fruiting body could reach 41.16 g. It was composed of high protein (28.5%), high fiber (34%), low fat (1.4%), and a high proportion of fresh amino acids and sweet amino acids. The EC_{50}

values of polysaccharides against $ABTS^+$ free radicals, DPPH free radicals, and hydroxyl free radicals were 0.06 mg/mL, 1.21 mg/mL, and 3.62 mg/mL, respectively, and the peak FRAP value was 4.24 mmol/L. The IC_{50} values of HepG2 cells and MDA-MB-468 cells were 1.69 mg/mL and 1.76 mg/mL, respectively. In summary, this strain has a high yield, rich nutrition, delicious taste, and a good antioxidant and cytotoxic effect, which provides a scientific basis for the development and application of anti-oxidation and anti-cancer products, as well as expanding the international edible mushroom germplasm resource base (Figure 6).

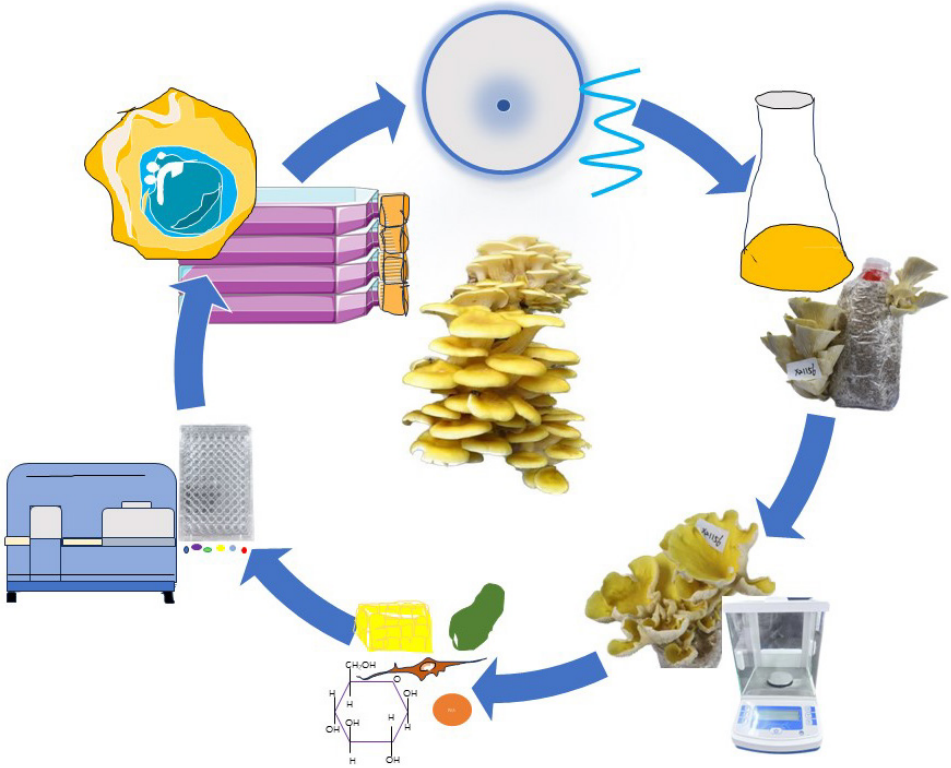

Figure 6. Technology roadmap.

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