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Research Article

# DNA Extraction from Oyster Mushrooms Fruiting Bodies without Mycelial Activation Process

**Nirutcha Suriya, Rutthaporn Chandet*, Parinyanuch Pinnil,**
**Tipsuda Tangtragoon, Chindanai Aurfurphan**
Faculty of Science, Maejo University, Nong Han, San Sai, Chiang Mai, Thailand
*Corresponding author E-mail: auanmolec@gmail.com

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## Abstract

**Background and Objectives:** Oyster mushrooms (*Pleurotus* spp.) are economically valuable mushrooms with high market demand. They are popularly consumed worldwide due to their highly palatable taste and numerous medicinal properties. Despite the many benefits of oyster mushrooms, information regarding the genetic diversity of these mushrooms is limited; investigation into their diversity is of utmost importance. While molecular marker techniques, along with the study of phenotypes, are popular approaches, the use of these techniques necessitates the use of high-quality DNA. However, the process of stimulating mycelium growth is complex and time-consuming. A direct DNA extraction method from oyster mushroom fruiting bodies was therefore developed in this present study.

**Methodology:** For the method of DNA extraction from oyster mushrooms, we adjusted the composition of the extraction reagents to suit the samples by modifying the original extraction methods. The quality and quantity of the DNA were assessed using a Nanodrop Lite spectrophotometer, while the DNA quality was evaluated in reactions using ITS and HAT-RAPD techniques.

**Main Results:** The results demonstrated clear and high-quality DNA band patterns, enabling the identification and classification of oyster mushroom species.

**Conclusions:** The direct DNA extraction method from oyster mushrooms, using a modified approach, demonstrated that the extracted DNA was of high quality and purity, suitable for PCR reactions. Furthermore, when the DNA quality was analyzed for classification using ITS and HAT-RAPD techniques, clear and appropriately sized bands were obtained.

**Practical Application:** This modified extraction method allows for rapid direct DNA extraction from oyster mushroom fruiting bodies. It is also suitable for rare, deteriorated and limited quantity samples. Consequently, this method can be used to investigate the genetic diversity of oyster mushroom strains.

**Keywords:** Oyster Mushrooms, Molecular Marker, ITS, HAT-RAPD, DNA

## Introduction

Currently, there are more than 50 species of mushrooms that have been reported to possess bioactive compounds with relevance to the human immune system. The 10 species of mushrooms that have the most beneficial and demand, which have been extensively studied for their potential uses, include *Agaricus subrufescens, Cordyceps sinensis, Ganoderma lucidum, Grifola frondose, Hericium erinaceus, Inonotus obliquus, Lentinula edodes, Pleurotus ostreatus, Poria cocos, Trametes versicolor* [1].

Oyster mushrooms are economically important mushrooms with a relatively high market value. It is widely consumed both in Thailand and internationally. Oyster mushrooms can be classified into two groups, namely those with white and gray caps, whose key characteristics include a smooth cap surface, a central concave on the cap, and slightly rolled-in edges when the mushrooms are fully mature, the underside of the cap has gills, moderate stalks and continuous, homogeneous to cap.

Additionally, oyster mushrooms are rich in nutritional value. Eventually, the chemical components of the edible portion of the oyster mushrooms 100 g have 24 Kcal and contain protein, carbohydrates, lipid, calcium, and fiber of 3.6 g., 7.4 g., 0 g., 1 g., and 4.3 g. respectively.

Additionally, it was found to be rich in β-glucan, a polysaccharide compound consisting of ß-glycosidic linkages, commonly found in mushrooms, yeast, oats, barley, and certain bacteria [2]. Nowadays, the genus Oyster (Pleurotus spp.) are acknowledged to be β-glucans source. [3]

β-glucan has been reported to possess immunomodulatory properties, reduce cholesterol levels in blood, and exhibit anti-cancer effects against various types of cancer cells. In terms of biological activity, b-1,3-D-glucans and b-1,6-D-glucans, which are contained in Oysters are the most effective. [4].

Although oyster mushrooms are economically valuable mushrooms known for their high nutritional value. However, information regarding the genetic diversity of these strains in Thailand is limited. Therefore, it is crucial to accurately identify the strains before using them for quality control of utilization as raw materials before processing them into products.

Currently, molecular markers have been applied in the study and classifying of organisms for considering the morphology of samples. To classify oyster mushrooms by molecular markers is particularly essential to require high-quality DNA. In most cases, DNA is extracted from mycelium [5]. Due to its simple method which yields high-quality DNA. However, the preparation of mycelium for DNA extraction involves complex and time-consuming steps.

The research investigated the genome sequence of oyster mushroom strain P9 using mycelium cultivated on two different growth media: yeast extract, malt extract, and glucose medium (YMG) and yeast nitrogen base (YNB) for 7 and 3 days [6]. Subsequently, the mycelium was extracted to obtain genomic DNA for further study and research.

