

Full Length Research Paper

Molecular ecological typing of wild type *Aspergillus terreus* from arid soils and screening of lovastatin production

Zaidan. K. Imran* and Azal Alaa Al Rubaiy

All Women College of Science, Babylon University, Hilla, Iraq.

Received 18 October, 2014; Accepted 8 December, 2014

Arid soils are complex ecosystem that maintains topographically distinct mycoflora populations. A total of 45 soil samples collected from the arid soils in Iraqi desert were cultured by dilution plate method and screened for *Aspergillus terreus*. The aim of this study was to enhance ecological knowledge of cultured colonies variation of *A. terreus* as well as environmental interactions in arid soils. An attempt is made to comprehensively screen desert soil for the wild type *A. terreus* producing lovastatin. The results show that the most frequent *Aspergillus* spp. included: *A. niger* (159 isolates), *A. terreus* (143), *A. flavus* (115) and *A. fumigates* (42) and other fungi. Genetically, the diagnoses of 19 isolates of *A. terreus* were in the scope of our interest. The specific primer pair had monomorphic bands of approximately PCR product of 450 bp. Ribotyping isolates of *A. terreus* with primer pairs (ITS1/ITS4 and ITS1/ITS2) were 19 isolates, with a single PCR product of 550-600 bp and 280-380 bp, respectively. RAPD-PCR was also used to distinguish between ecological patterns generated and allowed a distinction of very closely related environmental isolates. Lovastatin production was carried out with thin layer chromatography. Results suggest that phenotypic variations in *A. terreus* isolates were not useful for identifying them, and showed no significance in the identification in all the sites studied. However, using discriminatory molecular methods, such as amplification of the targeted regions by specific and universal characterization of the isolates could be pivotal in understanding ecological variation. Wild type soil isolates of *A. terreus* have the ability to produce lovastatin.

Key words: Molecular ecological typing, *Aspergillus terreus*, arid soils, lovastatin, Iraq.

INTRODUCTION

Aspergillus terreus is a common fungus, which has an important role in desert soils (Amin et al., 2010). Arid soil is complex ecosystem with topographically distinct mycofloras. Its microbial communities are subjected to surface heating, cycles of rainfall and extreme desiccation,

changing water table, CO₂ enrichment and UV light supplementation and attenuation; it is directly exposed to sun light due to the absence or abundance of vegetation variation (Lipson et al., 1999).

A. terreus is a widely dispersed species among the soil

*Corresponding author. E-mail: zaidan_omran@yahoo.com. Tel: 009607801309448.

mycoflora; it belongs to the genus *Aspergillus*, and of the sub-genus *Terrei Nidulantes* (Raper and Fennell, 1965; Varga et al., 2005). Many isolates of this fungus show morphological variation in growth criteria (Rath et al., 1999). Most of the *A. terreus* isolates produce a variety of secondary metabolites that are of pharmaceutical significance; they are used for producing lovastatin drug (Casas L'opez et al., 2003). Morphological characters include making weak classification at the species and genus levels, while molecular methods are powerful tools in species identification (Hong et al., 2008).

In advanced fungal molecular studies, ribosomal marker genes have been used, such as universal genes found in all fungi. Fungal communities are commonly genotyped by the multiple genes of rDNA (ITS, IGS, 18S and 25SrRNA, 25S and their introns) (Imran and Al-Asadi, 2014). These genes allow for phylogenetic analysis, and assist in taxonomic classification (Chase and Fay, 2009).

Various genotypic methods have been used to successfully do fingerprinting in several fungi such as *A. fumigatus*, *Fusarium solani* and *Candida* spp. (Crowhurst et al., 1991; Loudon et al., 1993; Nariasimhan and Asokam 2010, Imran and Al-Shukry 2014). Ribotyping analysis of the ITS region revealed detailed information about molecular analysis and showed reproducible polymorphism in several studies (Chase and Fay, 2009). Random Amplification of Polymorphic DNA (RAPD) assay revealed polymorphic DNA patterns of *Aspergillus* spp. (Anderson et al., 1995). Furthermore, RAPD-PCR assay is adapted for doing fingerprinting of fungi, as it reveals an accurate and simple method for differentiation among fungal isolates (Aubin et al., 1991; Symones et al., 2000; Nariasimhan and Asokan, 2010).

