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Diagnosis of Some Plant Pathogenic Fungi by PCR-ITS Regions

Nashwan Abdul Razzaq Mohsin[1], Alaa Mohsin Al-Araji[2]
Department of Biology, College of Sciences, Baghdad University, Baghdad, Iraq
[1](nashwanalbawii@gmail.com), [2](alaraji.alaa@sc.uobaghdad.edu.iq)

Abstract

This study aimed to diagnosis of plant pathogenic fungi by molecular technique Polymerase Chain Reaction-Internal Transcript Spacer (PCR-ITS). Seven plant pathogenic fungi isolates were isolates from infected Solanaceae plants and diagnosed depending on morphological characteristics. The pathogenicity test was performed for Seven of plant pathogenic fungi isolates. Genomic DNA was extracted from seven plant pathogenic fungi by using Wizard Genomic DNA Purification kit. ITS gene was amplified by using PCR-ITS. The results of the pathogenicity test showed that Macrophomina *phaseolina*, and *Rhizoctonia solani* isolates high significance pathogenicity more than other plant pathogenic fungi *Fusarium1*,*Fusarium 2*, *Fusarium 3*,*Sclerotinia*,andCurvularia which recorded 100%, 96.67%, 86.67%, 70%, 60%, 60%, and 56.67% respectively. The sequencing results of the amplified product of ITS gene using the ITS1 and ITS4 primers obtained were compared with NCBI GenBank by using BLAST analysis. The results of diagnosed depending on morphological characteristics revealed five genera of pathogenic fungi (Fusarium, *Curvularia*, *Sclerotinia*, *Rhizoctonia* and *Macrophomina*) and also the results of molecular diagnosis confirmed the genus and the species of plant pathogenic fungi as Rhizoctonia solani, Curvularia spicifera, Macrophomina phaseolina, Fusarium oxysporum, Sclerotinia sclerotiorum, Fusarium oxysporum f.sp. cumini and Fusarium oxysporum .
Keywords: plant pathogenic fungi; PCR; Sequence analysis; ITS gene.

Introduction

Plant pathogens caused by fungi are one of the most common biotic factors that trigger devastating disease in crops [1]. about 8,000 fungi and oomycetes species are associated with plant diseases [2, 3]. Pathogenic fungi may infect plants at any stage of development, from seedling to seed maturation, in normal environmental conditions, either alone or in combination with other types of phytopathogens [4].blight, damping off, leaf spot, rust, root rot, scab, and wilt are the most prevalent diseases that are caused byplant pathogenic fungi [5,6 and 7].

molecular approaches are usually more rapid and precise than those focused on colony morphology, microscopic characteristics, and physiological or biochemical characteristics of pure fungal cultures. Matter of fact, DNA-based methods have been used to detect pathogens during the development, harvest, and postharvest processing stages of crops [8]. In addition to, the use of species-specific primers in PCR for the identification of fungal pathogens has become common, particularly for economically significant plant pathogens (9,10 and11).The aim of the study was to diagnosis of some plant pathogenic fungi by PCR-ITS regions.

Methods and materials

Collected infected plants and Isolation of pathogenic fungi

The infected plants (100 plants) were collected from different fields of solanaceae in Al Muthana province randomly, by placing the infected plants in polyethylene bags individually with recoded notes (type of infected plant, date collected). After that, the plants were transferred to the laboratory, and then the roots, stems, and leaves of infected plants

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were washed by running water to get rid of the mud suspended. Thereafter, the stems, leaves, and roots were cut into small pieces (0.5 cm) and sterile by immersing them for 2 mines in the sodium hypochlorite solution (2% free chlorine), then washed by immersing them in sterilized distilled water three times, after that transferred on sterile filter paper for drying. Three pieces were cultured of each infected plant in Petri dishes (90mm) containing autoclaved PDA (Oxoid – England) and incubated for 5 days at 27±2 ˚C. The isolates fungi were purified by taking mycelial plugs with a 10 mm diameter from the growing margin, put overhead onto fresh PDA in the center of the petri dish, and incubated for 5 days at 27±2 ˚C. The purified isolates were diagnosed based on the morphological characteristicaccording to[12], [13], and [14] by Assistant Professor Dr. Alaa Mohsin Al-Aaraji.