A similar study analyzed the genome sequence of P. pulmonarias' using genomic DNA extracted from mycelium grown on YMG medium for a total of 3 days [7]. While the research examined the DNA barcode of 123 different strains of oyster mushrooms. Genomic DNA was extracted from mycelium cultivated on potato dextrose agar (PDA) medium [8].

So, if it were possible to directly extract DNA from the mushrooms fruiting bodies, it would significantly reduce the preparation steps and time required for analysis. Therefore, this study aims to develop a method for extracting genomic DNA directly from the oyster mushrooms' fruiting bodies, referencing the technique proposed by Park, M., et al. [9] due to this method is simple, with uncomplicated extraction steps, and yields high-quality DNA for analyzing the genetic diversity of oyster mushrooms using molecular marker techniques.

## Materials and Methods

### DNA extraction from oyster mushrooms fruiting bodies by using a modified method

Oyster mushroom samples, obtained from The Huai Hong Khrai Royal Development Study Centre, were initially cleaned by rinsing with tap water and subsequently air-dried. Subsequently, DNA extraction from oyster mushrooms fruiting bodies was performed using a modified method. [9] Which, the modification above method eliminates the mycelium stimulation step, thus reducing the sample preparation time and DNA extraction process.

Initially, oyster mushrooms fruiting body samples weighing 0.1 grams were ground with liquid nitrogen and transferred to a 1.5 mL test tube. Then, 500 μL of extraction buffer was added to the sample, and mixture was homogenized. The homogenized sample was then incubated at 65°C for 60 minutes, followed by centrifugation at 10,000 x g for 15 minutes at 4°C. The supernatant was carefully collected and transferred to a new 1.5 mL test tube. Then, a mixture of isoamyl alcohol, chloroform, and phenol in a ratio of 1:24:25 was added total volume of 500 μL. After that, inverted several times and centrifuged at 10,000 x g for 5 minutes at 4°C Then, repeated once.

The supernatant was carefully collected and transferred to a new 1.5 mL test tube. Then, mixture of isoamyl alcohol and chloroform in a ratio of 1:24 was added total volume 500 μL. After that, inverted several times and left at room temperature for 2-3 minutes. Subsequently, the mixture was centrifuged at 10,000 x g for 5 minutes at 4°C. The supernatant was carefully collected and transferred to a new 1.5 mL test tube. Then, absolute ethanol was added to 2 volumes of supernatant for precipitate the DNA.

Additionally, 5 M sodium acetate was added at 10% of the supernatant for enhance DNA precipitation. After that, inverted several times and incubated at -20°C for 8-24 hours. After incubation, the mixture was centrifuged at 10,000 x g for 10 minutes at 4°C and then, discarded the solutions and the DNA pellet was washed with 1 mL of 75% ethanol.

The DNA pellet was centrifuged at 10,000 x g for 1 minute at 4°C. Then, repeated once. After washing, the DNA pellet was air-dried for 20-30 minutes at 25°C and then dissolved in 30 μL of distilled water that had been sterilized. The quality and concentration of the extracted DNA from oyster mushroom fruiting bodies were assessed by using a Nanodrop Lite spectrophotometer (Thermo Scientific, USA). Subsequently, the DNA concentration was adjusted to a range of 5-10 ng/μL for optimal quality test for PCR reactions in the next step.

**Figure 1** Oyster mushrooms powder

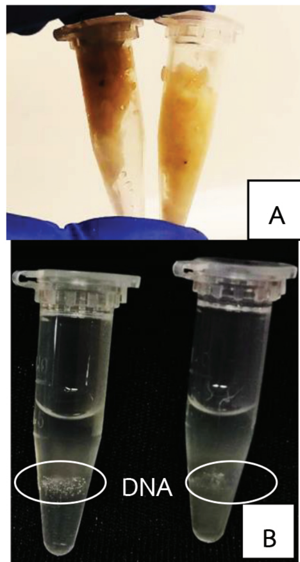


**Figure 2** Pre-extraction DNA sample (A) Post-extraction DNA sample (B) of the Oyster mushrooms ample

**Quality and quantification analysis of extracted oyster mushrooms DNA by using a modified method**

The quantity and quality of oyster mushroom DNA extracted using a modified method were examined. The absorbance values at 260 and 280 nm. were measured using a Nano-drop Lite spectrophotometer (Thermo Scientific, USA) to assess the light absorbance of proteins and nucleic acids. The DNA quality was further evaluated by electrophoresis by using a 1% agarose gel, comparing the band size with lambda/*PstI* ladder DNA standard. Electrophoresis was performed in a 1X TBE (Tris-Borate-EDTA) buffer at 100V. and 400mA. for 60 min. The gel was then stained with ethidium bromide and visualized under UV light at a wavelength of 254 nm.