It is not clear whether the variations in the characteristics of the colony of *A. terreus* isolates are due to environmental response or genetic variation. As a result, there is the need for a reliable molecular ecological typing of *A. terreus* isolates in arid soils. Furthermore, the important roles played by these fungi in the arid regions include biodegradation, drug biosynthesis, recycling of materials and microbial activities and are potential source of novel pharmaceuticals (Amin et al., 2010). Few comparable data in the literature are available for *A. terreus* in the world (Lass-Florl et al., 2007).

The role of genetic variants on phenotypic traits often depends upon environmental and physiological conditions, but such gene-environment interactions are poorly understood. The beneficial role of soil mycoflora is the focus of this study.

Several studies have been attempted to evaluate the ability of *A. terreus* isolates to produce lovastatin drug (Alberts et al., 1980; Juzlova et al., 1996; Casas L'opez et al., 2003; Miyake et al., 2006). The wild and mutant strain of *A. terreus* appears to be the most commonly producer of lovastatin, although it uses various ways to produce biological and significant product (Novak et al., 1997; Hajjaj et al., 2001).

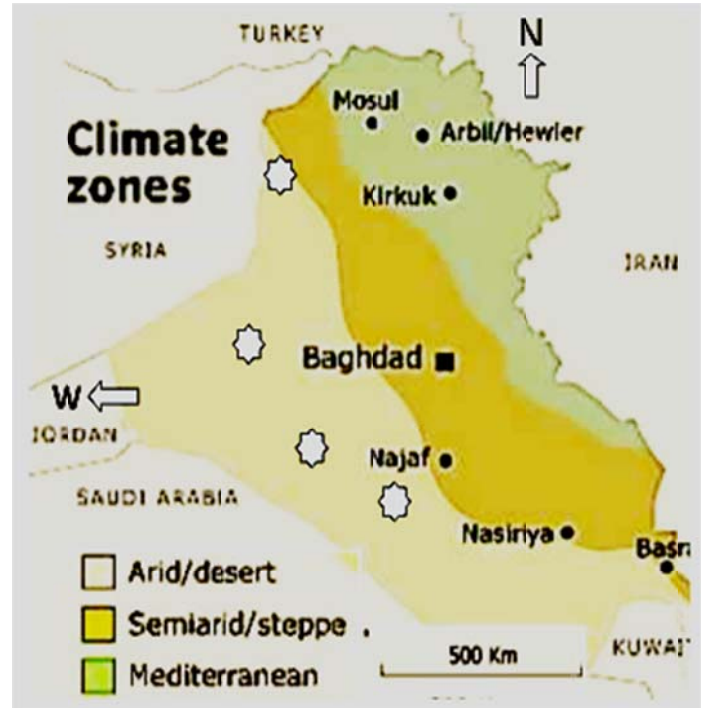


Figure 1. Climatic zones in the map of Iraq, () sampling sites of the arid soil for the survey of *A. terreus*.

Unfortunately, there is no information on the molecular ecology of fungal communities in the desert of Iraq and also there are no previous studies on the role of genetic variants on phenotypic traits of *A. terreus* based on environmental changes. The aim of our study was to evaluate the phenotypic and genotypic variations based on ITS typing region, using RAPD-PCR of the environmental isolates of *A. terreus*; it aimed to perform a phylogenetic analysis, explains their variations by molecular ecological diagnosis and how this understanding enables fungal diagnosis and screening of lovastatin produced by the wild type *A. terreus* cultures.

