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Loop-mediated isothermal amplification (LAMP) for rapid and easy identification of *Omphalotus japonicus*

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ABSTRACT

Omphalotus japonicus is a major toxic mushroom in Japan. When food poisoning caused by *O. japonicus* occurs, quick and accurate identification using a method that does not rely on morphological discrimination is required. Because the loop-mediated isothermal amplification (LAMP) method meets these requirements, we developed a LAMP method for detecting *O. japonicus*. Amplification occurred within 60 min, and the presence or absence of *O. japonicus* was confirmed within 2 h, including the DNA extraction protocol. The LAMP method did not show cross-reactivity with 13 species of edible mushrooms, had high specificity toward *O. japonicus*, and had sufficient detection sensitivity even in a mixed mushroom sample containing 1% *O. japonicus*. Additionally, *O. japonicus* could be detected in simulated food poisoning samples of heated and digested mushrooms, and in actual food poisoning residual samples.

1. Introduction

Many cases of mushroom food poisoning are caused by accidental ingestion because of the close resemblance of poisonous mushrooms to edible mushrooms. For example, *Omphalotus japonicus* and *Entoloma rhodophylla* are poisonous mushrooms that are often accidentally consumed, accounting for more than half of the food poisoning cases caused by mushrooms in Japan (Toda et al., 2012). In most food poisoning cases caused by *O. japonicus*, the mushrooms are mistaken for *Lentinula edodes*, *Pleurotus ostreatus*, and *Panellus edulis*, which are morphologically similar.

When food poisoning caused by *O. japonicus* occurs, it is difficult to morphologically identify the causative mushroom from a small amount of the causative food or vomitus. Therefore, a quick and accurate identification method that does not rely on morphological discrimination is required. The main toxic component of *O. japonicus* is illudin S (Nakanishi et al., 1965; Tada et al., 1964), which if ingested can cause toxic gastrointestinal symptoms, such as vomiting, diarrhea, and abdominal pain, after approximately 30 min to 1 h. Therefore, chemical methods for detecting illudin S using GC/MS or LC/MS/MS, for example, have been reported (Kanamori-Kataoka et al., 2006; Kasahara & Itou, 2009). Identification of *O. japonicus* using a molecular biological method in addition to a chemical discrimination method will increase the reliability of any results. Mushrooms are generally classified using molecular biology techniques by analyzing the nucleotide sequence information of the internal transcribed spacer (ITS) region (Kirchmair et al., 2004; Matheny et al., 2006). Analysis of the ITS region of *O. japonicus* is one of the most reliable identification methods (Schoch et al., 2012); however, it takes several days to weeks from DNA extraction to nucleotide sequence analysis. To overcome this limitation, a method for detecting *O. japonicus* using PCR–RFLP (Sugano et al., 2017) or real-time PCR (Maeta et al., 2008; Tsuruda et al., 2012), which can identify the organism within a shorter time than nucleotide sequence analysis, has been reported.

We have been working on constructing a rapid identification method for *O. japonicus* using loop-mediated isothermal amplification (LAMP). This method amplifies DNA under isothermal conditions of 60 °C–65 °C, and its amplification efficiency is reportedly 100 to 1000 times that of PCR (Notomi et al., 2000). The amplification reaction can be performed using a simple device, and the thermal cycler used for PCR can be used as

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a substitute. Additionally, the LAMP method is less sensitive to polymerase inhibitors than PCR (Zhang et al., 2019), and the target gene can be amplified even with a simple DNA extraction method. LAMP method amplification can be confirmed within a short time (≤60 min), and the presence or absence of amplification can be visually confirmed by adding a fluorescent detection reagent to the reaction solution, which avoids the need for agarose gel electrophoresis. Recently, battery-powered portable LAMP amplifiers have been become commercially available, and the LAMP method has been performed on-site. Against this background, this method has been further developed to detect various nucleic acids, such as viruses, bacteria, plants, and mushrooms (Kurosaki et al., 2007; Misawa et al., 2021; Ravan et al., 2016; Vaagt et al., 2013). In this study, we developed a method for identifying *O. japonicus* using the LAMP method to quickly identify the causative mushroom in food poisoning cases.

