Hindawi
Journal of Food Quality
Volume 2022, Article ID 5526810, 7 pages
https://doi.org/10.1155/2022/5526810

Research Article

Species-Specific Gene, *spt5*, in the Qualitative and Quantitative Detection of *Boletus reticulatus*

Zhan Lei, Chen Zhang, Yinjiao Li, Lunzhao Yi, and Ying Shang

Faculty of Agriculture and Food, Kunming University of Science and Technology, Kunming 650500, Yunnan, China

Correspondence should be addressed to Ying Shang; shangying1986@163.com

Received 27 January 2021; Accepted 7 March 2022; Published 1 April 2022

Academic Editor: Efstathios Giaouris

Boletus reticulatus is a wild edible fungus with high nutritional value in Yunnan Province. In this study, *B. reticulatus* was used as the research object to diagnose the species characteristics. A commercial kit was used to extract the DNA of various fungi, and the quality of DNA was determined by using universal fungus primers. Through sequence alignment, the *spt5* gene was selected as the species-specific gene of *B. reticulatus*. This gene was then qualitatively and quantitatively analyzed by PCR. In the qualitative detection, the *spt5* amplification products were only found in *B. reticulatus* which proved its good specificity. Meanwhile, SYBR Green I based quantitative PCR results were highly sensitive, and the limit of detection was 0.04 ng of genomic DNA. These experiments illustrated that *spt5* is an ideal species-specific gene for the quantitative and qualitative detection of *B. reticulatus*. This method is also suitable for the analysis of the processed samples of *B. reticulatus* and the determination of the adulteration of edible wild mushrooms.

1. Introduction

The fruiting body of *Boletus reticulatus* is thick and delicate [1], and the nutritional value is extremely rich [2, 3]. *B. reticulatus* is not only edible but also has certain health effects [4], such as the treatment of numbness of the hands and feet [5], lumbago and leg pain, and infertility [6]. In the market, the price of *B. reticulatus* is more expensive than that of other fungi, food adulteration may occur, and food poisoning may even be reported in severe cases. Therefore, *B. reticulatus* should be identified to avoid this phenomenon. To improve the current situation of adulteration and protect the rights and interests of consumers, an efficient and rapid wild edible fungus detection method should be established.

Traditional detection methods include morphological identification, zoological detection, and physical and chemical detection. Morphological identification is based on the determination of the shape, odor, color, and secretions of fruiting bodies [7]. However, many types of wild fungi exist in Yunnan, and many wild fungi are highly similar in shape and cannot be directly distinguished [8]. Some deep-processed products can also severely damage the morphology of wild fungi, so morphological identification has certain limitations. Zoological detection refers to the use of animals to test whether edible fungi are toxic, which is not representative. The physical and chemical techniques are based on the analysis of unique markers in food to achieve product identification, and it is widely used in the field of food detection. With the continuous development and application of various technologies such as chromatography, mass spectrometry, and spectroscopy, chemical methods have been rapidly developed and perfected, playing an active and effective role in monitoring poor manufacturers [9–11]. However, with the advancement of science and technology, food adulteration technology is becoming increasingly sophisticated [12, 13]. By artificially adding chemical components, ordinary physical and chemical detection methods cannot easily identify the authenticity of food ingredients [14].

With the rapid development of modern biotechnology, molecular biological detection has been widely used to identify food sources [15, 16]. DNA-based forgery detection mainly relies on polymerase chain reaction (PCR) to amplify highly specific DNA fragments in the genome to achieve

detection purposes [17, 18]. The techniques that fall under DNA-based forgery detection mainly include single-strand conformation polymorphism technology (SSCP), random amplified polymorphic DNA technology (RAPD), simple sequence repeat technology (SSR), intersimple sequence repeat technology (ISSR), and DNA amplification fingerprinting technology [19–21]. However, these technologies either need to design multiple pairs of primers and amplify multiple targets in the early stage or sequence the amplified products in the later stage. The purpose of detection can be achieved by sequence comparison, which takes a long time. Compared with the above techniques, detection technology based on amplifying species-specific gene (also called endogenous reference gene) is more convenient, specific, and economical [22].

