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

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### Article Info

### Abstract

#### Article History:

Received: July 3, 2023

Revised: January 30, 2024

Accepted: February 21, 2024

DOI:

#### Keywords:

Oyster Mushrooms /  
Molecular Marker / ITS /  
HAT-RAPD / DNA

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. The quality of the extracted DNA was evaluated using PCR with ITS and HAT-RAPD molecular markers. The results demonstrated clear and high-quality DNA band patterns, enabling the identification and classification of oyster mushroom species.

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### ข้อมูลบทความ

### บทคัดย่อ

#### ประวัติบทความ :

รับเพื่อพิจารณา : 3 กรกฎาคม 2566

แก้ไข : 30 มกราคม 2567

ตอบรับ : 21 กุมภาพันธ์ 2567

DOI:

คำสำคัญ : เห็ดนางรม /

เครื่องหมายโมเลกุล / ไอทีเอส /

แฮ็ทอาร์เอฟที / ดีเอ็นเอ

เห็ดนางรม (*Pleurotus* spp.) เป็นเห็ดเศรษฐกิจที่มีมูลค่าทางการตลาดสูง อีกทั้งยังเป็นเห็ดที่นิยมบริโภคทั่วโลกเนื่องจากมีรสชาติที่ทานง่ายและมีสรรพคุณทางยาที่หลากหลาย อย่างไรก็ตาม แม้ว่าเห็ดสกุลนางรมจะอุดมไปด้วยคุณประโยชน์ที่มากมายแต่ข้อมูลด้านพันธุกรรมนั้นยังมีอยู่อย่างจำกัด ดังนั้น การศึกษาความหลากหลายของเห็ดชนิดนี้จึงเป็นสิ่งสำคัญ ในปัจจุบัน นิยมประยุกต์ใช้เทคนิคทางเครื่องหมายโมเลกุลร่วมกับการศึกษาความหลากหลายด้วยลักษณะฟีโนไทป์ ซึ่งเทคนิคดังกล่าวจำเป็นอย่างยิ่งที่จะต้องใช้ดีเอ็นเอที่มีคุณภาพ แต่ด้วยขั้นตอนการกระตุ้นเส้นใยที่ค่อนข้างซับซ้อนและใช้ระยะเวลาอันยาวนาน คณะผู้วิจัยจึงพัฒนาวิธีการสกัดดีเอ็นเอจากดอกเห็ดนางรมโดยไม่ผ่านกระบวนการเพาะเลี้ยงเส้นใยบนอาหาร เมื่อตรวจสอบคุณภาพและประสิทธิภาพของดีเอ็นเอที่สกัดด้วยวิธีที่พัฒนาขึ้นด้วยปฏิกิริยาพีซีอาร์ (PCR) โดยใช้เครื่องหมายโมเลกุลไอทีเอส (ITS) และแฮ็ทอาร์เอฟที (HAT-RAPD) พบว่ามีการแสดงผลของแถบลายพิมพ์ดีเอ็นเอที่ชัดเจนและมีคุณภาพเพียงพอที่สามารถนำไปจำแนกสายพันธุ์เห็ดนางรมได้

## 1. 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  $\beta$ -glucan, a polysaccharide compound consisting of  $\beta$ -glycosidic linkages, commonly found in mushrooms, yeast, oats, barley, and certain bacteria [2]. Nowadays, the genus Oyster (*Pleurotus* spp.) are acknowledged to be  $\beta$ -glucans source. [3]

$\beta$ -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. pulmonarius* 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, 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.

## 2. Materials and methods

### 2.1 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  $\mu$ L 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  $\times$  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  $\mu$ L. After that, inverted

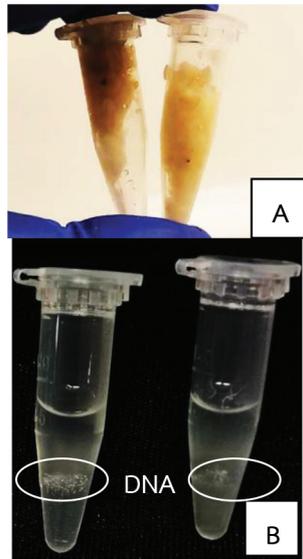
several times and centrifuged at 10,000  $\times$  g for 5 minutes at 4°C Then, repeated once.

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  $\mu$ L. After that, inverted several times and left at room temperature for 2-3 minutes. Subsequently, the mixture was centrifuged at 10,000  $\times$  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  $\times$  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  $\times$  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  $\mu$ L 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/ $\mu$ L 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 sample

### 2.2 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 Nanodrop 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.

### 2.3 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™ 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.

### 2.4 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 Thermo-cycler. The following components were used include DreamTaq™ 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.

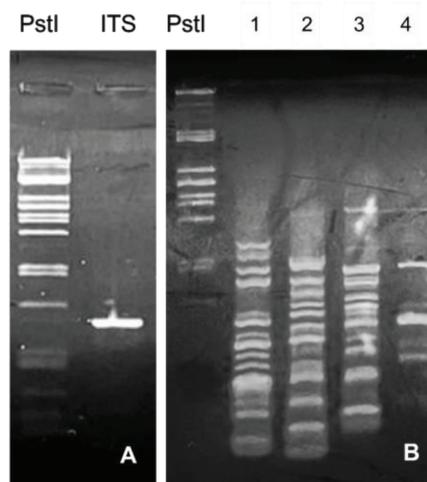
### 3. Results and discussion

#### 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/ $\mu$ l, 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  $\beta$ -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

#### 4. 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.

#### 5. Acknowledgements

This research has received financial support from the Plant Genetic Conservation Project Under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG) and we would also like to express our gratitude for the graduate scholarship funding provided by the Graduate School “ทุนศิษย์ก้นกุฏิ” from Maejo University during the academic year 2562.

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