MATERIALS AND METHODS

Site descriptions and samples collection

The study area is located around the geographical coordinates of 33° 20' N. 44° 23' E. in Iraq. Western and southern Iraq cover a vast desert area of about 168,000 square km: south-west (Najaf and Kerbela provinces) and north-west (Ramadi and South Mosul provinces) (Figure 1). The common vegetation observed in the area includes: *Alhaji*, *Tamarix* and *Salsols*. Soil pH ranges from 6.8 to 8.8 (Guest and Al-Rawi, 1966).

A total of 45 soil samples (150-200 g each) were collected from October to April 2011-2012, in different localities in the desert regions. Soil temperatures were approximately around 5°C in January, 56°C in August and 37°C in October at the time the collections were made. Soil samples were taken from a depth of 5 cm and stored in polythene bags at 4°C.

Culturing and isolating of *A. terreus*

The pure cultures of *A. terreus* were isolated by the serial dilution technique using Potato Dextrose Agar (PDA) medium. Suspected yellow isolates of *A. terreus* were sub cultured on the PDA medium in separate triplicate plates for each isolate and incubated at 28°C for 7 days (Suhail et al., 2007). Microscopic examination was performed using mounted hyphal inoculums from the colony margins. This was done by using adhesive transparent tape placed on a slide with a drop of lacto phenol cotton blue stain. *A. terreus* isolates were identified phenotypically using the taxonomic key created by Raper and Fennel (1965). They were maintained on PDA slants at 28°C for four days and were kept in refrigerator at 4°C until use; they were sub cultured every two weeks. The frequency of a fungus is denoted by the number of samplings in which it is recorded against the total: Frequency (%) = No. of observation in which colony appears / total number of observation recorded x 100 (Adhikari et al., 2004).

Genomic DNA extraction

The culture media for each of the 19 *A. terreus* isolates were frozen for 1 h and tiny portions of the mycelia mat were harvested into 1.5 ml tube. The harvested mats were suspended in 400 µl of lysis extraction buffer (400 mM Tris-HCl, 20 mM EDTA, 150 mM NaCl, and 0.5% SDS adjusted 8.5 pH) then vortexed for 5 min and added to 10 µL proteinase K. Tubes were incubated in 65°C water bath overnight. A mixture of phenol: chloroform: Isoamyl alcohol (25:24:1) was added to the tubes. Tubes were centrifuged at 5000 rpm for 10 min. The aqueous supernatant was transferred to a new tube. An equal volume of cool isopropanol was added and agitated many times; it was centrifuged at 1000 rpm for 10 min. The supernatant was poured out. The pellet containing DNA was rinsed with 70% ethanol. It was air dried; pellets were re-suspended with 100 µL TE and placed in 70°C water bath. 6 µL of RNase A was added, and incubated at 37°C for 30 min. The tubes were centrifuged at 5000 rpm for 2 min. The supernatant was transferred to a new tube and frozen at -20°C until use (Saghai-Marooof et al., 1984; Edwards et al., 1991).

Genotyping of *A. terreus*

The 19 *A. terreus* isolates were confirmed by amplification, using specific pair primer: ATE1:CTA TTG TAC CTT GTT GCT GGCG; ATE2 :AGT TGC AAA TAA ATG CGT CGG CGG (Logotheti et al., 2009). Ribotyping of the targeted rDNA (ITS1 -5.8S-ITS2 region) of the 19 isolates was done with primer pairs of ITS1/ITS4 and ITS1/ITS2 that amplified ITS1-5.8S-ITS2 and ITS1 regions. The reaction was performed in a thermal cycler (LABENAT, USA) with 1.2 µL of each primer (20 pmole) and 0.8 µL of genomic DNA; the water was adjusted to a final volume of 25 µL. PCR protocol consisted of the initial denaturation at 95°C for 5 min; followed by 35 cycles at 94°C for 1 min, 59°C for 1 min, and 72°C for 1.5 min; and a final elongation of 72°C for 5 min. Finally, 10 µL of PCR products was loaded onto 1.2% agarose gel that was premixed with ethidium bromide stain (0.5 µg/ml) and TBE running buffer for 45 min at 100 V at room temperature. The products were visualized under a UV transilluminator and then photographed.