Plant pathogenic fungi Pathogenicity test

Local cress seeds untreated with fungicide were surface sterilized by immersion in sodium hypochlorite (2 % free chlorine) for 2 min, rinsed 3 times with sterile distilled water, dried by sterile filter paper.Tenseedsforthreereplicates were placed on thesterile filter paperinsidea Petri dishcontainingmoistureby puttingdropsof sterile distilled water,thenleavesfor 7 days, then account the percentage of seeds germinationaccording to this formula:

$$p = \frac{\text{number of germinating seeds}}{\text{Total number of seeds planted}} \times 100$$

P = the percentage of seeds germination

The tested fungal isolates were grown on PDA plates for 7 days. 5ml of sterile water was powered on each plate containing fungal colony to obtain fungal suspension. Ten seeds of local cress seeds untreated with fungicide were surface sterilized by immersion in sodium hypochlorite (2 % free chlorine) for 2 min, rinsed 3 times with sterile distilled water, dried by sterile filter paper, after that the sterile seeds were soaked in the fungal suspensions for 30 min, then inoculated seeds were placed on thesterile filter paperinsidea petri dishcontainingmoisture (by puttingdropsof fungal suspension),andleavesfor7 days at room temperature, then the percentage of infection was account according to this formula [15].

$$p\ \% = \frac{\text{Number of non germinating seeds}}{\text{Total seeds number}} \times 100$$

P = the percentage of infection percentage

Genomic DNA Extraction

From seven fungi isolates which diagnosed based on the morphological characteristic to genus (*Fusarium 1, Fusarium 2, Fusarium 3, Curvularia, Sclerotinia, Rhizoctonia,* and *Macrophomina*) Genomic DNA was extracted by Wizard genomic DNA Purification kit (Promega, USA) according to manufactures protocol.

Primers of gene ITS preparation

The primer pair *ITS1* (5`-TCCGTAGGTGAACCTGCGG-3`) and *ITS4* (5`-TCCTCCGCTTATTGATATGC-3`) (Macrogen Company) using for amplification of *ITS* gene. The stock solution was prepared by dissolving lyophilized primers in nuclease-free water to a final concentration of 100pmol/l. To make a functioning primer solution of 10 pmol/µl, and 10µl of primer stock solution (stored at -20 C) was mixed with 90µl of nuclease-free water.

PCR amplification

The PCR amplification mixture of the specific reaction fordiagnosis gene was performed in a total volume of 25µl containing 12.5 µl GoTaq® Green Master Mix

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(Promega /USA), 3 µl DNA , 1µl of each primer (10 pmol) then 7.5 µl Nuclease Free Water was added into tube to a total volume of 25µl.

Amplification of ITS region

The universal primers (ITS-1 and ITS-4) were used to amplify the ITS regions, which is present in all eukaryotes as a conserved region [16]. The following conditions were used for thermal cycling: Initial denaturation at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 sec, 55°C for 30s and 72 °C for 30s with final extension at 72 °C for 7 min using a thermal cycler (Gene Amp, PCR system 9700; Applied Biosystem).

Agarose gel electrophoresis of DNA

All PCR products were separated by 1.5% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302nm) after staining by ethidium bromide**.**