Normal qualitative PCR has the advantages of low-cost, convenience, and wide applicability. Real-time fluorescent quantitative PCR technology has the advantages of high sensitivity, strong specificity and accuracy, short detection time, data visualization, and high automation. It is widely used in the fields of genetic modification detection, clinical medicine, and research and development of new disease-resistant crops. This study mainly used normal PCR and real-time fluorescent quantitative PCR technology to achieve qualitative and quantitative detection of species-specific gene of *spt5* in *B. reticulatus*.

In this study, the DNA of various edible fungi was extracted using a commercial kit, and the DNA quality was determined by PCR using universal fungus primers. Through multiple sequence alignment, the *spt5* gene is selected as the species-specific gene of *B. reticulatus*. This gene was then qualitatively and quantitatively analyzed by PCR using substrate genomic DNA of *B. reticulatus* and other species. The minimum detection limit was 0.04 ng of genomic DNA by SYBR Green fluorescence quantitative PCR, and the *spt5* amplification products were not found in the 10 other species. Allelic variation was not detected in *B. reticulatus*. These experiments proved that *spt5* is an excellent species-specific gene for the quantitative and qualitative detection of *B. reticulatus*. It can be used to analyze *B. reticulatus* and its processed samples and determine the adulteration of wild mushrooms.

2. Materials and Methods

2.1. Materials and Reagents. This study used 11 species of fungus samples, such as *Boletus reticulatus*, *Boletus magnificus* W.F.Chiu, *Lentinus edodes*, *Agrocybe aegirit*, *Leccinum extremiorientale* (L.Vass.) Singer, *Boletus griseus* Frost, *Boletus sinicus* W.F.Chiu, *Russula virescens*, *Collybia albuminosa*, *Lactarius volemus*, and *Pleurotus eryngii*. These samples were purchased from local markets in Yunnan Province, China.

The following reagents were used in the study: Ezup column fungal genomic DNA extraction kit purchased from Sangon Biotech (Shanghai) Co., Ltd.; 50× TAE buffer; EDTA (pH 8.0); 10 mg/mL RNase A, 20 mg/mL proteinase K, ethidium bromide (10 mg/mL), and DNA marker DL 2000 purchased from Tiangen Biotech (Beijing) Co., Ltd.; and 6× loading buffer purchased from Takara Biomedical Technology (Beijing) Co., Ltd. The primers used in the PCR reaction were synthesized by Sangon Biotech (Shanghai) Co., Ltd.

2.2. DNA Extraction and Purification. About 50–100 mg of each fresh fungus was ground into powder with liquid nitrogen and transferred into a 1.5 mL centrifuge tube. The steps in the kit were followed to perform the experiment. The purity and concentration of the extracted DNA were measured by using the microconcentration analyzer NanoDrop 2000. Each sample was measured three times. Finally, the extracted DNA was placed at −20°C till used for the subsequent experiments.

2.3. Qualitative Detection of the Extracted Genomic DNA Using ITS Universal Fungal Primer. To verify whether the extracted DNA can be used for PCR amplification, the universal primers of fungal internal transcribed spacer (ITS) rDNA (Table 1) were used to amplify the genomic DNA of the 11 samples.

PCR assays were conducted in a total volume of 25 μL on an ABI SimpliAmp thermal cycler (Applied Biosystems, USA) (Table 2). The PCR amplification conditions were as follows: predenaturation (95°C, 5 min); 30 cycles of denaturation (95°C, 30 s), annealing (58°C, 30 s), and extension (72°C, 30 s); and termination extension (72°C, 10 min). The 5 μL of each PCR product was then analyzed by 2% agarose gel electrophoresis (containing 0.1 g/mL EB) [23].

2.4. Screening of Species-Specific Gene in B. reticulatus. The gene information of *B. reticulatus* was searched in the nucleotide database of NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and BLASTN alignment analysis was performed. The gene with low homologous sequences and high species specificity was selected as the candidate of endogenous reference gene.

