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Research Article

Rapid Differentiation of Pleurotus Ostreatus from Pleurotus Sapidus Using PCR Technique

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Abstract: *Pleurotus ostreatus* and *Pleurotus sapidus* are difficult to differentiate using standard morphologically based characteristics. This study, using two taxon-selective primers for the Internal Transcribed Spacer (ITS) region in the nuclear ribosomal repeat unit. These primers, ITS1-F & ITS4, were intended to be specific for the higher fungi, respectively. RAPD-PCR technique was used to reveal DNA polymorphism in DNA of the two fungi *Pleurotus ostreatus* and *Pleurotus sapidus* in order to search for the sources of differences that could be used as a DNA marker represent the differentiation between this two species. In RAPD-PCR, eight different decamer primers chosen randomly were employed to detect the genetic polymorphisms among studied strains.

Keywords: Pleurotus ostereatus, Pleurotus sapidus, Higher Fungi, PCR; (ITS) region primers, RAPD-PCR, Edible mushrooms

INTRODUCTION

Mushrooms are identified by morphological characteristics; however, the morphological characteristics are inconsistent and unstable criteria because they are strongly influenced by the environmental conditions (Bresinsky *et al*., 1977; Petersen and Hughes, 1999). *Pleurotus* is a genus of gilled mushrooms which includes one of the most widely edible mushrooms, *P. ostreatus*. Species of *Pleurotus* may be called oyster, abalone, or tree mushrooms and are some of the most commonly cultivated edible mushrooms in the world (Shu-Ting and Miles, 2004). *Pleurotus pulmonarius*(*Sapidus*), commonly known as the Indian Oyster, Phoenix Mushroom, or the Lung Oyster, is a mushroom very similar to *Pleurotus ostreatus*, the pearl oyster, but with a few noticeable differences. The caps of *pulmonarius* are much paler and smaller than *ostreatus* and develops more of a stem. *P. pulmonarius* also prefers warmer weather than *ostreatus* and will appear later in the summer. Otherwise, the taste and cultivation of the two species is generally described as largely the same (Stamets, 2000). Genetic profiles and polymorphic sequences are important tools for a rapid and effective characterization of these species, namely in certification processes. On the other hand, molecular approaches to establish relationships between different regions and their characteristic strains can constitute valuable tools. Literature reports some approaches such as RAPD and ITS analyses to characterize and identify edible mushrooms (Moncalvo *et al*., 2000; Firenzuoli *et al*., 2007; Pacioni *et al*., 2007; Ro *et al*., 2007). The RAPD technique can simply and rapidly detect genetic alteration in the entire genome without knowledge of specific DNA sequence information and it works effectively with tiny amount of DNA. Therefore RAPD-PCR become a particularly popular technique and thus was applied in genetic mapping, for plant and animal breeding applications, DNA fingerprinting, study of population genetics, epidemiological studies and typing of micro-organisms and for the identification of pathogenic strain of bacteria and fungi (Williams *et al*., 1990).

Molecular analysis was performed by using random markers in (RAPD-PCR) technique and the ITS region to characterize the Pleurotus two species (Albores *et al*., 2006). The standard ITS1+ITS4 primers used by most labs, several taxon-specific primers have been described that allow selective amplification of fungal sequences (Gardes and Bruns, 1993). The study aims to use rapid differentiation of Pleurotus ostreatus from Pleurotus sapidus using PCR technique , characterise the Pleurotus two species with molecular biomarkers by RAPD and the ITS region, in order to establish different genetic profiles of these edible mushrooms. It focuses on the molecular identification of the Pleurotus two species, a kind of identification

METHODOLOGY

- **Fungal isolates:** Pleurotus ostreatus and Pleurotus sapidus were obtained from Biotechnology Department/College of Science/ Baghdad University/Baghdad/Iraq.
- **DNA extraction:** DNA was extracted from 0.5 g (wet weight), fungal mycelia harvested from 3 days

Table 1: The names of the random primers used in the study and their sequences

No.	Primer's name	*Sequence 5'--- 3'
1	OPA-2	TGCCGAGCTG
2	OPA-04	AATCGGGCTG
3	OP-R12	ACAGGTGCGT
4	OP-N16	AAGCGACCTG
5	OP-M14	AGGGTCGTTC
6	OP-L20	TGGTGGACCA
7	OP-K01	CATTCGAGCC
8	OP-IO2	GGAGGAGAGG

growing cultures in potato dextrose broth (PDB). The mycelium were transferred to a mortar, frozen in liquid nitrogen and were ground well. Steps of extraction had been completed using EZ-10 Spin Column Fungal Genomic DNA Mini-preps Kit, BIO BASIC INC., Markham Ontario, Canada.