RAPD-PCR ecological typing

The primer R108 (5'-GTATTGCCCT-3'), described by Aufauvre-Brown et al. (1992), was used for RAPD-PCR typing of the 19 environmental isolates of *A. terreus*. Amplification reactions were done in a final volume of 20 µL containing 0.5 µL of genomic DNA, 1 µL Primer (50 pmole), and 12.5 µL master mix PCR buffer. Water

was adjusted to the final volume reaction. PCR was performed in a thermal cycler (LABENAT, USA) with the following temperature profile: 1 cycle of 5 min at 94°C, followed by 35 cycles of 45 s at 94°C, 45 s at 36°C and 1 min at 72°C and a final extension step at 72°C for 10 min. 10 µL of amplification products were loaded onto 2% agarose gel, which was premixed with ethidium bromide stain (0.5 µg/ml) and TBE running buffer for 1.30 h at 80 V at room temperature. The products were photographed using a UV transilluminator. (Lass-Flori et al., 2007).

Phylogenetic analysis

A phylogenetic tree dendrogram (UPGMA) of the 19 environmental isolates of *A. terreus* was constructed by using UVI band software, and the similarity coefficient factor was evaluated according to Ute et al. (1994).

Cultivation of the *A. terreus* isolates for production lovastatin

Three *A. terreus* isolates were selected to produce lovastatin. They were selected based on their color variation on agar plate. Production was performed in two complex media: The first culture was prepared in 250-ml Erlenmeyer flask containing 40 ml of medium A (10 g of glucose, 5 g of corn steep liquor, 40 g of tomato paste, 10 g of oatmeal, and 10 ml of trace elements, 1 g of FeSO₄·7H₂O, 1 g of MnSO₄·4H₂O, 200 mg of ZnSO₄·7H₂O, 100 mg of CaCl₂·2H₂O, 25 mg of CuCl₂·2H₂O, 56 mg of H₃BO₃, and 19 mg of (NH₄)₆Mo₇O₂₄·4H₂O per liter of solution (Alberts et al., 1980); it was inoculated with 1·10⁷ conidiospores. The flasks were shaken at 200 rpm for a day at 28°C. The second culture was prepared by inoculating 200 ml of medium B (containing [per liter] 45 g of glucose, 24 g of peptone, 2.5 g of yeast extract) (Alberts, 1990) with 6 ml of the previous culture in a 1-liter Erlenmeyer flask. The flasks were shaken at 200 rpm and incubated at 28°C for 12 days. For cultivations in fermentors, a 1-litre Erlenmeyer flask containing 200 ml of medium A was inoculated with 4 ml (10⁷) of conidiospore suspension. The flask was shaken at 200 rpm for 1 day and then transferred to the fermentor (Alberts, 1990).

RESULTS

Cultural and microscopic characteristics of *A. terreus*

A. terreus grew fast on PDA medium. Macroscopic characters of the colonies included difference in their colors, from pale-yellow to dark yellow. There was reverse pigmentation, from yellow to dark gray color. The texture of the colonies showed raised surfaces which are velvety, yet tough, yellowish and powdery (Figure 2a). Microscopic characters did not show more variations among the isolates; conidiophores were typically long, hyaline and smooth giving rise to sub-spherical vesicles that were biseriate. Conidia had smooth wall, and were slightly elliptical) Figure 2b).

Isolation and Identification

A total of 143 desert *A. terreus* isolated from South-west Najef Province (n=28 isolates), Ramadi Province (n=40), South Mosul Province (n=46 isolates), and Western Kerbela province (n=25 isolates) (Table 1) were studied.

