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Identification of Nematode Trapping Fungus *Monacrosporium eudermatum* Based on Genetic Diversity Using RAPD Technique

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Abstract Nematode-trapping fungus *Monacrosporium eudermatum* a predacious fungus of nematodes, has been very useful in understanding the most relationship between nematophagous fungi and their nematode hosts. *M.eudermatum* is by far the common nematode-trapping fungus with the characteristic ability of forming adhesive trapping nets once in contact with nematodes. Total DNA was extracted from *M.eudermatum* sera and amplified by RAPD-PCR using three random primers (OPA-16, OPB-08, OPF-05). The results show amplification of all markers (OPF-05, OPB-08, OPA-16) with the genomic DNA studied. As evidenced by the results, all the markers covered in the study showed high polymorphism (up to 60%) with the exception of the primer (OPB-08) which showed no polymorphism.

Keywords: *nematophagous, Monacrosporium eudermatum, RAPD technique, PCR*

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1. Introduction

In recent years, different Polymerase Chain Reaction (PCR) based molecular markers including Random Amplified Polymorphic DNA (RAPD) have proved to be useful tools for studying genetic diversity and monitoring of soil borne fungi [1,2]. RAPD-PCR has the advantage of being quick and easy and requires minute quantities of genomic DNA for amplification. Furthermore, DNA finger print can be generated with RAPD by using short nucleotide (10-16 nucleotides bases) sequence of arbitrary nature as primers and does not require any prior knowledge of the target site sequence [3]. Several DNA-based molecular markers such as; Randomly Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphisms (RFLPs), Simple Sequence Repeats (SSRs) and Amplified Fragment Length Polymorphism (AFLP) have been successfully used to estimate genetic diversity of fungi [4,5,6].

Nematophagous fungi are a diverse group of fungal species that use refined mycelial structures or their conidia to trap and capture nematodes and other small invertebrates. This unique ability it made it possible to use these fungi as agents of biological control against plant-and animal-parasitic nematodes. However, effective application of biological control factors in the field requires a more information to understand the ecology and population genetics of the nematophagous fungi in natural environments [7].

Several studies using molecular markers to study the diversity and evolution of nematode-trapping fungi. Restriction fragment length polymorphism (RFLP) and DNA sequencing of the internal transcribed spacer region of the ribosomal RNA gene (ITS) region have found genetic diversities among isolates within species in the genera *Arthrobotrys* and *Monacrosporium*, showing cryptic species among morphologically similar isolates [8,9,10]. Intraspecific differentiation derived from RFLP data and polymorphisms in serine protease genes were also revealed revealing among ecotypes of the nematophagous fungus *Pochonia chlamydospora* based on host preferences [5,11].

The nematode-trapping fungus *M.eudermatum* belong to the Orbiliomycetes, order Orbiliales and family Orbiliaceae [12]. It is ubiquitous in a variety of habitats such as a agricultural soils, farmed, fields, forests. The present study was undertaken to determine if the three markers (OPF-05, OPB-08 and OPA-16) amplify the genomic DNA of the nematode-trapping fungus *M.eudermatum* (monophyletic group Orbiliales) that isolated from farmed soil of Misan governorate of Iraq.

2. Material and Methods

2.1. Isolates of *M.eudermatum*

Isolates of *M.eudermatum* were obtained from microbiology laboratory at Biological Department, College of Sciences, Misan University, Iraq and were isolated from farmed soil of Misan, Governorate of Iraq).

M.eudermatum isolates were grown on corn meal agar media.

2.2. DNA Isolation and RAPD Primers

Total DNA was extracted from sample using salting-out procedure (13). Promega fungi kit was also used to isolate genomic DNA from *M.eudermatum* sera. Three random primers (Promega, USA) were used for DNA amplification. Each random primer was a 10-mer with GC content varying from 60% to 70%. Three orbilial specific primers (OPA16, OPB08 and OPF05) were selected according to the recommendation of NCBI because these primers are most commonly used for studying polymorphism in most organisms and for further use in genotyping. The base sequence and length of the primers is given in Table 1.

2.3. RAPD-PCR Analysis

RAPD-PCR was carried out on genomic DNA from individual *M.eudermatum* as well as pooled DNA samples (mixture of individual DNA samples within the same breed) from each breed. Breed–specific genomic DNA samples were prepared by pooling the same amount of genomic DNA from each individual of the respected breed. RAPD-PCR amplifications of each *M.eudermatum* were performed in 13.2 µl reaction mixtures containing; 0.2 mM of primer, 1.25 U TaqTM polymerase, 25 mM $MgCl_2$, 10 mM dNTP and 200 ng of genomic DNA reached to final concentration of 10µL. Amplifications were performed using a Eppendorf thermal cycler that was programmed for 45 cycles at 94°C for 1 min, at 35°C for 40 sec and at 72°C for 45 sec, and a final extension at 76°C for 6 min for elongation. RAPD-PCR amplifications of each fungus were performed at least twice for confirmation of the accuracy and the repeatability of the products. Amplification products were separated by agarose gel (1.4%) electrophoresis instead of 2% due to the very close and minimum differences between PCR-PAPD bands it was more clear to separate on 1.4% gelthan 2% geland detected by ethidium bromide staining results were analyzed using Quantity One software.