2. Materials and methods

2.1. Materials

The experimental samples used in this study are shown in Table 1. Samples of *O. japonicus* were collected in Yamagata and Shimane prefectures in Japan. Samples from cases of food poisoning with *O. japonicus* were collected at public health centers in Akita and Yamagata. Samples of edible mushrooms sold in Japan, including *L. edodes*, *P. ostreatus*, and *P. edulis,* were also collected. To prepare mixed samples, edible mushrooms and *O. japonicus* were homogenized separately in a food processor (MK-K78; Panasonic, Tokyo, Japan). Then, the homogenized *O. japonicus* was added to the homogenized edible mushrooms at 1%–100%(w/w) (Table S1).

2.2. DNA extraction

DNA was extracted for gene analysis and the LAMP method was performed using a DNeasy Plant Mini kit (Qiagen, Hilden, Germany). Each sample was washed to avoid staining with distilled water. First, 200 μL of AP1 buffer was added to 100 mg of the sample, and the mixture was crushed with a BioMasher II homogenizer (Nippi, Tokyo, Japan). Next, 400 μL of AP1 buffer and RNase A 4 μL were added and the mixture was incubated at 65 °C for 15 min. P3 buffer (260 μL) was added and the sample was allowed to stand on ice for 10 min before centrifuging at room temperature at 14,000×*g* for 10 min. The supernatant was loaded on a QIAshredder spin column and centrifuged at room temperature at 14,000×*g* for 1 min, and the eluent was collected. Then, AW1 buffer was added at 1.5 times the volume of the eluent. Next, 650 μL of the sample was loaded onto the mini spin column and it was centrifuged at 10,000×*g* for 1 min at room temperature. The same operation was repeated until the entire solution was loaded. For washing, 500 μL of AW2 buffer was loaded on the mini spin column and it was centrifuged at 10,000×*g* at room temperature for 1 min. This washing operation was performed twice.

Furthermore, the mini spin column was centrifuged at room temperature at 10,000×*g* for 15 min and then transferred to a new centrifuge tube. After adding 40 μL of TE(pH 8.0)(Nippon gene, Tokyo, Japan) to the mini spin column and letting it stand for 5 min, it was centrifuged at room temperature at 10,000×*g* for 1 min to recover the eluent. The same procedure was repeated to obtain a total of 80 μL of DNA extract. The obtained DNA extract was prepared such that the DNA concentration was quantified to be 10 ng/μL using NanoDrop One. Additionally, for rapid and easy DNA extraction, 400 μL of PrepMan Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, MA) was added to 100 mg of the mushroom sample (20 mg of the dry sample), and the mixture was crushed using the BioMasher II. After heating at 100 °C for 10 min, the resultant mixture was centrifuged at room temperature (13,000×*g*) for 2 min, and the supernatant was used as a DNA extract.

2.3. Gene analysis and primer design

To analyze the nucleotide sequence of the ITS region of the mushroom samples, PCR was performed using ITS Universal Primers ITS 1 (White et al., 1990) and ITS 4B (Gardes & Bruns, 1993) using KOD FX DNA polymerase (Toyobo, Osaka, Japan). The forward primer (ITS1) was 5′-CTTGGTCATTTAGAGGAAGTAA-3′, and the reverse primer (ITS 4B) was 5′-TCCTCCGCTTATTGATATGC-3′.

The PCR products were sequenced using the BigDye 3.1 kit and loaded on a 3500xL Genetic Analyzer (Thermo Fisher Scientific). The obtained nucleotide sequence was analyzed using GENETYX ver 13 (Genetyx, Tokyo, Japan) and BLAST. The nucleotide sequence characteristics of *O. japonicus* were selected by comparing the nucleotide

Table 1
Omphalotus japonicus and edible mushrooms used in this study.