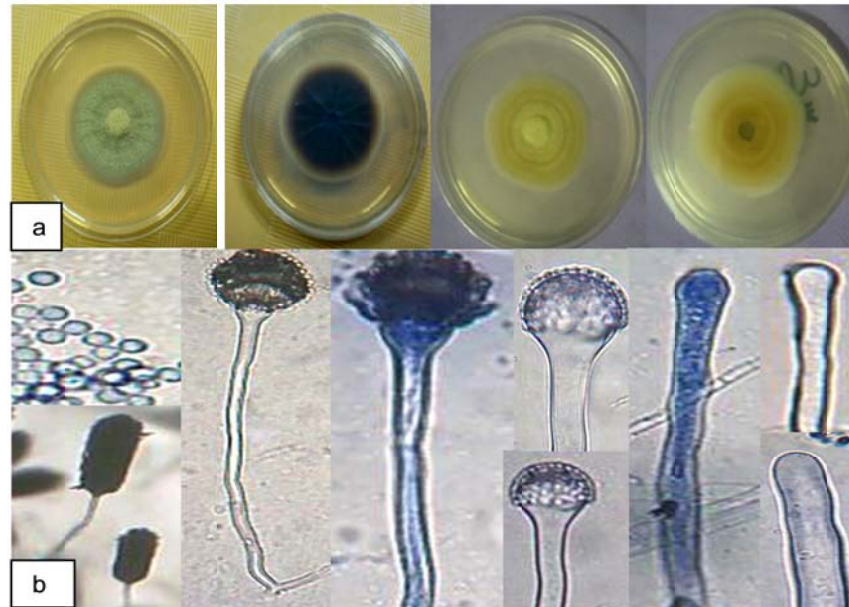


Figure 2. The appearance of the characteristics of *A. terreus* on the PDA medium incubate at 28 °C for 7 days: a-colony characters superficial and reverse view , b-microscopic characters (conidia, conidial heads, conidiophores development stages).

Table 1. Fungal species and their frequency. Occurrence percentage and number of isolates drawn from the arid soils of Iraq.

Fungal specie	Number of isolates	Occurrence (%)	Frequency (%)
<i>A. niger</i>	159	13.81	24.68
<i>A. terreus</i>	143	13.25	20.8
<i>A. fumigates</i>	42	9.39	6.52
<i>A. flavus</i>	115	11.04	17.31
<i>P. chrysogenum</i>	52	6.35	8.15
<i>P. digitatum</i>	53	6.36	8,15
<i>A. alternate</i>	5	4.41	0.7
<i>Cladosporium</i> spp.	4	1.14	0.62
<i>Monilia</i> spp.	16	4.97	2.48
<i>Trichoderma</i> spp.	10	3.31	1.55
<i>Rhizopus</i> spp.	28	12.7	4.34
<i>Rhodoterla</i> spp.	3	1.65	0.46
<i>Candida</i> spp.	8	3.31	1.24
White mycelium	6	8.83	0.9
Total isolates	644		

Molecular ecological typing

Molecular diagnosis of *A. terreus* isolates

The specific pair of primers for *A. terreus* ATE1 and ATE2 was successfully annealed, and the targeted regions of the 19 environmental isolates of *A. terreus* were amplified. The PCR product showed monomorphic bands of 450 bp in length (primer included) (Figure 3a).

These results confirmed the diagnosis that all the isolates used in this study belonged to *A. terreus*.

Ribotyping the ITS region for environmental isolates of *A. terreus*

The targeted rDNA (ITS1 -5.8S-ITS2 region) of the 19 isolates was amplified with primer pairs: ITS1/ITS4 that