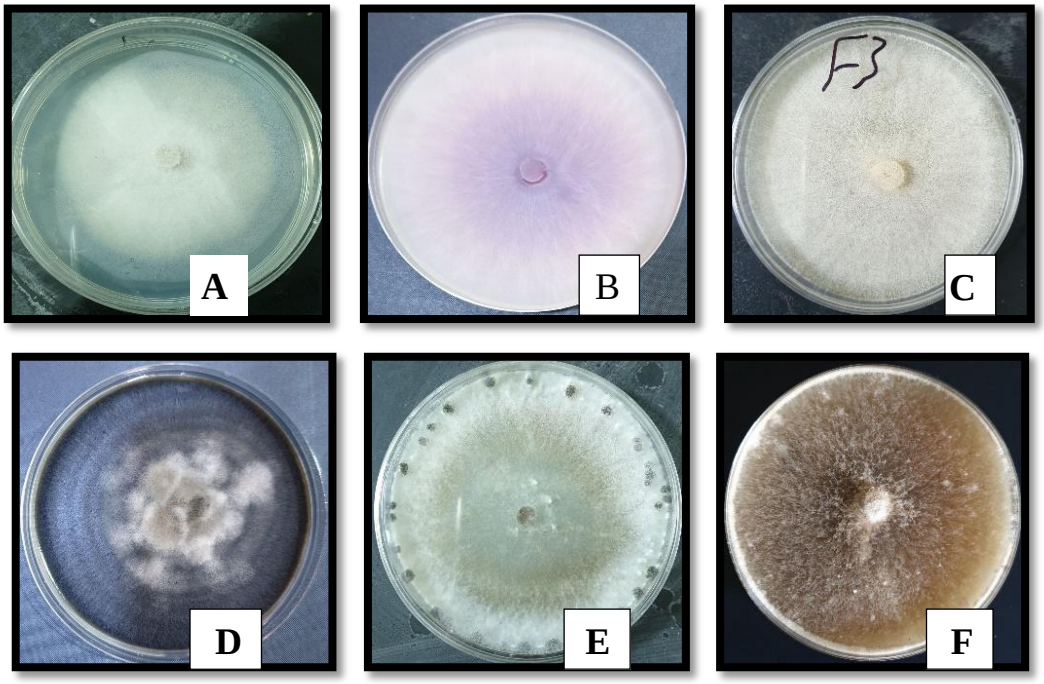
Sequencing and sequence alignment

Sequencing of ITS region from 7 fungi was performed by ABI3730XL, applied biosystem, Macrogen Corporation – Korea. Sequence analyzed by using basic local alignment search tool (blast) program, which is available at the national center biotechnology information (NCBI) online at (http:// www.ncbi.nlm.nih.gov). The results were received by email then analyzed using geneious software.

Results and Discussion

Collected infected plants and Isolation of pathogenic fungi

Seven isolates of pathogenic fungi(three isolates of *F. oxysporum*, *Rhizoctonia solani, Macrophomina phaseolina, Sclerotinia sclerotiorum, and Curvularia spicifera*) were isolated from infected solanaceae plants, and identified depending on morphological (Figure 1**)** and microscopically characters according to [12], [13] and [14].



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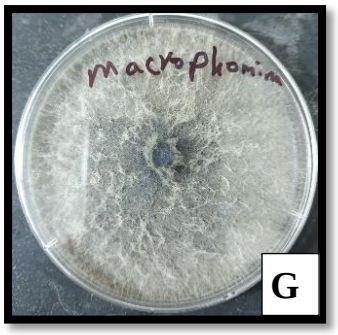

Figure.1. Colonies of fungal isolates on PDA at 27±2 ˚C after 7 days: **A**. *Fusarium oxysporum1***B**. *Fusarium oxysporum*2, **C**. *Fusarium oxysporum*3, **D**. *Curvularia spicifera*, **E**. *Sclerotinia sclerotiorum*, **F**. *Rhizoctonia solani*, and **G**. *Macrophomina phaseolina*.

Pathogenicity Test of pathogenic fungi

Results showed that *Macrophomina phaseolina*, and *Rhizoctonia solani*high significance pathogenicitymore than *Fusarium1*,*Fusarium 2*, *Fusarium 3*,*Sclerotinia*,and*Curvularia* which recorded 100%, 96.67%, 86.67%, 70%, 60%, 60%, and 56.67% respectively(Table 1).

Table 1. Pathogenicity test of pathogenic fungi on local cress seeds

Fungi isolates	Mean of infection
Fusarium 1	86.67 ± 6.67
Fusarium 2	70.0 ± 5.77
Fusarium 3	60.0 ± 5.77
Curvularia	56.67 ± 3.33
Sclerotinia	60.0 ± 5.77
Rhizoctonia	96.67 ± 3.33
Macrophomina	100.0 ± 0.0
LSD	8.60

each number represents three replicates.