ID	Japanese name	Species	Locality	Date of collection	Remarks
OJ-1	Tsukiyotake	*Omphalotus japonicus*	Shimane, Japan	2008/Oct.	Freeze-dried fruiting body isolated from the field
OJ-2			Yamagata, Japan	2006/Oct.	Freeze-dried fruiting body isolated from the field
OJ-p1			Yamagata, Japan	2015/Oct.	Frozen fruiting body cooked in miso soup that caused food poisoning
OJ-p2			Yamagata, Japan	2015/Nov.	Frozen fruiting body cooked in oil that caused food poisoning
OJ-p3			Yamagata, Japan	2015/Sep.	Frozen fruiting body that caused food poisoning
OJ-p4			Yamagata, Japan	2015/Sep.	Frozen fruiting body that caused food poisoning
OJ-p5			Akita, Japan	2015/Oct.	Frozen fruiting body that caused food poisoning
OJ-p6			Akita, Japan	2015/Oct.	Frozen fruiting body that caused food poisoning
OJ-p7			Akita, Japan	2015/Oct.	Frozen fruiting body that caused food poisoning
LE-1	Shiitake	*Lentinula edodes*	Hokkaido, Japan	2013/May.	Commercial cultivar
LE-2			Hokkaido, Japan	2013/Nov.	Commercial cultivar
PO-1	Hiratake	*Pleurotus ostreatus*	Hokkaido, Japan	2013/Jun.	Commercial cultivar
PO-2			Hokkaido, Japan	2013/Nov.	Commercial cultivar
PE-1	Mukitake	*Panellus edulis*	Saga, Japan	2014/Feb.	Commercial cultivar
HM	Bunasimeji	*Hypsizygus marmoreus*	Niigata, Japan	2013/Nov.	Commercial cultivar
GF-1	Maitake	*Grifola frondosa*	Hokkaido, Japan	2013/Dec.	Commercial cultivar
GF-2			Niigata, Japan	2013/Dec.	Commercial cultivar
PEr	Eringi	*Pleurotus eryngii*	Hokkaido, Japan	2013/Dec.	Commercial cultivar
AB-1	Mushroom (Tsukuritake)	*Agaricus bisporus*	Hokkaido, Japan	2013/Dec.	Commercial cultivar
AB-2			Hokkaido, Japan	2013/Dec.	Commercial cultivar
PN	Nameko	*Pholiota nameko*	Ibaraki, Japan	2013/Nov.	Commercial cultivar
FV	Enoki	*Flammulina velutipes*	Hokkaido, Japan	2013/Dec.	Commercial cultivar
PC	Tamogitake	*Pleurotus citrinopileatus*	Hokkaido, Japan	2013/Dec.	Commercial cultivar
ES	Urabenihoteisimeji	*Entoloma sarcopum*	Fukushima, Japan	2012/Oct.	Fruiting body isolated from the field

sequences of the ITS regions of *O. japonicus*, *L. edodes*, *P. ostreatus*, and *P. edulis*. The primers for the LAMP method that specifically recognize *O. japonicus* were designed using PrimerExplorer V5 (Table 2).

2.4. LAMP method

The LAMP method was performed using a Loopamp DNA amplification kit (Eiken Chemical, Tokyo, Japan). When it was necessary to confirm the amplification visually, the Loopamp fluorescent detection reagent (Eiken Chemical) was added to the reaction solution. The composition of the reaction solution was as follows: 10 ng of template DNA, 1 × reaction mix, 5 pmol of F3 primer, 5 pmol of B3 primer, 40 pmol of FIP primer, 40 pmol of BIP primer, 20 pmol of LF loop primer (optional), 20 pmol of LB loop primer (optional), 1 μL of fluorescent detection reagent (optional), and 1 μL of *Bst* DNA polymerase. The total volume was 25 μL. The amplification reaction was maintained at 63 °C for 1 h and then at 80 °C for 5 min to inactivate the enzyme. A turbidity measurement apparatus (LA-320C, Eiken Chemical) was used to confirm amplification in real-time. A fluorescent detection reagent was added to the reaction solution confirm amplification according to the presence or absence of fluorescence in the reaction solution after completion of the reaction under visible light and UV irradiation.

2.5. Construction of a positive control plasmid

As a positive control, a plasmid containing the ITS region of the *O. japonicus* was created. The ITS region of the *O. japonicus* amplified by PCR was inserted into pCR2.1-TOPO (Thermo Fisher Scientific) using the TOPO TA cloning Kit (Thermo Fisher Scientific) and cloned in the *Escherichia coli* Top10 strain. Plasmids were collected using the QIAprep Spin Miniprep Kit (Qiagen).

