MOLECULAR STUDY ON CLADOSPORIUM SPECIES ISOLATED FROM AIR OF CAIRO, USING UNIVERSALLY PRIMED-PCR (UP) AND INTERNAL TRANSCRIBED SPACER (ITS) PCR TECHNIQUES

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ABSTRACT

Universally Primed-PCR (UP-PCR) and Internal Transcribed Spacer-PCR (ITS-PCR) based genomic fingerprinting techniques are considered a good methods that rely on specifically targeted primers. These techniques, which analyse the rDNA, have been shown to be relatively robust and discriminatory. This study was designed to investigate and characterize the molecular variation among Cladosporium strains collected at different sites in Cairo by using two different fingerprinting methods, Universally Primed-PCR (UP-PCR) and Internal Transcribed Spacer (UP-PCR) technique. The Cladosporium isolates investigated were isolated from air of Cairo by settle plate method. The samples were then purified and identified by using culture based techniques, microscopical methods, and biochemical reactions followed by confirmation in the regional center for mycology and biotechnology (RCMB). Molecular fingerprinting, and genetic similarities among Cladosporium species populations depending on microsatellites-polymerase chain reaction (ITS-PCR). Primers used are ITS4, and ITS5. PCR products were digested with 3 restriction enzymes and separated by agarose electrophoresis. Restriction patterns generated by CfoI, MspI and RsaI. In addition, we have applied the Universally Primed PCR (UP-PCR) technique using two primers L21 and Fok1. The current work showed prominent discriminatory power given by amplification of internal transcribes spacers PCR regions followed by restriction with CfoI enzyme than other endonucleases. moreover, Fok1 primer revealed minor variability among Cladosporium strains using UP-PCR genotyping technique.

Key words: Molecular study, Cladosporium, Universally Primed-PCR (UP-PCR), Internal Transcribed Spacer-PCR (ITS-PCR)
INTRODUCTION

*Cladosporium* species are well known for their production of substances with antimicrobial activities, several of which have formed the basis for the development of new clinically important antimicrobial agents.

The genus *Cladosporium* is one of the most important group of fungi which includes many saprophytic and some pathogenic species. Also, its ability to biodegrade some aromatic compounds in industry has been well established. The dematiaceous fungi have been recognized as a possible useful source of bioactive secondary metabolites, especially in anticancer application (Ibrahim *et al.*, 2018). Universally primed PCR (UP-PCR) is a PCR fingerprinting method similar to the well-known RAPD technique in that it is possible to amplify DNA from any organism without previous knowledge of DNA sequences and to generate multibanding profiles (fingerprints) following gel electrophoresis. Some of the advantages of UP-PCR are the use of relatively high annealing temperatures, fast ramping and relatively long primers, features that seem to enhance the reproducibility which many have found to be problematic with RAPDs (Lubeck *et al.*, 2005). Other advantages are the resulting banding profiles which consist of higher numbers of bands than most RAPDs, facilitating identification of specific markers, and at the same time showing species conservative bands (Demissie *et al.*, 2019). A variant of the UP-PCR technique is UP-PCR product cross hybridization assay that facilitates investigation of sequence similarity (homology) of UP-PCR products. This allows grouping of strains into UP-PCR hybridization groups which we use to separate the strains into genetic entities featured by high genetic similarity (DNA homology) (Bulat *et al.*, 1998). The internal transcribed spacer (ITS) region is well suited for comparison of closely related organisms. This non-coding region is highly polymorphic and provides a useful tool for taxonomic and phylogenetic studies. ITS regions vary between species within the same genus, but show little or no intraspecific variation. Hence, they have been used in many phylogenetic studies of various fungi (White *et al.*, 1990). The internal transcribed spacer (ITS) region is well suited for comparison of closely related organisms. Analysis of the ITS region has been used to distinguish species and higher taxonomic divisions. DNA fingerprint has been used to study several genera. Restriction Fragment Length Polymorphism (RFLP) of the ITS region has been used to distinguish between species of the same or among different genera. Many species among different genera have been studied (Bernier *et al.*, 1994). ITS1 and ITS2 separates the 18S, 5.8S and 28S genes and an external transcribed spacer (ETS) that is located up stream of the 18S gene. The transcribed spacers contain signals for processing rDNA transcript. Nearby copies of the rDNA repeat unit are separated by a nontranscribed spacer (NTS), also called intergenic spacer (IGS) by some workers. This region contains subrepeating elements that serve as enhancers of transcription. The ETS and ITS are removed during rRNA processing (Kamle and Ali, 2013). The aim of this study to explore the genetic variation between various *Cladosporium* strains isolated through this investigation using DNA target such as internal transcribed spacer - polymerase chain reaction (ITS-PCR) and Universally primed-PCR (UP-PCR) techniques.
MATERIALS AND METHODS