Aiming at the sequence of candidate genes, Primer Premier 5.0 software (PREMIER Biosoft, San Francisco, USA) was used to design primers. BLASTN comparative analysis was performed on the designed primers to ensure the specificity, and the detailed sequences are given in Table 1.

2.5. Verification of the Specificity of the Selected Species-Specific Gene. The genomic DNA of the 11 mushroom species was used as the template to qualitatively verify the interspecies specificity of the species-specific gene in *B. reticulatus*. The PCR amplification system and conditions were the same with those of ITS amplification.

2.6. Detection Sensitivity of the Species-Specific Gene in B. reticulatus. The genomic DNA of *B. reticulatus* was selected as the target, and the sample was diluted tenfold in a gradient of 20, 2, 0.2, 0.02, 0.002, and 0.0002 ng/μL; ddH_2O was used as a negative control. The qualitative and

Table 1: Primers used in qualitative and quantitative PCR.

Primer name	Primer sequence (5′⟶3′)	Length	Product size (bp)	Reference
ITS-F	TCCGTAGGTGAACCTGCGG	19	189	This study
ITS-R	TCCTCCGCTTATTGATATGC	20		
spt5-F	GGTCTTGTTGTGTCTGTTTCGG	22		
spt5-R	TTGCCTACAATGTTTGTGCCA	21		

Table 2: PCR amplification system.

Reagent	Final concentration	Volume (μL)
10× buffer	1×	2.5
dNTPs	0.2 mM	2
Forward primer	0.4 μM	1
Reverse primer	0.4 μM	1
Taq DNA polymerase	2.5 units	0.2
DNA template	5 ng/μL	2
Water		16.3
Total volume		25

quantitative detection was performed to determine the detection sensitivity.

In the qualitative detection, the PCR conditions were the same with those of ITS amplification. The quantitative detection sensitivity of the species-specific gene was evaluated in a final volume of 25 μL. Each reaction mixture contained 1× SYBR Green Mix, 200 nm of each primer, and 2 μL of genomic DNA. Real-time PCR reactions were performed on an ABI Step One Plus detection system (Applied Biosystems, Foster City, USA) with the following program: 2 min at 50°C, 10 min at 95°C, 40 cycles of 30 s at 95°C, 30 s at 60°C, and 30 s at 68°C. Then, melting curve analysis was performed as follows: 15 s at 95°C, 20 s at 60°C, and 95°C at a heating rate of 0.5°C/s. The specificity of the species-specific gene can be verified by checking whether the melting curve has a single peak at the appropriate Tm value. The samples of each biological replicate were quantified, and the process was replicated twice. A standard curve relating the DNA quantities to the detection thresholds (Ct) was constructed.

3. Results and Discussion

3.1. Qualitative Verification of the Extracted Genomic DNA. In this experiment, the genomic DNA of 11 species of wild mushrooms was extracted by using the kits and detected by electrophoresis in 1% agarose gel. The test results are shown in Figure 1(a); although there are no visible bands in some lanes, this does not mean that DNA has not been successfully extracted. The lack of bands in electrophoresis results may be due to the low DNA concentration. As long as the target genomic DNA can be successfully amplified by PCR using universal primers of fungal, the extracted DNA can be used for the subsequent experiments.

The purity of DNA extraction plays a prerequisite role for subsequent PCR reactions. Extracting high-quality DNA is a key step in molecular biology tests. NanoDrop 2000 was used to determine the concentration and purity of the genomic DNA of the 11 wild bacteria, and each sample was repeatedly measured three times. The test results are given in Table 3. In general, the $OD_{260/280}$ values among 1.7–1.9 can be judged as DNA, but the small fragment of degraded DNA cannot be observed in the electrophoretic diagram, and the concentration can still be measured when analyzed the absorbance value. When the $OD_{260/280}$ value is out of the range mentioned above, it may be generated by other substances, such as protein, RNA, or impurities. In this table, the $OD_{260/280}$ of the DNA of these 11 wild bacteria was between 1.7 and 2.0, indicating that the purity of the DNA was relatively high.