DNA Extraction:

The DNA was extracted efficiently from seven plant pathogenic fungi isolates by using Wizard Genomic DNA Purification kit. Purity (1.6 – 1.8) and concentration of DNA (200-270µg) were measured using Nanodrop [17]. Then the product of DNA was confirmed by agarose gel electrophoresis (Figure 2).

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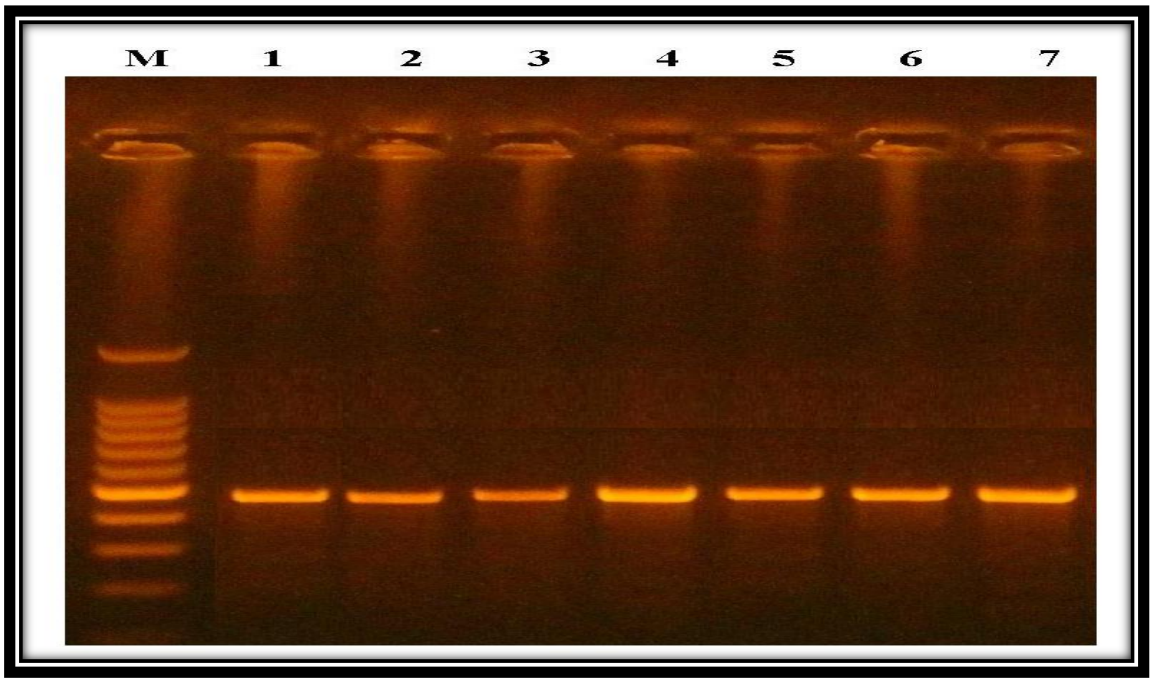

Figure.2.Agarose gel Electrophoresis of total genomic DNA for 7 fungal isolates.

Detection of ITS Gene in plant pathogenic fungi

The PCR products were separated by 1.5% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302nm) after ethidium bromide staining(Figure3)**.**