2.6. Heating and digestion treatment to simulate food poisoning

To identify the causative food of food poisoning, mushrooms were heated and subjected to artificial digestion, and detection of *O. japonicus* was attempted using the LAMP method. First, 500 μL of distilled water was added to 100 mg of the mushroom sample. This was followed by heating at 100 °C for 30 min and centrifuging at room temperature (6,000×*g*) for 1 min, after which the supernatant was removed. Next, 500 μL of Disintegration Test Solution 1 (pH 1.2; Nacalai Tesque, Kyoto, Japan) was added and the mixture was incubated at 37 °C for 1 h, followed by centrifugation at room temperature at 6,000×*g* for 1 min to remove the supernatant. For washing, distilled water was added and the mixture was centrifuged at room temperature at 6,000×*g* for 1 min, after which the supernatant was removed. The washing operation was repeated twice. DNA was extracted from the remaining precipitate and used as a sample for the LAMP method. PrepMan Ultra Sample Preparation Reagent (Thermo Fisher Scientific) was used as a simple DNA extraction reagent for DNA extraction. For the simple DNA extraction reagent, 1 μL was used as a template in the reaction solution of the LAMP method.

3. Results and discussion

3.1. Design of primers for LAMP to detect O. japonicus

The nucleotide sequences of the ITS region in *O. japonicus*, *L. edodes*, *P. ostreatus*, and *P. edulis* were compared to design primers for the LAMP method that could specifically recognize the characteristic sequence of *O. japonicus*. The ITS region is expected to have improved detection sensitivity because many copies are present in the genome. Additionally, databases of the ITS region are substantial, making it possible to design primers with high versatility. Primer design for the LAMP method was performed using PrimerExplorer V5. Primers that could amplify the target region (about 230 bp) in the ITS 2 region of *O. japonicus* were selected. Because the LAMP method has the property of being less susceptible to polymerase inhibitors (Zhang et al., 2019a, 2019b), this method targets a relatively short region of approximately 230 bases. To confirm the selectivity of the LAMP method for *O. japonicus* detection, we analyzed DNA extracted from *O. japonicus* and edible mushrooms (*L. edodes*, *P. ostreatus*, and *P. edulis*) (Fig. 1a). Clear amplification was confirmed only for *O. japonicus* and occurred approximately 35 min after the start of the reaction (Fig. 1b). The amplification could be determined both from the increase in turbidity (measured using the LA-320C) and the presence or absence of green fluorescence under visible and UV light with the addition of Loopamp fluorescent detection reagent (Fig. 1 c and d). To confirm that the LAMP did not show non-specific amplification, we analyzed DNA extracted from various edible mushrooms (13 species). None of the edible mushrooms showed amplification, whereas the DNA derived from *O. japonicus* showed clear amplification (Fig. S1). These results confirmed that this method had high selectivity for *O. japonicus* and no cross-reactivity with edible mushrooms.

3.2. Change in amplification rate using a loop primer

A loop primer with a sequence complementary to the single-stranded portion of the loop (between B1 and B2 or between F1 and F2) was added to improve the amplification rate by increasing the number of origin sites of DNA synthesis in the LAMP method. Loop primers were designed for the LAMP method for *O. japonicus* detection. The influence of the presence or absence of a loop primer on the amplification rate was confirmed using a plasmid (positive control plasmid) incorporating the ITS region of *O. japonicus*. With a loop primer, amplification started approximately 10–18 min earlier than without a loop primer, and the total determination time was approximately 40 min (Fig. S2). Furthermore, the detection sensitivity improved. Without loop primer (Fig. 2a), amplification was confirmed in only one sample (10^4 copies of the sample reacted in triplicate). With loop primer (Fig. S2b), amplification was confirmed in all 10^4 copies. Because the detection sensitivity was improved using the loop primer, there was no non-specific amplification for edible mushrooms and the high *O. japonicus* selectivity was maintained (Fig. 2).

3.3. Detection of mixed samples of O. japonicus

Mixed samples containing 1%–100%(w/w) *O. japonicus* and edible mushrooms were prepared and analyzed by the LAMP method for

Table 2
Primers for detecting O. japonicus for use in LAMP.