The genomic DNA of the 11 species of wild fungi was amplified by PCR using universal primers of fungal ITS rDNA, and the amplification results were detected by 2% agarose gel electrophoresis. The test results are shown in Figure 1(b). The ITS rDNA was effectively amplified from the genomic DNA of the 11 wild fungi, showing electrophoretic bands different from the blank. The bands were clear and tidy, which meets the requirements of PCR experiments. The electrophoretic results of *A. aegirit* (lanes 7-8) were shallow but not absent.

3.2. Selection of Species-Specific Genes in B. reticulatus. A qualified species-specific gene should have a low and constant copy number because low-copy genes tend to be relatively conserved, and their mutation rates are low; meanwhile, it also should exhibit low heterogeneity within the same species. The nucleotide of *B. reticulatus* was searched in NCBI, and 209 results were produced; every DNA sequence was analyzed by BLAST alignment. After the analysis, the *spt5* gene (accession number: LC084605.1, *Boletus reticulatus spt5* genes for *spt5* protein) was selected as the candidate of species-specific gene. As shown in Figure 2, the *spt5* gene exhibits low homology, and no other DNA sequences were aligned.

3.3. Species Specificity Verification of spt5 Gene in B. reticulatus. To verify the species specificity of the *spt5* gene in *B. reticulatus*, the genomic DNA of 11 of mushrooms

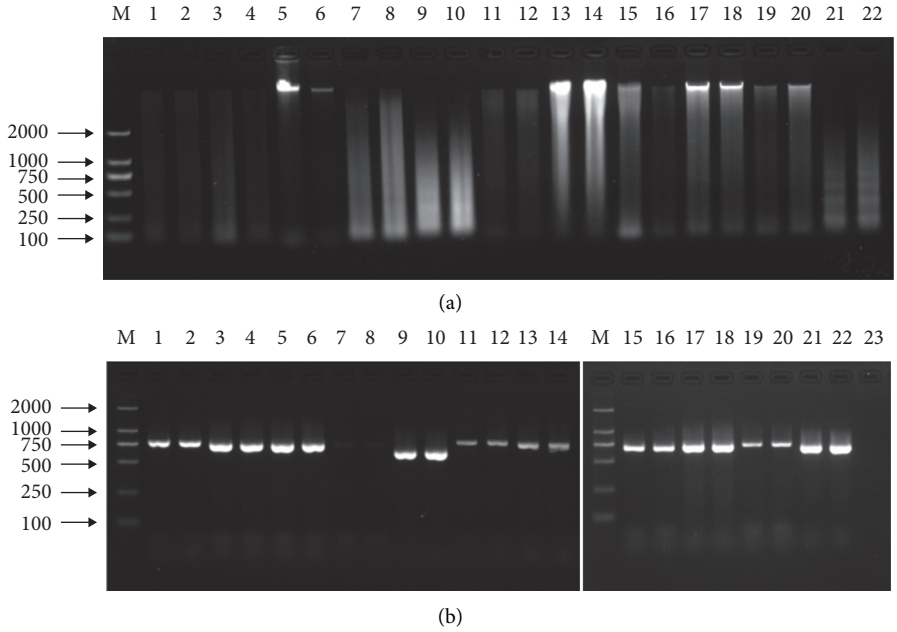

Figure 1: The DNA extraction results by the kit method. (a) The electrophoresis profile of genomic DNA extracted from the 11 tested species of fungi. (b) The electrophoresis profile of the PCR products amplified by ITS primers. M DNA marker DL 2000; lanes 1-2, *Boletus magnificus* W.F.Chiu; 3-4, *Lentinus edodes*; 5-6, *Boletus reticulatus*; 7-8, *Agrocybe aegirit*; 9-10, *Leccinum extremiorientale* (L.Vass.) Singer; 11-12, *Boletus griseus* Frost; 13-14, *Boletus sinicus* W.F.Chiu; 15-16, *Russula virescens*; 17-18, *Collybia albuminosa*; 19-20, *Lactarius volemus*; 21-22, *Pleurotus eryngii*.