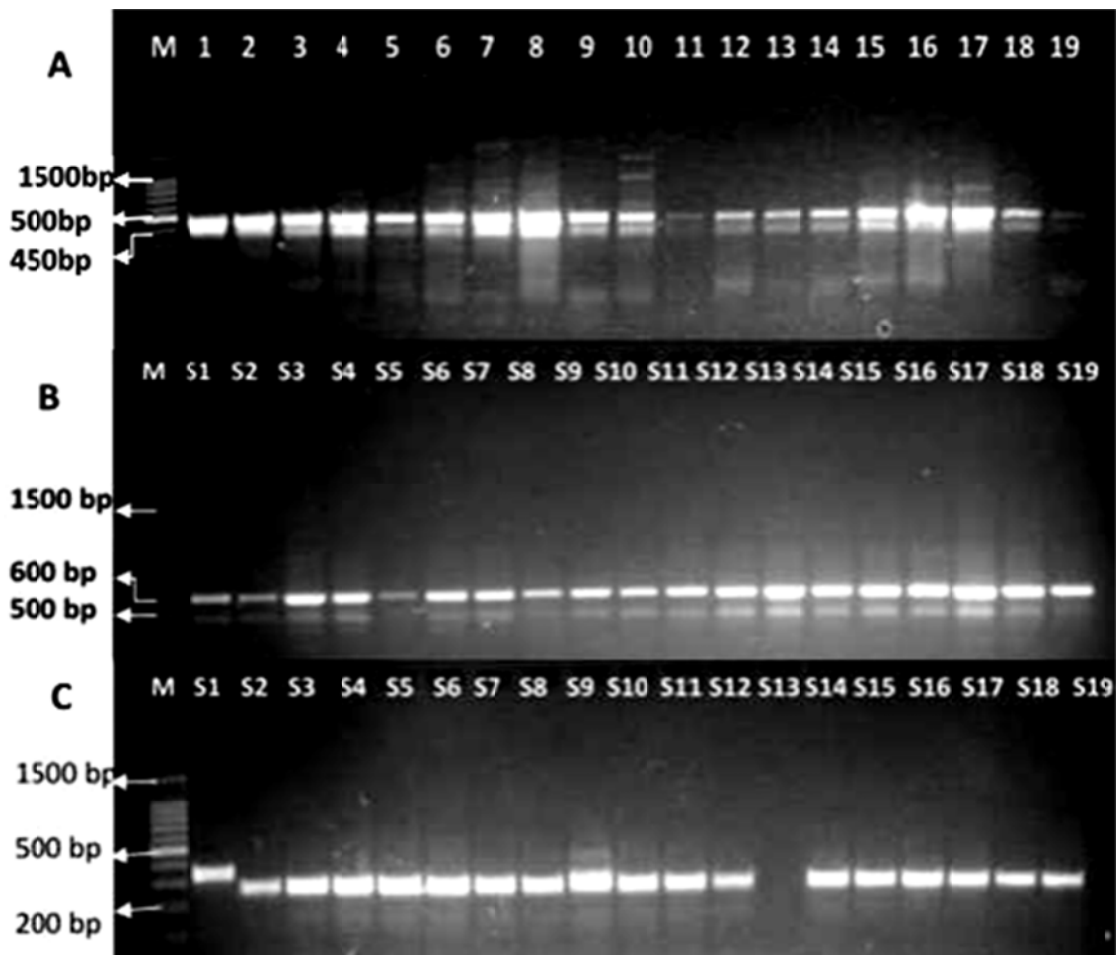


Figure 3. Agarose gel electrophoresis of the PCR products for *A. terreus* isolates amplified by: A-pair primers ATE1 and ATE2; B, amplified ITS1-5.8S-ITS2 region by pair primers 1TS1 - ITS4; C, amplified ITS1-ITS2 region by pair primers 1TS1 -ITS2. Lane M= Molecular marker 100 bp; lanes 1- 19 *A. terreus* isolates.

produced an amplicon length of approximately 550 -600 bp, which was obtained for all the tested isolates of *A. terreus* (Figure 3b); and ITS1/ITS2 primer that amplified the ITS1 region, which produced an amplicon length of approximately 280-380 bp (Figure 3c).

RAPD-PCR ecological typing

Several bands (1 to 5 bands) in various sizes ranging from about 100 to 1000 base pairs were obtained by using primer R108. This primer generated by RAPD-PCR patterns can discriminate between very closely related environmental isolates, but incidental similarities among the typing of distantly related isolates may also occur. We highlighted that similar typing patterns of bands correspond to the same ecological genotype having the same locus or loci and generating similar or different patterns with identical band sizes in the different environmental isolates of *A. terreus* (Figure 4).