3. Results

The PCR-RAPD results showed that all markers (OPF-05, OPB-08, OPA-16) has amplified genome DNA of *M.eudermatum* studied (Figure 1). All markers covered by the study showed high polymorphism with the exception of the primer (OPB-08) that has shown no polymorphism. The results of electrophoresis of the PCR-RAPD products showed that a total of 10 RAPD bands were obtained from the fungi breeds. Amplified products ranged from 50 bp of OPF-05 primer to 750 bp of OPA-16. The maximum number of bands was obtained with the primer OPA-16 (Figure 1, Figure 2).

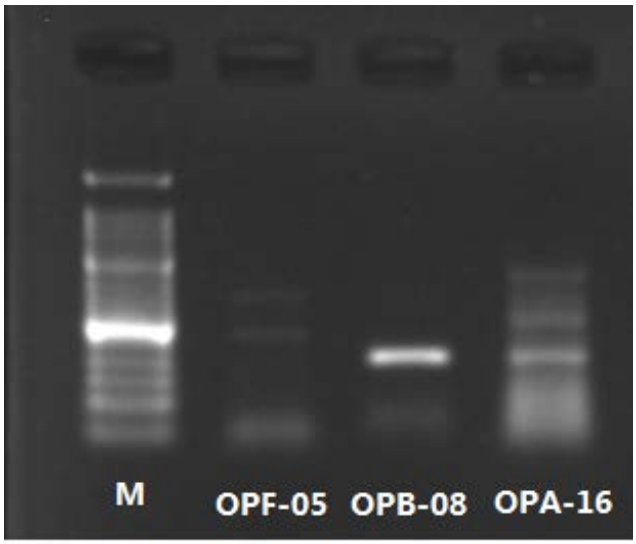

Figure 1. RAPD-PCR amplification products were separated by agarose gel (1.4%) electrophoresis and detected by ethidium bromide staining. M marker (50-1500), amplified bands ranged between minimum50 bp in OPF-05 to maximum 750bp in OPA-16

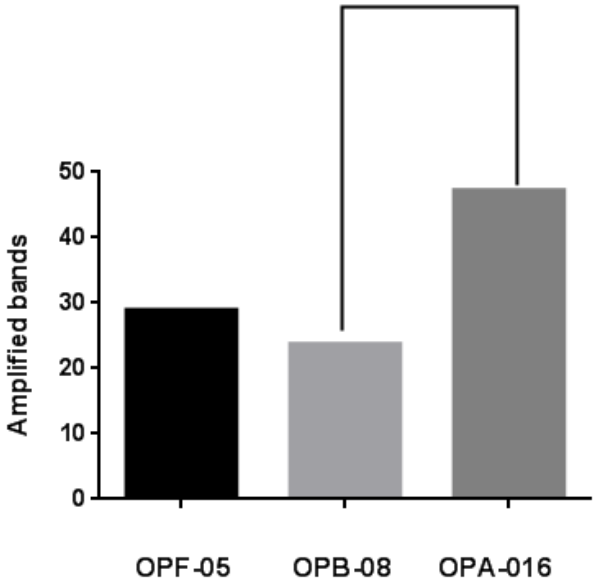

Figure 2. Amplified bands were analyzed using Quantity one software to analyze size of the bands and relative relationship between primer APO-16 showed higher amplification as well as higher number of bands, while APB-08 was the less one

Table 1. DNA bands amplified and polymorphism genotypes using 3 RAPD markers

Name of the primer	Sequence	GC%	Total Bands	Polymorphism% of primers	Polymorphic bands (Na. of amplified bands)	Size rang (bp)	
						Minimum	Maximum
OPA-16	AGCCAGCGAA	60	5	60	3	50	750
OPB-08	GTCCACACGG	70	2	0	0	100	200
OPF-05	CCGAATTCCC	60	3	33	1	50	650
TOTAL			10	40	4	200	1600

4. Discussion

The taxonomy and diversity of nematode-trapping fungi have been primarily studied using traditional morphological methods and rRNA sequences. These studies identified that the type of nematode-trapping devices was a highly reliable indicator about the evolutionary relationships among these species [14,15,16,17]. Currently, available genomic sequences of nematode-trapping fungi have made it possible for the evolutionary study at genomic level [18]. The combined use of Orbiliales specific primers and culture-based techniques may benefitfuture studies of nematophagous fungi [19,20].

Randomly Amplified Polymorphic DNA (RAPD) is a relatively simple technique and has been commonly used for genetic characterization and identification of individual isolates of fungi [21]. The objective of the present study was to characterize *M.eudermatum* isolate, collected from farmed soil of Misan governorate of Iraq, at molecular level. For this purpose one isolate of *M.eudermatum* was analyzed using 3 RAPD primers. All the tested genotypes in their RAPD assay generated variety of amplification products. Level of genetic polymorphism among the genotypes detected during present study varied from primer to primer. The quantification of PCR products by conventional PCR is limited, because during exponential amplification of the template, small variations in amplification efficiency can drastically change the yield of the PCR product, resulting in a non-quantitative assay [22]. Co-amplification of the molecule of interest with a known amount of competitor molecules bearing primer sites identical to those of the target allows reproducible quantification of templates [23].The difference in size of the original PCR [22].

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