Table 3: The purity and concentration of the DNA extracted by the kit method ($\overline{a} \pm$ SD, $n = 3$).

Fungi	OD260/280	Concentration (ng/μL)
Boletus magnificus W.F.Chiu	1.79	24.5 ± 2.5
Lentinus edodes	1.98	31.05 ± 1.7
Boletus reticulatus	1.82 ± 0.01	23.1 ± 2.1
Agrocybe aegirit	1.86	36.3 ± 1.6
Leccinum extremiorientale (L.Vass.) Singer	1.78 ± 0.02	24.8 ± 1.1
Boletus griseus Frost	1.85 ± 0.01	23.6 ± 1.6
Boletus sinicus W.F.Chiu	1.97	33.5 ± 1.2
Russula virescens	1.88	36.7 ± 1
Collybia albuminosa	1.96	39.6 ± 0.6
Lactarius volemus	2.00 ± 0.01	40.8 ± 1.2
Pleurotus eryngii	1.69 ± 0.02	67.2 ± 3.1

was used as samples, and the primer pair *spt5*-F/R was used for common PCR amplification detection. The PCR products were analyzed by 2% agarose gel electrophoresis (containing 0.1 μg/mL EB). The results are shown in Figure 3.

The *spt5* gene was successfully amplified from the genomic DNA of *B. reticulatus*, and the band was obvious, while the other mushrooms did not obtain the amplified products.

3.4. Detection Sensitivity of spt5 Gene in B. reticulatus

3.4.1. Qualitative Detection Results of spt5 Gene in B. reticulatus. To obtain the qualitative detection limit of the *spt5* gene of *B. reticulatus*, the genomic DNA of *B. reticulatus* was series diluted. The amplified products were analyzed by 2% agarose gel electrophoresis, and the result is shown in Figure 4.

When the DNA template contents of *B. reticulatus* were 40, 4, and 0.4 ng, a clear and specific band appeared on the electrophoresis map, and the brightness gradually decreased. When the template content decreased to 0.04 ng, no product was appeared, which indicated that the detection limit of qualitative PCR was 0.4 ng.

3.4.2. Quantitative Detection Results of spt5 Gene in B. reticulatus. The SYBR Green real-time PCR amplification curve and standard curve are shown in Figure 5. In Figure 5(a), the amplification curve still occurred when the template content was as low as 0.04 ng, and the Ct value was significantly lower than the negative control. Thus, the detection limit of SYBR Green quantitative PCR was 0.04 ng.

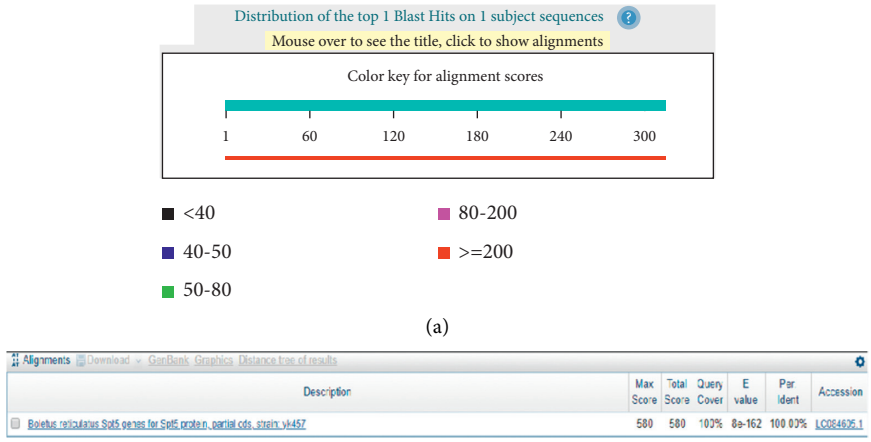

Figure 2: The homology analysis of *spt5* gene. (a) The BLAST analysis of *spt5* in the nucleotide collection database. (b) The detail information about the BLAST result.