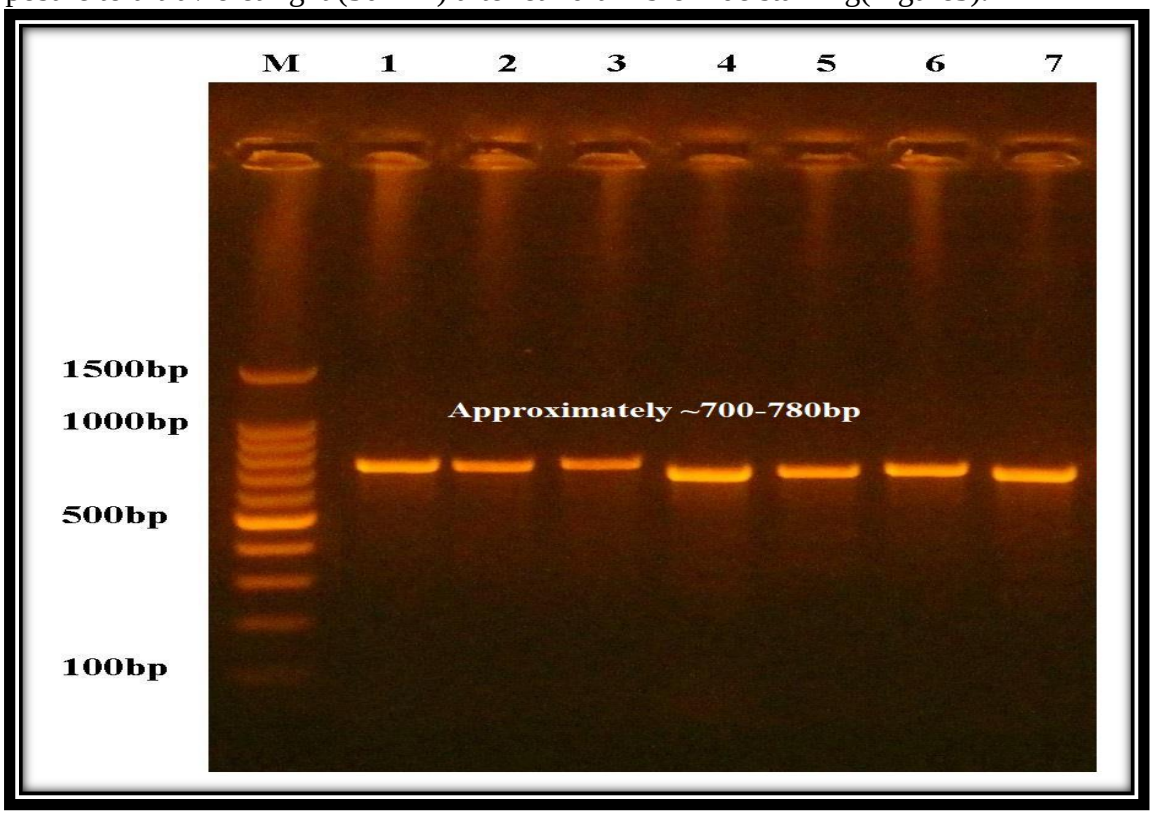

Figure.3. The PCR product of 7 fungal isolates performed by ITS gene that electrophoresis on 1.5% agarose at 100v/mAmp.1x TBE buffer for 60min.M: 100bp ladder marker. Lanes 1-7.

Sequencing and alignment of NCBI

The seven PCR product samples were sent for sequence analysis.The result of the sequence analysis was analysed by blast search in the National Centre Biotechnology Information

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(NCBI) online at (http:// www.ncbi.nlm.nih.gov).Results obtained from the BLAST database showed that 100% nucleotide identities with Rhizoctonia solaniisolateGPB, Curvularia spiciferastrainCBS 125738, Macrophomina phaseolina strain BRIP 68039and Fusarium oxysporum isolate AFIC15; 99.70% nucleotide identitieswith Sclerotinia sclerotiorum; 99.85% nucleotide identities with Fusarium oxysporum f.sp. cumini and Fusarium*oxysporum* isolate S106. shown in shown in figure 4

BLAST results ofSample 1

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhizoctonia solani isolate GPB small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer …	Rhizoctonia solani	1157	1157	100%	0.0	100.00%	664	MK621284.1
Ceratobasidium sp. AG-Fa isolate Y1053 18S ribosomal RNA gene, partial sequence; internal transcribed spacer…	Ceratobasidium …	1157	1157	100%	0.0	100.00%	685	JX913818.1
Ceratobasidium sp. AG-Fa isolate Y1058 18S ribosomal RNA gene, partial sequence; internal transcribed spacer…	Ceratobasidium …	1151	1151	100%	0.0	99.84%	685	JX913820.1
Ceratobasidium sp. AG-Fa isolate Y1055 18S ribosomal RNA gene, partial sequence; internal transcribed spacer…	Ceratobasidium …	1151	1151	100%	0.0	99.84%	686	JX913819.1
Rhizoctonia solani isolate RSzA6.seq small subunit ribosomal RNA gene, partial sequence; internal transcribed s…	Rhizoctonia solani	1151	1151	100%	0.0	99.84%	683	MW369735.1
Rhizoctonia solani isolate RhCh-14 small subunit ribosomal RNA gene, partial sequence; internal transcribed sp…	Rhizoctonia solani	1149	1149	99%	0.0	100.00%	658	MK027051.1
Rhizoctonia solani isolate AV4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int…	Rhizoctonia solani	1144	1144	100%	0.0	99.68%	668	MH517362.1
Rhizoctonia solani isolate IQ49 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int…	Rhizoctonia solani	1142	1142	98%	0.0	100.00%	641	KF372653.1
Rhizoctonia solani strain MML4001 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene an…	Rhizoctonia solani	1142	1142	98%	0.0	100.00%	623	JX535004.1
Ceratobasidium sp. AG-Fa isolate Y1064 18S ribosomal RNA gene, partial sequence; internal transcribed spacer…	Ceratobasidium …	1138	1138	100%	0.0	99.52%	684	JX913821.1