Primer	Sequence
F3 primer	5′-GAAGCTTGGACTGTGGAG-3′
B3 primer	5′-GTGAAAACAGACGATTAGAGAG-3′
FIP primer	5′-ACACCAAGGCTTAGGTCCGAACTAGATGTTCTCAGCTCCT-3′
BIP primer	5′-ATCTACGCCTTGGTGGTTTGATTTGAAATGAAAGCAGACAGA-3′
LF loop primer (optional)	5′-TAATCCGGTTTCCGCTAATGC-3′
LB loop primer (optional)	5′-CTCTTTGGTTGGGATAGCTGCAAC-3′

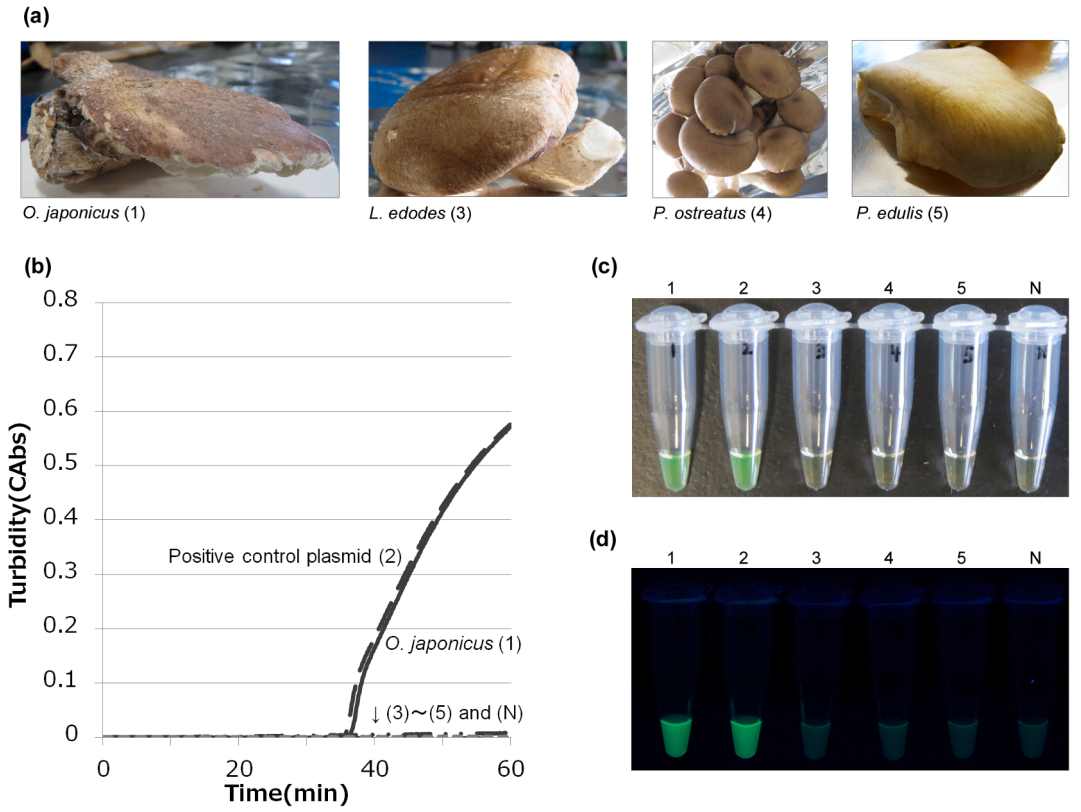

Fig. 1. The LAMP method for *O. japonicus* detection using mushrooms (a), and a real-time turbidity measurement apparatus (LA-320C) (b), or under visible light (c), and UV irradiation (d) with addition of a fluorescent detection reagent. For the LAMP method, *O. japonicus* (1), *L. edodes* (3), *P. ostreatus* (4), and *P. edulis* (5) were used as samples. A plasmid with ITS regions of *O. japonicus* was used as a positive control (2), and water was used as the NTC (N).