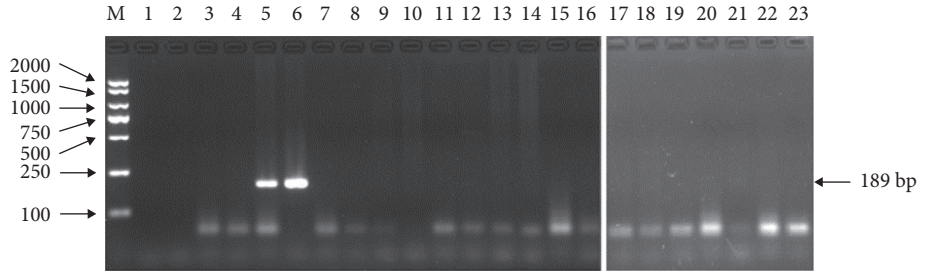

Figure 3: Specificity of the *spt5* gene detected by qualitative PCR. M DNA marker DL 2000; lanes 1-2, *Boletus magnificus* W.F.Chiu; 3-4, *Lentinus edodes*; 5-6, *Boletus reticulatus*; 7-8, *Agrocybe aegirit*; 9-10, *Leccinum extremiorientale* (L.Vass.) Singer; 11-12, *Boletus griseus* Frost; 13-14, *Boletus sinicus* W.F.Chiu; 15-16, *Russula virescens*; 17-18, *Collybia albuminosa*; 19-20, *Lactarius volemus*; 21-22, *Pleurotus eryngii*; 23, negative control.

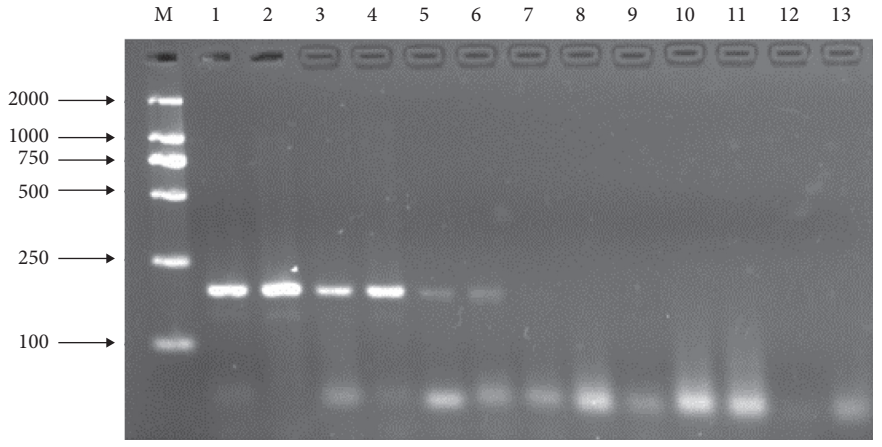

Figure 4: The detection sensitivity of the spt5 gene detection in qualitative PCR. The initial amount of DNA in each PCR reaction is as follows: 1-2, 40 ng; 3-4, 4 ng; 5-6, 0.4 ng; 7-8, 0.04 ng; 9-10, 0.004 ng; 11-12, 0.0004 ng; 13, negative control; M DNA marker DL 2000.