1. Collection of Cladosporium samples

Air samples were collected in Cairo city at 5 distinct geographical regions during all climate seasons from June 2017 to May 2019, using Passive sampling “settle plates” method, the petri dish containing Malt Extract Agar (MEA) and Sabouraud Dextrose Agar (SDA) and supplemented with chloramphenicol (100 mg/L) and gentamicin (40 mg/L) (Schubert et al., 2007 and Bensch et al., 2012).

2. Culture media used in the current study

In the current study, multiple culture media are used for collection, isolation, purification and preservation of Cladosporium species including, Dichloran glycerol agar DGA (MERCK) Sabouraud Dextrose Agar (SDA) (Oxoid), Malt Extract Agar (MEA) (MERCK), Potato Dextrose Agar (PDA) (Oxoid-USA) or Czapek Dox Agar (CDA) (MERCK), were prepared according to the supplier’s instructions at pH 6.8. Culture media were incubated at 30 °C for 7-14 days and examined after 4 days (Asl et al., 2017).

3. Identification of Cladosporium species

Cladosporium isolates were identified based on culture characterization, macroscopic, microscopic properties according to Crous et al. (2007), and biochemical reactions such as starch hydrolysis test, casein hydrolysis test and gelatin hydrolysis test, according to Rodarte et al. (2011) the identification was confirmed at the Regional Center of Mycology and Biotechnology (RCMB), Al-Azhar University.

4. Purification and preservation of Cladosporium species

The purification procedure of the fungal isolate under investigation was carried out by the agar streak plate method. All expected colonies of Cladosporium forms on the growth medium were picked up and re-streaked onto the agar surface of plates containing the same medium. Pure colony of Cladosporium isolates only were sub-cultured and stored on slants of Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA) media at 5 °C and kept for further investigation. Pure cultures were stored in refrigerator at 4 °C and sub-cultured periodically each 3 months (Jin et al., 2012).

5. Cladosporium DNA extraction

DNA was extracted using fungal DNA extraction kit (PrepSEQ, Thermo-Scientific, USA) according to developer instructions as follow: Extraction Buffer was warmed to 65 °C at water bath. Fifty mg of the grounded mycelia were transferred to 2 ml microtube. This microtube contains 400µl warmed extraction buffer and 6µl RNAse A. The microtube was incubated at 65°C for 10 minutes. The microtube was gently shaken every 5 minutes intervals. After that, 130µl from sodium acetate solution was added and the microtube was incubated at -20°C for 10 minute. The lysate was centrifuged at 10000 rpm at 4°C for 15 min. The upper aqueous phase was decanted into fresh centrifuge tubes. An equal volume of isopropanol was added, the microtube was
stored at room temperature for 7 minutes and then spun at 6000 rpm for two minutes at 4°C. The DNA pellet was washed two times with 70% Ethanol (700 µl) and centrifuged at 8000 rpm for 1 minute, vacuum dried and dissolved in 100µl of warmed TE buffer. DNA concentration and purity was checked on an 1.5% agarose containing 0.05 μg ml⁻¹ ethidium bromide, using (5µl of each sample+3µl gel loading dye + 3µl ultrapure water), 10µl was loaded at each well, 5-6µl of DNA marker 100bp was run. Photograph were taken after 30 minute. Finally, extracted DNA was stored at -20 °C (Weising et al., 1995; Hajkova et al., 2006)