- **PCR amplification:** The ITS region was amplified by PCR from DNA isolated from pure cultures of each of the fungi under study. Primers ITS1-F (CTT GGT CAT TTA GAG GAA GTA A) which is specific for the higher fungi and ITS4 (TCC TCC GCT TAT TGA TAT GC) (Cinnagen) the universal primer, were used together to amplify the ITS region from higher fungi.
  The polymerase chain reaction was performed in 25 mL, Each reaction mixture was heated to 95 C for 10 min. A total of 30 PCR cycles, each cycle at 0.3 min at 94 C for denaturation, annealing (0.45 min at 50ºC for reactions with ITS1-F and ITS4 and at 55ºC for reactions with ITS1-F and ITS4-B), 1.15 min at 72 C for extension and a 10 min final extension at 72 C) and extension at 72ºC for 1 min. The PCR products were analysed by electrophoresis on a 1.5% agarose gel in (1x) TBE buffer (50 mM Tris-acetate, 1 mM EDTA, pH 8.0) stained in 0.5 mg/mL ethidium bromide.
- **Rapid-PCR amplification:** Eight different decamer primers chosen randomly were employed to detect the genetic polymorphisms among studied fungi. The polymerase chain reaction was performed in 25 mL, Each reaction mixture was heated to 95 C for 10 min. A total of 35 PCR cycles, each cycle at 0.3 min at 94 C for denaturation, 0.45 min at 36 C for annealing, 1.45 min at 72 C for extension and a 10 min final extension at 72 C). The PCR products were analysed by electrophoresis on a 2% agarose gel in (1x) TBE buffer (50 mM Tris–acetate, 1 mM EDTA, pH 8.0) stained in 0.5 mg/mL ethidium bromide. The random PCR primers as indicated in Table 1.

RESULTS AND DISCUSSION

When making the amplification with primers ITS1F and ITS4, general for higher fungi, the same restriction profile for fungi of the two species (*P. ostreatus* and *P. sapidus*) is obtained. (Fig. 1). Sequence comparison of the ITS region is widely used in taxonomy and molecular phylogeny because it a is

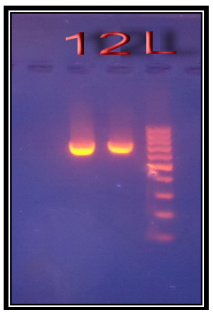

Fig. 1: 1.5 % Agarose gel analysis of PCR profile obtained on Amplification with primers, ITS1F and ITS4, general for higher fungi, (1) *P. Ostreatus*; (2) P. *sapidus*; (L) DNA Ladder 100 bp (DNA molecular weight marker)

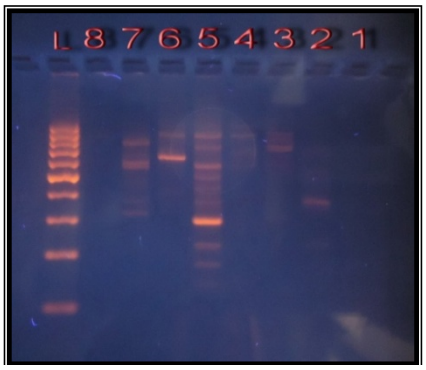

Fig. 2: Agarose gel electrophoresis of RAPD-PCR reaction screening eight random primers on *P.Ostreatus* DNA sample (under optimum conditions). Bands were fractionated by electrophoresis on a 2% agarose gel (2hr, 5V/cm, 1XTris-borate buffer) and visualized under U.V. light after staining with ethidium bromide. (L): DNA Ladder 100 bp (DNA molecular weight marker) Primers; (1): OPA-2, (2): OPA-04 , (3) OP-R12 , (4) OP-N16 , (5): OP-M14 , (6): OP-L20, (7): OP-K01 and (8): OP-IO2

(due to the high copy number of rRNA genes) easy to amplify even from small quantities of DNA) has a high degree of variation even between closely related species. This can be explained by the relatively low evolutionary pressure acting on such non-functional sequences, For example, ITS has proven especially useful for elucidating relationships among congeneric species and closely related genera in Asteraceae (Baldwin, 1992).