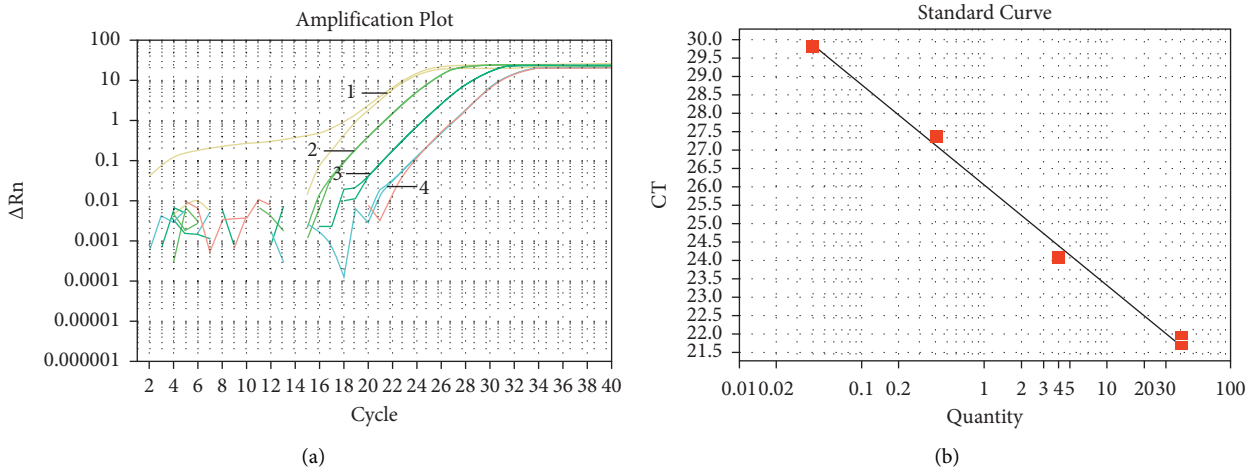

Figure 5: The detection sensitivity of the spt5 gene detection in SYBR Green I quantitative PCR. (a) The final content of DNA in each reaction is as follows: 1, 40 ng; 2, 4 ng; 3, 0.4 ng; 4, 0.04 ng. (b) The standard curve of the quantitative PCR.

As shown in Figure 5(b), we could obtain the linear formula of the Ct value and the content of the DNA template: $Y = -2.728X + 26.048$ (The value range of X is: > 0.04), and the correlation coefficient R^2 was 0.995.

4. Conclusion

In this study, we screened and verified the species-specific gene, *spt5*, of *B. reticulatus*, and the results showed that the *spt5* gene exhibited excellent species specificity, which are suitable for practical use in the qualitative and quantitative monitoring of *B. reticulatus* component. This method showed good specificity and sensitivity, and limit of detection was 0.04 ng of genomic DNA by SYBR Green fluorescence quantitative PCR. The results showed that this method can be used to evaluate the processed products of *B. reticulatus* or other types of products containing small amounts of *B. reticulatus*, and the DNA quantities of it reached the detection thresholds.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31801635) and the Yunnan Education Department Scientific Research Fund Project (2018JS020).

References

[1] G. Jaworska, E. Bernaś, A. Biernacka, and I. Maciejaszek, "Comparison of the texture of fresh and preserved Agaricus bisporus and Boletus edulis mushrooms," *International Journal of Food Science and Technology*, vol. 45, no. 8, pp. 1659–1665, 2010.

[2] I. Janco, M. Snirc, M. Hrstkova, I. Tirdil'Ova, T. Losak, and J. Arvay, "The relationship between risk elements contamination of wild edible mushrooms (Boletus reticulatus schaeff.) and underlying soil substrate," *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 9, pp. 657–660, 2019.

[3] M. Rasalanavho, R. Moodley, and S. B. Jonnalagadda, "Elemental distribution including toxic elements in edible and inedible wild growing mushrooms from South Africa," *Environmental Science and Pollution Research*, vol. 26, no. 8, pp. 7913–7925, 2019.

[4] N. Nikolic, J. Stojanovic, J. Mitrovic, M. Lazic, I. Karabegovic, and G. Stojanovic, "The antioxidant activity and the composition of free and bound phenolic acids in dough of wheat flour enriched by Boletus edulis after mixing and thermal processing," *International Journal of Food Science and Technology*, vol. 51, pp. 2019–2025, 2016.

[5] S. Santoyo, A. C. Ramírez-Anguiano, G. Reglero, and C. Soler-Rivas, "Improvement of the antimicrobial activity of edible mushroom extracts by inhibition of oxidative enzymes," *International Journal of Food Science and Technology*, vol. 44, no. 5, pp. 1057–1064, 2009.

[6] X. Yuan, *Molecular Identification and Phylogeny Research on Commercial Boletes in Yunnan*Yunnan University, 2016.

[7] K Plouznikoff, M. J Asins, H. D De Boulois, E. A Carbonell, and S Declerck, "Genetic analysis of tomato root colonization by arbuscular mycorrhizal fungi," *Annals of Botany*, vol. 124, pp. 933–946, 2019.