6. Universally Primed PCR (UP-PCR) amplification condition

Universally Primed-PCR (UP-PCR) was conducted in a 25-µl reaction volume. Three µl of DNA template (1 ng quantified with a spectrophotometer) was added to a 7µl Master mix of thermostable DNA polymerase, Jena Bioscience GmbH, Cat.-No.PCR-101S. Taq Master/high yield contains all reagents required for PCR (except template and primer) in a premixed 5x concentrated ready-to-use solution for PCR (Thermostable DNA polymerase, dATP, dCTP, dGTP, dTTP, reaction buffer with (NH₄)₂SO₄, MgCl₂ and Triton X-100, stabilizers), 13µl of PCR grade water (Jena Bioscience GmbH), 2µl of 20 pmol Universally Primed primer. The used Universally Primed primers were; L21 (5`-GGATCCGAGGGTGGCTCT-3`), and FOK-1 (5`-GGATGACCCACCTCCTAC-3`). Primers were brought in from Metabion International AG GmbH. Using a DNA thermal cycler (Techne TC-312, Techne, Stone, UK) thermal cycling parameters were initial denaturation at 94°C for 4 min, followed by 35 cycles consisting of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 2 min. A final extension at 72°C for 7 min followed. PCR product was checked on an 1.5% agarose containing 0.05 μg ml⁻¹ ethidium bromide, using (5µl of each sample + 3µl gel loading dye + 3µl ultrapure water), 10µl was loaded at each well, 5- 6µl of DNA marker 100 bp was run. Photographs were taken after 30 minute (Lubeck et al., 2005).

7. Internal Transcribed Spacer-PCR (ITS-PCR) amplification condition.

The same as in UP-PCR with exception of using 2µl of 10 pmol of ITS4 primer, and 2 µl of 10 pmol of ITS5 primer. Primers were supplied by GE Healthcare. Using a DNA thermal cycler (Techne TC-312, Techne, Stone, UK) thermal cycling parameters were initial denaturation at 94°C for 5 minutes, followed by 35 cycles consisting of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 2 minutes, then final extension at 72°C for 10 minutes. PCR product was checked on an 1.5% agarose containing 0.05 μg ml⁻¹ ethidium bromide, using (5µl of each sample + 3µl gel loading dye + 3µl ultrapure water), 10µl was loaded at each well, 5- 6µl of DNA marker 100 bp was run. Photographs were taken after 30 minutes (Abd-Elsalam et al., 2007).

7.1. Digestion of ITS-PCR Product with restriction enzymes.

Internal transcribed spacer fragments were digested with three base cutter restriction enzymes: CfoI, MspI, and RsaI. Fifteen microliters of amplified DNA were digested for 2 hours at 37°C using a DNA thermal cycler (Techne TC-312, Techne, Stone, UK) as follow; 15 µl of ITS-PCR Product, 1 µl of enzyme (U), 2.5 µl of buffers provided
by the manufacturer, and 2.5 µl of PCR grade water (Jena Bioscience GmbH). The resulting DNA restriction was electrophoresed on 1.5% agarose containing 0.05 µg ml⁻¹ ethidium bromide (Agarose 25). DNA patterns were visualized and photographed under ultraviolet light (UV) (Troche, 1997).

8. Statistical analysis and software used in the current investigation.

Levels of genetic similarity between Microsatellite Primed (MP) and Universally Primed (UP) PCR fingerprints were calculated by using Pearson product-moment correlation coefficient. The samples were clustered using the unweighted pair group method of arithmetic average (UPGMA) which resulted in a Dendrogram by website [http://genomes.urv.es/UPGMA/](http://genomes.urv.es/UPGMA/), genetic similarity between Cladosporium strains calculated using Pearson product-moment correlation coefficient (Aamir et al., 2015).

**RESULTS**

1. **Isolation and identification of Cladosporium species**

A total number of 886 fungal colonies were collected, of 212 Cladosporium isolates of 10 different species with isolation rate 23.9%, depending on culture properties, microscopical examination and biochemical properties, a total 10 species of Cladosporium were identified which are: C. asterinae, C. uredinicola, C. acacicola, C. macrocarpum, C. herbarum, C. nigrillum, C. oxysporum, C. cladosporioides, C. sphaerospermum and C. chlamydosporis. In the current work, the most predominant species was C. herbarum, with 38 isolates represented by 17.9% of total collected isolates, followed by C. cladosporioides (28 isolates) 13.2% and C. oxysporum (27 isolates) 12.7 %, followed by followed by C. acaciicola and C. sphaerospermum that were identified with rate 10.4 % (22 isolate) for each, while C. chlamydosporis, C. nigrillum and C. uredinicola were isolated in lowest isolates rates 5.66 %, 6.6 % and 7.8 % (12, 14 and 15 isolates) respectively, at collection sites as showed in table (1).