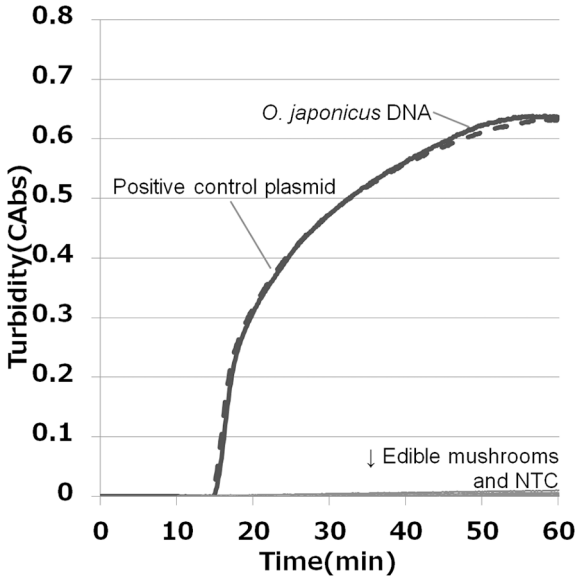

Fig. 2. The LAMP method with a loop primer for detecting *O. japonicus* using a real-time turbidity measurement apparatus (LA-320C) to confirm cross-reactivity with edible mushrooms. The LAMP method using a loop primer was performed on *O. japonicus* and the following edible mushrooms: *L. edodes*, *P. ostreatus*, *P. edulis*, *H. marmoreus*, *G. frondosa*, *P. eryngii*, *A. bisporus*, *P. nameko*, *F. velutipes*, *P. citrinopileatus*, and *E. sarcopum* (Table 1).

O. japonicus detection (Table S1). Amplification was confirmed in all samples containing up to 1% *O. japonicus* (Fig. S3). Therefore, this method can be applied to samples that contain multiple species of mushrooms to confirm the presence or absence of *O. japonicus*.

3.4. Analysis of food poisoning samples of *O. japonicus*

To evaluate detection of *O. japonicus* in food poisoning samples, heated and digested mushroom samples were subjected to the LAMP method (Fig. S4a). For this method, a DNA solution was obtained from heated and digested mushrooms [*O. japonicus* (two samples), *L. edodes*, *P. ostreatus*, and *P. edulis*] and extracted rapidly and easily using PrepMan Ultra Sample Preparation Reagent. When food poisoning occurs, speed is of the essence when identifying the cause for determining treatment. With this in mind, we extracted DNA using the PrepMan Ultra Sample Preparation Reagent, which is rapid and easy. Amplification was confirmed only with *O. japonicus*, and *O. japonicus* could be detected even in residual foodstuffs and vomitus when food poisoning occurred. Therefore, the LAMP method was performed on remnants of samples of from food poisoning cases caused by *O. japonicus* (Fig. S4b). The LAMP method was conducted by heating and digesting the sample and then rapidly and easily performing DNA extraction. An increase in the *O. japonicus* gene in the LAMP method was observed in all samples. For sample OJ-p2, previously reported PCR–RFLP, real-time PCR, and ITS sequencing results (Sugano et al., 2017) identified the causative mushroom was *P. edulis*. The *P. edulis* was cooked with *O. japonicus*, and we considered that amplification of this sample detected a trace amount of *O. japonicus* DNA. This result shows that the detection ability of the LAMP method is high. In addition, with the rapid and easy DNA extraction method, it is possible to prepare a sample in approximately 30 min. Combined with the 60 min reaction time of the LAMP method, this allows for the presence or absence of *O. japonicus* to be determined within 2 h. Therefore, the LAMP method is much faster than PCR–RFLP or real-time PCR for *O. japonicus* detection.

4. Conclusion

The LAMP method detected only *O. japonicus* and false positives were not observed for many edible mushrooms, including *L. edodes*, *P. ostreatus*, and *P. edulis*. Furthermore, using the loop primer, amplification was achieved in a short reaction time of approximately 40 min. When combined with rapid and simple extraction of DNA, it was possible to identify *O. japonicus* within 2 h. It is expected that this method could be reliably used to identify the cause of food poisoning cases in which *O. japonicus* is suspected. In the near future, as the availability of portable LAMP amplifiers improves, application of the LAMP method at mushroom collection sites could be used to identify *O. japonicus* before consumption. This could actively prevent food poisoning cases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2022.100115.

References

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology, 2*(2), 113–118.

Kanamori-Kataoka, M., Seto, Y., & Kuramoto, M. (2006). Development of a method for determining illudin S in food by gas chromatography-mass specrtometry. *Journal of Health Science, 52*, 237–242.