[8] J. Zhang, D. Barałkiewicz, A. Hanć, J. Falandysz, and Y. Wang, "Contents and health risk assessment of elements in three edible ectomycorrhizal fungi (boletaceae) from polymetallic soils in yunnan Province, SW China," *Biological Trace Element Research*, vol. 195, no. 1, pp. 250–259, 2020.

[9] J. Gayo and S. A. Hale, "Detection and quantification of species authenticity and adulteration in crabmeat using visible and near-infrared spectroscopy," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 3, pp. 585–592, 2007.

[10] Y Peng, R Gan, H Li et al., "Absorption, metabolism, and bioactivity of vitexin: recent advances in understanding the efficacy of an important nutraceutical," *Critical Reviews in Food Science and Nutrition*, vol. 61, pp. 1–16, 2020.

[11] A. I. Ruiz-Matute, S. Rodríguez-Sánchez, M. L. Sanz, and I. Martínez-Castro, "Detection of adulterations of honey with high fructose syrups from inulin by GC analysis," *Journal of Food Composition and Analysis*, vol. 23, no. 3, pp. 273–276, 2010.

[12] I. Khalil, W. A. Yehye, N. Muhd Julkapli et al., "Dual platform based sandwich assay surface-enhanced Raman scattering DNA biosensor for the sensitive detection of food adulteration," *The Analyst*, vol. 145, no. 4, pp. 1414–1426, 2020.

[13] S. Lohumi, S. Lee, H. Lee, and B.-K. Cho, "A review of vibrational spectroscopic techniques for the detection of food authenticity and adulteration," *Trends in Food Science & Technology*, vol. 46, no. 1, pp. 85–98, 2015.

[14] J. Tian, H. Chu, Y. Zhang et al., "TiO2 nanoparticle-enhanced linker recombinant strand displacement amplification (LRSDA) for universal label-free visual bioassays," *ACS Applied Materials & Interfaces*, vol. 11, no. 50, pp. 46504–46514, 2019.

[15] S. Gianì, V. Di Cesare, F. Gavazzi, L. Morello, and D. Breviario, "Tubulin-based polymorphism genome profiling: a novel method for animal species authentication in meat and poultry," *Food Control*, vol. 110, Article ID 107010, 2020.

[16] Y. Liu, N. Liu, and Y. Zhang, "Research progress of food authentication technology," *Science and Technology of Food Industry*, vol. 37, pp. 374–383+393, 2016.

[17] E. Bonerba, A. Di Pinto, L. Novello et al., "Detection of potentially enterotoxigenic food-related Bacillus cereus by PCR analysis," *International Journal of Food Science and Technology*, vol. 45, no. 6, pp. 1310–1315, 2010.

[18] K. Hong, "Application of nucleic ACID sequence analysis in fungi taxonmy," *Journal of Tropical Biology*, vol. 2, pp. 39–43, 2006.

[19] G. P. Danezis, A. S. Tsagkaris, F. Camin, V. Brusic, and C. A. Georgiou, "Food authentication: techniques, trends & emerging approaches," *TRAC Trends in Analytical Chemistry*, vol. 85, pp. 123–132, 2016.

[20] W. Powell, M. Morgante, C. Andre et al., "The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis," *Molecular Breeding*, vol. 2, no. 3, pp. 225–238, 1996.

[21] J. Welsh and M. Mcclelland, "Fingerprinting genomes using PCR with arbitrary primers," *Nucleic Acids Research*, vol. 18, no. 24, pp. 7213–7218, 1990.

[22] Y Xu, W Xiang, Q Wang et al., "A smart sealed nucleic acid biosensor based on endogenous reference gene detection to screen and identify mammals on site," *Scientific Reports*, vol. 7, pp. 43453–43510, 2017.

[23] X. Liu, J. Guo, S. Wang, Y. Li, and R. Na, "Identification of several edible mushrooms in Inner Mongolia by rDNA-ITS," *Edible and Medicinal Mushrooms*, vol. 23, pp. 301–306, 2015.