| Serial | Cladosporium Species | Number of isolates | Frequency (%)* |
|--------|-----------------------|--------------------|----------------|
| 1      | C. herbarum           | 38                 | 17.9           |
| 2      | C. cladosporioides    | 28                 | 13.2           |
| 3      | C. oxysporum          | 27                 | 12.7           |
| 4      | C. acacicola          | 22                 | 10.4           |
| 5      | C. sphaerospermum     | 22                 | 10.4           |
| 6      | C. macrocarpum        | 18                 | 8.49           |
| 7      | C. asterinae          | 16                 | 7.55           |
| 8      | C. uredinicola        | 15                 | 7.08           |
| 9      | C. nigrillum          | 14                 | 6.6            |
| 10     | C. chlamydosporis     | 12                 | 5.66           |
| **Total** |                     | **212**            | **100%**       |

*Percentages were correlated to the total number of Cladosporium isolates (212)

2. **Molecular characterization of Cladosporium strains**

Thirty Cladosporium strains selected on the basis of biodiversity in which they selected from different geographical, climate and biological conditions, as shown in table (2).
Table 2: *Cladosporium* strains used in the molecular study.

| No. | *Cladosporium* Species | Region of collection | Site of collection | Time of collation |
|-----|------------------------|----------------------|-------------------|-------------------|
| 1   | *C. chlamydosporis* HMA-13 | East Cairo | El Salam city | December |
| 2   | *C. herbarum* HMA-36 | East Cairo | El Salam city | March |
| 3   | *C. chlamydosporis* HMA-M | Downtown | Mokattam | December |
| 4   | *C. sphaerospermum* HMA-265 | West Cairo | El Zamalek | November |
| 5   | *C. macrocarpum* HMA-109 | East Cairo | Ain shams | June |
| 6   | *C. asterinae* HMA-188 | East Cairo | Nasr City | March |
| 7   | *C. oxyssporum* HMA-10 | East Cairo | El Salam city | March |
| 8   | *C. asterinae* HMA-300 | North Cairo | El Matarya | April |
| 9   | *C. herbarum* HMA-N | Downtown | Mokattam | March |
| 10  | *C. oxyssporum* HMA-M2 | Downtown | Mokattam | October |
| 11  | *C. cladosporioides* HMA-731 | Southern Cairo | Dar el salam | January |
| 12  | *C. asterinae* HMA-566 | North Cairo | Shobra | October |
| 13  | *C. cladosporioides* HMA-132 | East Cairo | Ain shams | November |
| 14  | *C. uredinicola* HMA-216 | West Cairo | El Zamalek | October |
| 15  | *C. acaciicola* HMA-235 | West Cairo | Bolaq | March |
| 16  | *C. nigrillum* HMA-225 | North Cairo | Shobra | December |
| 17  | *C. nigrillum* HMA-221 | West Cairo | El Zamalek | January |
| 18  | *C. uredinicola* HMA-59 | East Cairo | El Nozha | July |
| 19  | *C. oxyssporum* HMA-680 | Downtown | Mokattam | September |
| 20  | *C. herbarum* HMA-348 | North Cairo | Shobra | April |
| 21  | *C. cladosporioides* HMA-232 | West Cairo | Kasr El Nile | February |
| 22  | *C. macrocarpum* HMA-144 | West Cairo | Bolaq | February |
| 23  | *C. chlamydosporis* HMA-870 | Downtown | Mokattam | November |
| 24  | *C. sphaerospermum* HMA-62 | East Cairo | El Nozha | September |
| 25  | *C. macrocarpum* HMA-410 | Southern Cairo | El Maadi | December |
| 26  | *C. sphaerospermum* HMA-268 | West Cairo | El Zamalek | July |
| 27  | *C. acaciicola* HMA-238 | West Cairo | Kasr El Nile | April |
| 28  | *C. cladosporioides* HMA-285 | West Cairo | El Zamalek | December |
| 29  | *C. sphaerospermum* HMA-182 | West Cairo | Bolaq | October |
| 30  | *C. cladosporioides* HMA-407 | Southern Cairo | El Maadi | January |