BLAST results ofSample 2

Description	Scientific Name	Common Name	Taxid	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Curvularia spicifera strain CBS 125738 small subunit ribosomal RNA gene, partial sequence; interna…	Curvula…	NA	145392	1267	1267	100%	0.0	100.00%	707	MH863648.1
Curvularia spicifera genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2,…	Curvula…	NA	145392	1253	1253	98%	0.0	100.00%	698	LT631349.1
Curvularia spicifera genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S…	Curvula…	NA	145392	1253	1253	98%	0.0	100.00%	698	HF934915.1
Curvularia spicifera genomic DNA sequence contains ITS1, 5.8S rRNA gene, ITS2, strain CBS 198.31	Curvula…	NA	145392	1238	1238	98%	0.0	99.85%	696	LT631348.1
Curvularia spicifera genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S…	Curvula…	NA	145392	1238	1238	98%	0.0	99.85%	696	HF934916.1
Curvularia spicifera genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S…	Curvula…	NA	145392	1197	1197	95%	0.0	99.85%	674	HF934914.1
Curvularia spicifera strain AY992b small subunit ribosomal RNA gene, partial sequence; internal tran…	Curvula…	NA	145392	1101	1101	86%	0.0	100.00%	605	MG250435.1
Curvularia spicifera voucher SCFUN3038 small subunit ribosomal RNA gene, partial sequence; inter…	Curvula…	NA	145392	1086	1086	85%	0.0	100.00%	588	MG780411.1
Curvularia spicifera strain CsP1 small subunit ribosomal RNA gene, partial sequence; internal transc…	Curvula…	NA	145392	1074	1074	85%	0.0	99.83%	593	MF193492.1
Curvularia spicifera strain CsP2 small subunit ribosomal RNA gene, partial sequence; internal transc…	Curvula…	NA	145392	1061	1061	83%	0.0	100.00%	574	MF193489.1

BLAST results ofSample 3

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Macrophomina phaseolina strain BRIP 68039 small subunit ribosomal RNA gene, partial sequence; internal trans…	Macrophomina p…	1286	1286	100%	0.0	100.00%	722	MK968305.1
Macrophomina phaseolina strain BRIP 68051 small subunit ribosomal RNA gene, partial sequence; internal trans…	Macrophomina p…	1279	1279	99%	0.0	99.86%	720	MK968306.1
Macrophomina phaseolina strain WAC:14342 small subunit ribosomal RNA gene, partial sequence; internal trans…	Macrophomina p…	1277	1277	99%	0.0	100.00%	714	MW591675.1
Macrophomina phaseolina strain WAC:14341 small subunit ribosomal RNA gene, partial sequence; internal trans…	Macrophomina p…	1277	1277	99%	0.0	100.00%	714	MW591674.1
Macrophomina phaseolina strain WAC:14340 small subunit ribosomal RNA gene, partial sequence; internal trans…	Macrophomina p…	1277	1277	99%	0.0	100.00%	714	MW591673.1
Macrophomina phaseolina strain WAC:14338 small subunit ribosomal RNA gene, partial sequence; internal trans…	Macrophomina p…	1277	1277	99%	0.0	100.00%	714	MW591671.1
Macrophomina phaseolina strain WAC:7296 small subunit ribosomal RNA gene, partial sequence; internal transcr…	Macrophomina p…	1277	1277	99%	0.0	100.00%	714	MW591660.1
Macrophomina phaseolina strain BRIP:71621 small subunit ribosomal RNA gene, partial sequence; internal trans…	Macrophomina p…	1277	1277	99%	0.0	100.00%	714	MW591649.1
Macrophomina phaseolina strain BRIP:71620 small subunit ribosomal RNA gene, partial sequence; internal trans…	Macrophomina p…	1277	1277	99%	0.0	100.00%	714	MW591648.1
Macrophomina phaseolina strain BRIP:71612 small subunit ribosomal RNA gene, partial sequence; internal trans…	Macrophomina p…	1277	1277	99%	0.0	100.00%	714	MW591640.1