**Quality analysis of DNA from oyster mushrooms by using Internal Transcribed Spacer (ITS).**

Oyster mushroom extracts obtained from the modified extraction method were subjected to amplification of the ITS region. The components used for the amplification reaction include DreamTaq$^{TM}$ Hot Start Green DNA Polymerase (0.5 units), 10 mM ITS4, ITS5 primers, and 5-10 nanograms of DNA template.

The amplification was carried out using a Thermo cycler with the following cycling conditions: denaturation at 95 °C for 30 sec., annealing at 55 °C for 30 sec., and extension at 72 °C for 1 min. A total of 35 cycles were performed to complete the amplification process. The PCR results were analyzed using the electrophoresis technique.

**Quality analysis of DNA from oyster mushrooms by using High annealing temperature-random amplified polymorphic DNA (HAT-RAPD)**

Quality analysis was performed on oyster mushroom extracts by using the HAT-RAPD technique by Thermocycler. The following components were used include DreamTaqTM Hot Start Green DNA Polymerase (0.5 units), 10 mM Random primers (OPT-01, OPT-08, OPT-14, and OPT-12), and 5-10 nanograms of DNA template.

The reaction consisted of a denaturation stage at 95 °C for 30 sec., an annealing stage at 50 °C for 30 sec., and an extension step at 72 °C for 10 min. The entire reaction was repeated for a total of 35 cycles. The PCR products were analyzed by using electrophoresis.

## Results and Discussion

**Quality of DNA extracted from oyster mushrooms fruiting bodies by using a modified method**

The extracted DNA from oyster mushroom fruiting bodies by using the modified method exhibited a concentration of 5154.88 ng/µl, with an absorbance of A260/280 and A260/230 at 1.80 and 1.94, respectively. Which stated that DNA absorbance ratios should have an A260/280 equal to or greater than 1.8 and an A260/230 equal to or greater than 1.8 [10]. A lower ratio would indicate contamination with organic compounds that can denatured protein. Furthermore, the extraction buffer of the modified method, has been increasing the concentration of β-mercaptoethanol, which a role played in inhibiting the oxidation reaction of DNA and removes the polyphenols [11-12].

In addition, adding phenol, chloroform, and isoamyl alcohol after extraction buffer can remove polysaccharide and secondary metabolite content from supernatant. Including, eliminating protein contamination, and effectively eliminating interfering substances that could negatively affect the quality and stability of DNA [4]. In the quality assessment of the extracted DNA from oyster mushroom fruiting bodies by using the modified method, PCR reactions were performed using molecular markers ITS and HAT-RAPD.

The results showed clear DNA bands, as depicted in Figure 3, due to the presence of SDS (Sodium dodecyl sulfate) in the extraction process served to disrupt cell membranes and aid in the precipitation of DNA, reducing the contamination of proteins and polysaccharides. The presence of these organic compounds can degrade proteins and interfere with the activity of Taq polymerase during PCR, thus inhibiting the amplification of DNA. Which the DNA extracted from these samples exhibited sufficient concentration and quality for use in PCR reactions, as determined using a Nanodrop Lite spectrophotometer.

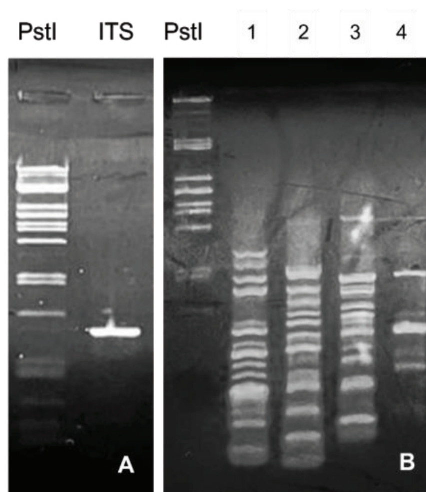


**Figure 3** PCR amplification by using ITS4 – ITS 5 primer (A), random primers: OPT01 (1), OPT08 (2), PT14 (3) and OPT12 (4) primer (B) and lambda/PstI 100bp ladder

## Conclusions

In conclusion, the direct extraction method of DNA from oyster mushrooms by modified method from Park, et al. [9] by increasing the concentration of compound and extraction steps found that the DNA extracted indicated good quality and purity suitable for PCR reaction.

Furthermore, when DNA was quality analyzed for classification by using the ITS and HAT-RAPD techniques found that DNA profiles were obtained with Clear and appropriate size bands (Figure 3).

Therefore, this modified extraction method enables direct DNA extraction from oyster mushroom fruiting bodies in a short period, reducing the complexity of mycelial stimulation but while the DNA extraction method from mycelium still requires time-consuming and

complex mycelium stimulation steps, this approach may not be suitable for rare and nearly deteriorated samples and limited quantity mushrooms samples.

Moreover, the modified extraction method can be employed for further investigations on the genetic diversity of oyster mushroom strains in Thailand by considering the morphological characteristics of the samples, resulting in more accurate classification data.

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