2.1. Molecular variation of *Cladosporium* species using Universal primed PCR (UP-PCR) technique.

2.1.1. UP-PCR patterns using L21 primer

The dendrogram revealed 8 well-separated clusters, each one corresponding to a different species. The genetic similarity between *Cladosporium* strains calculated using Pearson product-moment correlation coefficient, genetic similarities oscillated between 42% to 71% for inter-specific and 71% to 100% for intra-specific comparisons. UP-PCR markers detected a very high level of polymorphism between and among *Cladosporium* strains as shown in figure (1).
Figure 1: The Dendrogram of Cladosporium isolates was constructed after cluster analysis of L21-PCR marker with the UPGMA

2.1.2. UP-PCR patterns using Fok1 primer

The obtained dendrogram depicts that all isolates were separated from each other into 2 major distinct groups and 4 minor groups with genetic similarity between Cladosporium species isolates ranged from 26 to 53 % for inter-specific and 53 to 100% for intra-specific comparisons as shown in figure (2).
2.2. Molecular variation of *Cladosporium* species using ITS-PCR technique

2.2.1. ITS-PCR Patterns of *Cladosporium* species digested with *MspI* Restriction Enzyme.

In the current investigation, using a universal fungal rRNA primer pair, a 743 bp fragment was successfully amplified from all *Cladosporium* isolates. Results of the current investigation showed moderate genetic similarity between *Cladosporium* isolates that calculated using Pearson product-moment correlation coefficient which ranged from 50-70 % for inter-specific and 70-100 % for intra specific comparisons. The dendrogram constructed with ITS-PCR digested with *MspI* revealed that all isolates of *C. cladosporioides* and almost *C. herbarum* were grouped into a major 9 cluster delimited from other *Cladosporium* species comprising four molecular groups with genetic dissimilarity 30% as represented in figure (3).
Figure 3: The Dendrogram of *Cladosporium* isolates was constructed after cluster analysis of the digitized amplicons with *Msp*I using UPGMA

2.2.2. ITS-PCR patterns of *Cladosporium species* digested with *Rsa*I restriction enzyme.

The genetic similarity between *Cladosporium* strains calculated using Pearson product-moment correlation coefficient was ranged from 20 to 42% for inter-specific and 42 to 100% for intra-specific comparisons. The application of UPGMA clustering produced two large clusters within the population with a branched–off at genetic similarity 20%, each consisting of several subclusters (phenons). Dendrogram of the ITS-PCR patterns digested with *Rsa*I separated the isolates of *Cladosporium* species into 3 main clusters, as represented by figure (4).
2.2.3. ITS-PCR Patterns of *Cladosporium* species digested with *Cfo*I restriction enzyme.

The genetic similarity between *Cladosporium* species isolates ranged from 30 to 53% for inter-specific and 53 to 100% for intra-specific comparisons. The application of UPGMA clustering produced two large clusters within the population with a branched-off at genetic similarity of GS=29%, each consisting of several subclusters (phenons). Dendrogram of the ITS-PCR patterns digested with *Cfo*I separated the isolates of *Cladosporium* species into two main clusters as shown in figures (5).
Figure 5: The Dendrogram of Cladosporium isolates was constructed after cluster analysis of the digitized ITS4/ITS5-PCR and digested with CfoI using UPGMA