BLAST results ofSample 4

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Sclerotinia sclerotiorum chromosome 7 sequence	Sclerotinia scler…	1210	5935	100%	0.0	99.70%	2434682	CP017820.1
Botrytis cinerea B05.10 chromosome BCIN04, complete sequence	Botrytis cinerea…	1177	1177	100%	0.0	98.79%	2468882	CP009808.1
Uncultured ascomycete ITS region including 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA ge…	uncultured Asco…	1160	1160	98%	0.0	98.92%	712	AM901713.1
Botrytis cinerea strain CBS 261.71 small subunit ribosomal RNA gene, partial sequence; internal transcribed …	Botrytis cinerea	1147	1147	97%	0.0	98.91%	1199	MH860108.1
Uncultured fungus clone CMH170 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, …	uncultured fungus	1147	1147	100%	0.0	98.04%	749	KF800261.1
Monilinia fructicola strain CBS 127259 small subunit ribosomal RNA gene, partial sequence; internal transcri…	Monilinia fructicola	1142	1142	100%	0.0	97.89%	680	MH864497.1
Uncultured ascomycete ITS region including 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA ge…	uncultured Asco…	1142	1142	98%	0.0	98.46%	710	AM901946.1
Botryotinia ranunculi CBS 178.63 ITS region; from TYPE material	Botryotinia ranu…	1136	1136	97%	0.0	98.60%	690	NR_164278.1
Botryotinia ranunculi strain CBS 178.63 small subunit ribosomal RNA gene, partial sequence; internal transcr…	Botryotinia ranu…	1136	1136	97%	0.0	98.60%	1304	MH858258.1
Botrytis porri strain CBS 190.26 small subunit ribosomal RNA gene, partial sequence; internal transcribed sp…	Botrytis porri	1131	1131	97%	0.0	98.45%	1202	MH854885.1

BLAST results ofSample 5

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Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Fusarium oxysporum f. sp. cumini partial 18S rRNA gene for 18S ribosomal RNA, strain F11	Fusarium oxysp...	1240	1240	100%	0.0	99.85%	7705	LT841208.1
Fusarium oxysporum Fo47 chromosome IV	Fusarium oxysp...	1234	3704	100%	0.0	99.70%	4731052	CP052041.1
Fusarium oxysporum strain 149 small subunit ribosomal RNA gene, partial sequence; internal transcribed spa...	Fusarium oxysp...	1234	1234	100%	0.0	99.70%	2056	MK828120.1
Fusarium oxysporum f. sp. dianthi partial 18S rRNA gene for 18S ribosomal RNA, strain Fod008	Fusarium oxysp...	1234	1234	100%	0.0	99.70%	7875	LT841236.1
Fusarium oxysporum f. sp. dianthi partial 18S rRNA gene for 18S ribosomal RNA, strain Fod001	Fusarium oxysp...	1234	1234	100%	0.0	99.70%	7875	LT841222.1
TPA: Fusarium oxysporum f. sp. cubense race 4 strain B2 rDNA repeat region	Fusarium odorati...	1234	1234	100%	0.0	99.70%	7872	LT571434.1
Fusarium oxysporum Fo5176 chromosome 2	Fusarium oxysp...	1229	2458	100%	0.0	99.56%	5036271	CP053261.1
Uncultured Basidiomycota clone MO1_A01 18S ribosomal RNA gene, partial sequence; internal transcribed s...	uncultured Basid...	1229	1229	100%	0.0	99.55%	1375	EU490155.1
Fusarium sp. GUf-2 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, ...	Fusarium sp. G...	1221	1221	100%	0.0	99.41%	1131	LC150822.1
Fusarium oxysporum strain CBS 127149 small subunit ribosomal RNA gene, partial sequence; internal transc...	Fusarium oxysp...	1216	1216	98%	0.0	99.70%	682	MH864441.1