Phylogenetic tree of the *A. terreus* isolates based on RAPD-PCR

The results show that there was a wide range of degrees of similarity among the 19 isolates of *A. terreus*: J and L isolates showed 100% similarity coefficient value (0% distance coefficient), C and D showed 80% similarity, both S, T and N, O showed 68% similarity; H,I isolates showed 50% similarity coefficient value, while the R isolate showed a distinct ecological genotype with coefficient value of 0% similarity. Figure 5 shows all the similarity coefficient values.

Screening of fungal cultures for lovastatin production

The fungal cultures were grown under submerged fermentation conditions to assess their potential to produce lovastatin. From the results (data not shown), it is clear that all the three *A. terreus* cultures were able to

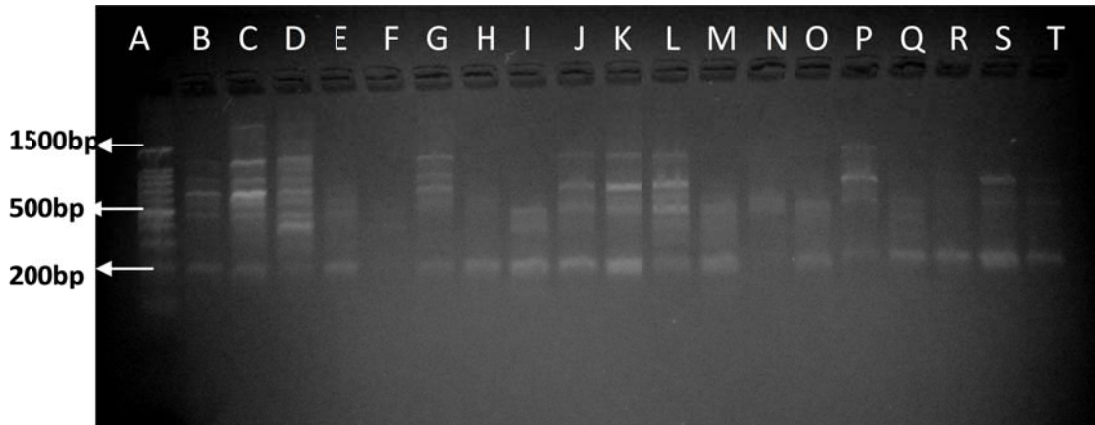


Figure 4. Agarose gel electrophoresis of the RAPD-PCR products for environmental isolates of *A. terreus* by primer (R108). The PCR products of the isolates were analysed in 2% agarose gel. A=Molecular marker 100bp, ecological genotypes were J-R.