DISCUSSION

UP-PCR markers were used to estimate the genetic relatedness among Cladosporium isolates. Two Universal Primed (UP) primers, individually were tested for the ability to distinguish Cladosporium strains. In the present study, molecular variation using Universal Primed PCR (UP-PCR) obtained by L21 primer revealed two main well-separated clusters, each one corresponding to a different species. The genetic similarity between Cladosporium strains, similarities oscillated between 42% to 71% for inter-specific and 71% to 100% for intra-specific comparisons. UP-PCR markers detected a
very high level of polymorphism between and among *Cladosporium* strains. The genetic similarity by UP-PCR using Fok1 primer was more than 55% between *Cladosporium* species isolates ranged from 26 to 53 % for inter-specific and 53 to 100% for intra-specific comparisons. Our observations were comparable with that of Maymon *et al.*, (2004), who reported 3 main different clusters by 76 *Trichoderma* species (genus of dematiaceous fungi closely related to *Cladosporium*) In the same context, Bulat *et al.* (2000), concluded that there is another possible direction for development of gene-specific typing should be noted. In addition Hafez *et al.* (2013); Chakdar *et al.* (2017), reported that universally primed PCR (UP-PCR) has been used to discriminate isolates of *Cladosporium lecanii* and *Fusicladium effusum*. This allows grouping of strains into hybridization groups which can be used to separate the strains into genetic entities with high genetic similarity. Moreover, based on similarity values, UP-PCR is a PCR characterization method that it is possible to amplify DNA from any organism without previous knowledge of DNA sequences and to generate multibanding profiles following gel electrophoresis (Ashfaq *et al.*, 2020). Furthermore these results was much comparable with that reported by Demissie *et al.* (2019), who reported that nonspecific primers reveal variability of some isolates, the corresponding PCR technique can be used to fingerprinting *Cladosporium* isolates.

In the current study, we have used the internal transcribed spacer (ITS) to aid compare ITS in length and restriction patterns. The internal transcribed spacer (ITS) was amplified using polymerase chain reaction combining primers ITS4 and ITS5. PCR products were digested with three restriction enzymes and separated by agarose electrophoresis. In the current investigation, using a universal fungal rRNA primer pair, a 743 bp fragment was successfully amplified from all *Cladosporium* isolates. This result was consistent with that of Asl *et al.* (2017) by using a universal fungal rRNA primer pair, a 700–800bp fragment was successfully amplified from all the isolates, while no PCR amplification was observed in negative controls. In addition, these observations were in the same line with that of Dean *et al.* (2005), who analyzed the genera *Stachybotrys*, *Penicillium*, *Aspergillus*, and *Cladosporium* in order to identify and characterize by simple ITS method, in which each organism underwent ITS-PCR that amplified ribosomal sequences generating products from 550 to 600 bp followed by enzymatic digestion with *EcoRI*, *HaeIII*, *MspI*, and *Hinfl*, and show that using this combination of restriction enzymes enables the identification of these fungal organisms at the species level. Results of the current investigation showed moderate genetic similarity between *Cladosporium* isolates ranged from 50-70 % for inter-specific and 70-100 % for intra-specific comparisons. The present results indicate that, the dendrogram constructed with ITS-PCR digested with *MspI* revealed that all isolates of *C. cladosporioides* and almost *C. herbarum* were grouped into a major 9 cluster delimited from other *Cladosporium* species comprising four molecular groups with genetic dissimilarity 30%. These results were similar to that of Kawasaki *et al.* (1993) that was conducted on *Cladosporium carrionii* and classified the 38 isolates into 4 mtDNA types (Type I to Type IV) based on the restriction patterns with *MspI*, *Sau3AI* and *HaeIII*. ITS-PCR digested with *RsaI* revealed that the genetic similarity between *Cladosporium* species isolates ranged from 20 to 42 % for inter-specific and 42 to 100 % for intra-specific comparisons. The application of UPGMA clustering produced 3 large clusters within the population with a branched–off at genetic similarity of GS=20 %, each consisting of several sub clusters (phenons). Dendrogram of the ITS-PCR patterns
digested with Rsal separated the isolates of Cladosporium species into two main clusters. The genetic similarity between Cladosporium isolates ranged from 50 to 68% for inter-specific and 68 to 100% for intra-specific comparisons, these results were inconsistent with Segers et al. (2015) who reported very low genetic similarity (23% for inter-specific comparisons) among Cladosporium species using Rsal restriction enzyme after amplification of ITS. The average of genetic similarity based on ITS-PCR patterns digested with CfoI was approximately 50%. Dendrogram of the ITS-PCR patterns digested with CfoI separated the isolates of Cladosporium species into 8 main clusters. The first main cluster included all Cladosporium cladosporioides isolates at the genetic similarity (GS=76 %). These observations were consistent with Moslem et al. (2010) study that revealed moderate genetic similarity among Cladosporium species using some restriction enzymes included CfoI. On contrary, Messini et al. (2017) study that showed very low genetic similarity 27% and high molecular heterogeneity among Cladosporium species using CfoI for restriction of ITS amplicons. Our results were consistent with those based on biological characteristics and morphological features. In harmony with our results, ITS molecular marker technique of ITS region represents a possible method for the classification of Cladosporium species (Troche, 1997). Additionally, the ITS restrictions profiles showed a great genetic similarity between C. cladosporioides and almost of C. sphaerospermum isolates. In the study carried out by Park et al. (2004) the sequences of the D1/D2 regions of the LSU rDNA genes and the ITS regions of the rDNA were employed in order to establish molecular standards for the demarcation of the common airborne species C. herbarum, C. cladosporioides and C. sphaerospermum.