BLAST results ofSample 6

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Fusarium oxysporum isolate AFIC15 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1...	Fusarium oxysp...	1138	1138	100%	0.0	100.00%	1052	KU872849.1
Fusarium cf. udum strain GFR19 small subunit ribosomal RNA gene, partial sequence; internal transcribed sp...	Fusarium cf. udum	1127	1127	100%	0.0	99.68%	679	MT447524.1
Fusarium udum f. sp. crotalariae isolate F-2 18S ribosomal RNA gene, partial sequence; internal transcribed s...	Fusarium udum ...	1127	1127	100%	0.0	99.68%	655	KY706082.1
Fusarium udum f. sp. crotalariae isolate F-1 18S ribosomal RNA gene, partial sequence; internal transcribed s...	Fusarium udum ...	1127	1127	100%	0.0	99.68%	679	KY706081.1
Fusarium oxysporum isolate AFIC3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, ...	Fusarium oxysp...	1127	1127	100%	0.0	99.68%	802	KU872814.1
Hypocreales sp. LM171 18S ribosomal RNA gene, partial sequence	Hypocreales sp. ...	1107	1107	100%	0.0	99.03%	999	EF060524.1
Fusarium fujikuroi strain Augusto2 chromosome II	Fusarium fujikuroi	1105	1105	100%	0.0	99.03%	5014829	CP023090.1
Fusarium fujikuroi strain I1.3 chromosome II	Fusarium fujikuroi	1105	1105	100%	0.0	99.03%	5092212	CP023102.1
Fusarium fujikuroi strain CSV1 chromosome II	Fusarium fujikuroi	1105	1105	100%	0.0	99.03%	5023961	CP023078.1
Fusarium sp. strain SMG04 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer ...	Fusarium sp.	1105	1105	100%	0.0	99.03%	832	MK355724.1

BLAST results of Sample 7

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Fusarium sp. isolate S106 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Fusarium sp.	1232	1232	99%	0.0	99.85%	947	MF076600.1
Fusarium equiseti strain A-JUN_17_011 small subunit ribosomal RNA gene, partial sequence; internal tra...	Fusarium equiseti	1230	1230	99%	0.0	99.85%	778	MK334366.1
Fusarium sp. strain GFR06 small subunit ribosomal RNA gene, partial sequence; internal transcribed spa...	Fusarium sp.	1225	1225	99%	0.0	99.70%	898	MT447511.1
Cladosporium sp. strain 50 small subunit ribosomal RNA gene, partial sequence; internal transcribed spa...	Cladosporium sp.	1225	1225	99%	0.0	99.70%	2069	MK828117.1
Fusarium sp. RGT-S4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S rib...	Fusarium sp. R...	1225	1225	99%	0.0	99.70%	898	HQ674657.1
Hypocreales sp. LM512 18S ribosomal RNA gene, partial sequence	Hypocreales sp. ...	1225	1225	99%	0.0	99.70%	937	EF060807.1
Uncultured fungus clone CMH023 18S ribosomal RNA gene, partial sequence; internal transcribed spacer...	uncultured fungus	1223	1223	99%	0.0	99.70%	757	KF800114.1
Uncultured Ascomycota clone 4S2_D01 18S ribosomal RNA gene, partial sequence; internal transcribed...	uncultured Asco...	1219	1219	99%	0.0	99.55%	1629	EU490021.1
Fusarium sp. 08006 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribos...	Fusarium sp. 08...	1219	1219	99%	0.0	99.70%	910	EU750676.1
Fusarium sp. 13002 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribos...	Fusarium sp. 13...	1218	1218	99%	0.0	99.70%	921	EU750679.1

Fig.4: BLAST Alignment

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