Optimization of PCR conditions, that including reagents, temperature, number of cycles and other parameters are very necessary to get a successful RAPD-PCR reaction. (McPherson and MØller, 2001). In RAPD-PCR reaction eight primers had been tested with same DNA sample under standard or optimum conditions of programming and reagent concentration that results different RAPD patterns for each primer (Fig. 2 and 3). Among the seven decamer

Table 2: Distinct characteristic of random primers included in the study: primer's name, total number of bands, number of polymorphic bands and percentage of polymorphism

No.	Primer	Total number of bands	Number of polymorphic bands	Polymorphism %
1	OPA-2	---	---	---
2	OPA-04	8	6	75
3	OP-R12	4	2	50
4	OP-M14	17	5	29
5	OP-L20	4	2	50
6	OP-N16	3	1	33
7	OP-KOl	9	3	33
Total		45	19	---

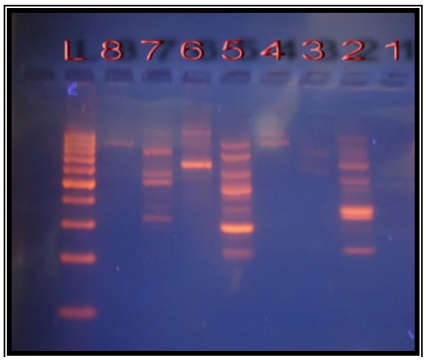

Fig. 3: Agarose gel electrophoresis of RAPD-PCR reaction screening eight random primers on *P.Sapidus* DNA sample (under optimum conditions). Bands were fractionated by electrophoresis on a 2% agarose gel (2hr, 5V/cm, 1XTris-borate buffer) and visualized under U.V. light after staining with ethidium bromide (L): DNA Ladder 100 bp (DNA molecular weight marker) Primers; (1): OPA-2, (2): OPA-04, (3): OP-R12, (4): OP-N16, (5): OP-M14, (6): OP-L20, (7): OP-K01 and (8): OP-IO2

oligonucleotide primers, there were one primer (OPA-2) that did not amplify the DNA, hence, they were eliminated from the analysis. Each of the remaining eight primers varied greatly in their ability to resolve variability among two species. Some primers generated several bands, while others generated only a few. The eight random primers produced a total of (45) useful bands across two species (Table 2). Of these (45) PCR products generated 4.4% (2 bands) were monomorphic across two species. The remaining (43) bands (95.6% of the total products scored) were polymorphic among the studied strains. The primer OPA-04 gave the highest number of polymorphic bands (6), while the minimum number of polymorphic bands (1) by using OP-L20 primer. The variation in the number of bands amplified by different primers influenced by variable factors such as primer structure, template quantity and less number of annealing sites in the genome (Kernodle *et al*., 1993). The size of the amplified fragments had ranged from 1000 bp (OPA-04) to 150 bp (OP-L20). Polymorphism of each primer was calculated as the percentage of polymorphic bands to the number of total bands produced by the designated primer (Ali, 2003).

The RAPD assay generated variety-specific products in some of the genotypes. These may be used as DNA fingerprints for variety identification. It would be of immense use for the establishment of proprietary rights and the determination of variety purity.

Concluding remarks: From the results obtained, we can conclude that:

- *P.Ostreatus* and *P. sapidus* are higher fungi
- Approximately all of the seven random primers used in this study could be used as a markers distinguishing the studied two species
- Genetic polymorphism values of random primers were almost alike, high levels of polymorphisms reached to 75% by the universal primer OPA-04

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