CONCLUSION

From the current work, we can conclude that ITS molecular marker technique of ITS region represents a possible method for identification of Cladosporium on genus and species level, in addition, subsequent digestion with Rsal was more beneficial than CfoI, MspI, enzymes as a tool for discrimination between Cladosporium strains within the same species. Moreover, UP-PCR is a simple method for genotyping of Cladosporium species especially when L21 primer used, over than Fok1.

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دراسة ميكروبيولوجية على سلالات الكلاذوسبوريوم المعزول من هواء القاهرة. 

تعني تقنيات طرق تفاعل البلمرة المتسلسل للوحدات

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فطر كلادوسبوريوم هو واحد من أكبر أجناس الفطريات و أكثرها انتشاراً. وذلك بسبب إنتاجها أ[Boolean]وبها متفرعة ووفرة و أيضاً بسبب القدرة على الهبوط على أدق المعطيات الغذائية تستطيع الطموح في أي مكان تجريبي. ومن أشهر السلالات في هذه الدراسة تم عزل C. elatum و C. herbarum, sphaerospermum, C. cladosporioides و C. elatum.

تم استخدام نماذج مختلفة وظروف متباينة من القاهره، وعرضت هذه النماذج للعزل والتكاثر والتعريض على فطر كلاذوسبوريوم باستخدام الوسائط الالتهابية والجهازية و أيضاً اعتماداً على نتائج التفاعلات الكيميائية. وتم تاكيد تعريف الفطريات في المركز الإقليمي للفطريات والتقنية الحيوية بجامعة الأزهر. تهدف الدراسة الحالية لبحث النتائج البيولوجية والجزئية بين سلالات فطر كلاذوسبوريوم وذلك باستخدام تقنيات تفاعل البلمرة المتسلسل (UP-PCR) ومعرفة مدى الاستخدام في استخدام نماذج بادئ L21 و Fok1. كما يشير العمل إلى أن فريق MP-PCR و UP-PCR مناسب للمقارنة بين الكائنات الحية ذات الصلة السغالة. استخدمت هذه النماذج غير المشتركة للغة وتتوفر آداً لمقدمة للدراسات الكيميائية والتاريخية. تم استخدام تحليل منطقة نمتان الذكية لتمييز أنواع الفطريات المختلفة. تم استخدام تحليل منطقة ITS (ITS-PCR) في الدراسة لدراسة عدة أنواع. تم استخدام تعدد أشكال طول القطع (RFLP) لتمييز بين أنواع من نفس النوع ITS. كانت هناك تفاعلات PCR مختلفة في الطول ومناطق الفصل. باستخدام تقنيات البلمرة المتسلسل (ITS-PCR) تبين أن النماذج تمكنت كائنات أصلية من إنشاء تفاعلات PCR في ITS4 و IT5.

في الدراسة ITS-PCR، كانت النتائج متباعدة مع ذلك القائمة على الخصائص البيولوجية والسمات الطبيعية. و هذا أدت إلى جيدة واضحة للзвездات على مستوى الأنواع. تم إنشاء ملفات تعريف سمات جينية التي تم تميزها بسهولة بين كل من الأنواع المتميزة.

الكلمات المفتاحية: دراسة ميكروبيولوجية. تفاعل البلمرة المتسلسل. Internal Transcribed Spacer. Universally Primed.