Kasahara, Y., & Itou, T. (2009). Determination of illudin S in Omphalotus guepiniformis and foods that caused food poisoning by liquid chromatography with tandem mass spectrometry. *Shokuhin Eiseigaku Zasshi (Food Hyg. Saf. Sci.), 50*, 167–172.

Kirchmair, M., Morandell, S., Stolz, D., Pöder, R., & Sturmbauer, C. (2004). Phylogeny of the genus Omphalotus based on nuclear ribosomal DNA-sequences. *Mycologia, 96*, 1253–1260.

Kurosaki, Y., Takada, A., Ebihara, H., Grolla, A., Kamo, N., Feldmann, H., … Yasuda, J. (2007). Rapid and simple detection of Ebola virus by reverse transcription-loop-mediated isothermal amplification. *Journal of Virological Methods, 141*, 78–83.

Maeta, K., Ochi, T., Tokimoto, K., Shimomura, N., Maekawa, N., Kawaguchi, N., … Aimi, T. (2008). Rapid species identification of cooked poisonous mushrooms by using real-time PCR. *Applied and Environment Microbiology, 74*, 3306–3309.

Matheny, P. B., Curtis, J. M., Hofstetter, V., Aime, M. C., Moncalvo, J. M., Ge, Z. W., … Hibbett, D. S. (2006). Major clades of Agaricales: A multilocus phylogenetic overview. *Mycologia, 98*, 982–995.

Misawa, S., Natsuhara, D., Kiba, Y., Yamamuro, T., Suzuki, R., Shibata, T., & Kitamura, M. (2021). Rapid identification of *Colchicum autumnale* based on loop-mediated isothermal amplification (LAMP) assay. *Forensic Toxicology, 39*(1), 259–265.

Nakanishi, K., Ohashi, M., Tada, M., & Yamada, Y. (1965). Illudin S (lampterol). *Tetrahedron, 21*, 1231–1246.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic acids research, 28*(12), e63–e.

Ravan, H., Amandadi, M., & Sanadgol, N. (2016). A highly specific and sensitive loop-mediated isothermal amplification method for the detection of *Escherichia coli* O157: H7. *Microbial Pathogenesis, 91*, 161–165.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., Chen, W., & Fungal Barcoding Consortium, and Fungal Barcoding Consortium Author List. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. *Proceedings of the National Academy of Sciences of the United States of America, 109*(16), 6241–6246.

Sugano, Y., Sakata, K., Nakamura, K., Noguchi, A., Fukuda, N., Suzuki T., Kondo, K., (2017) Rapid Identification Method of *Omphalotus japonicus* by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). *Shokuhin Eiseigaku Zassi* (Food Hyg. Saf. Sci.), 58, 113-23.

Tada, M., Yamada, Y., Bhacca, N. S., Nakanishi, K., & Ohashi, M. (1964). Structure and reactions of illudin S (lampterol). *Chemical & Pharmaceutical Bulletin, 12*, 853–855.

Toda, M., Uneyama, C., Toyofuku, H. Morikawa, K. (2012). Trends of food poisonings caused by natural toxins in Japan, 1989-2011. *Shokuhin Eiseigaku Zassi (Food Hyg. Saf. Sci.)*, 53, 105-20.

Tsuruda, S., Akaki, K., Hiwaki, H., Suzuki, A., & Akiyama, H. (2012). Multiplex real-time PCR assay for simultaneous detection of Omphalotus guepiniformis and Lentinula edodes. *Bioscience, Biotechnology, and Biochemistry, 76*, 1343–1349.

Vaagt, F., Haase, I., & Fischer, M. (2013). Loop-mediated isothermal amplification (LAMP)-based method for rapid mushroom species identification. *Journal of agricultural and food chemistry, 61*(8), 1833–1840.

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications, 18*(1), 315–322.

Zhang, H., Xu, Y., Fohlerova, Z., Chang, H., Iliescu, C., & Neuzil, P. (2019). LAMP-on-a-chip: Revising microfluidic platforms for loop-mediated DNA amplification. *Trends in Analytical Chemistry: TRAC, 113*, 44–53.