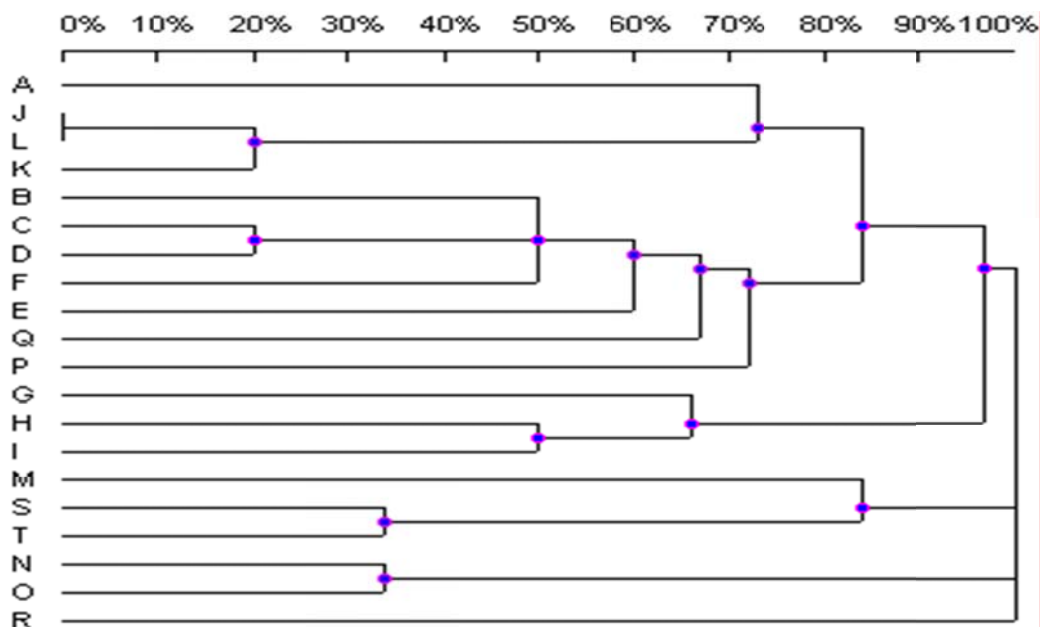


Figure 5. Phylogenetic tree dendrogram for 19 environmental isolates of *A. terreus* constructed using UVI band software based on the RAPD-PCR bands. A=Molecular marker; J-R= isolates of *A. terreus*. Scale 0%-100% =distance coefficient (UPGMA).

produce lovastatin. Lovastatin production was confirmed by using thin layer chromatography. It was observed that, both commercial lovastatin and the sample spots had approximately same R_f value = 0.32, with light brown in both treatments and standard drug.

DISCUSSION

Our study enhances understanding of the impact of

extreme environmental factors and shows high variation in the characteristics of the colonies of the *A. terreus* isolate in each site of study. We also concluded that variations in the colonies in all the soil samples were governed by dominant environmental characteristics of the desert soil such as soil texture (sandy – gravel texture), dry areas, and sites containing varied densities and low diversity of vegetation as well as scarcity of rain (Guest and Al-Rawi, 1966). The results of this study described the taxonomic criteria that allow rapid typing of

A. terreus isolate from the arid soils, including the cultural and microscopic features used for the identification of *A. terreus* isolates. These phenotypic variations correlated with the ecological effects and genetic interactions. In the desert environment, intensive mutagenic actions are expected from UV radiation.

The results of our study shows that no much genetic variations were observed in the ITS region typing of the 19 isolates of *A. terreus*. These markers were designed to identify defined strains of *A. terreus* in arid soils. They also showed that the monomorphic PCR bands of *A. terreus* can be used to explain why phenotypic variations do not correlate with the intron region but may correlate with the dynamics of the genomes at the exon regions in *A. terreus* populations. Our results do not agree with those of Varga et al. (2005), who elucidated some species: *A. alabamensis*, *A. terreus* var. *floccosus*, *A. terreus* var. *africanus*, *A. terreus* var. *aureus* as well as *A. aureoterreus*, according to Balajee et al. (2009); they presented that seven lineages were observed among isolates that have previously been treated as *A. terreus* based on ITS region (Chase and Fay, 2009) and parts of the β -*tubulin* and *calmodulin* genes.

Our results agree with the explanation of Sniegowski et al. (1997) and Wilke et al. (2001), that most mutations are deleterious. Mutation rates are generally low and reduce individual fitness, so increasing the probability of an adaptive mutation to appear.

The results based on RAPD-PCR marker showed the suitable values for identifying genotypes and described the differentiation of *A. terreus* populations (Figures 4 and 5). These results agree with those of Crowhurst et al. (1991). Unfortunately, previous studies on Iraqi soil fungi used only microscopic and culture based methods (Ismail and Abdullah, 1977; Haleem et al., 2013); so there are no molecular results to compare our work with.

Based on the phenotypic variations in the colonies' character, we postulate that unique *A. terreus* genotypes may occupy particular environmental habitats of desert soils in Iraq. Results of this study showed a great diversity of genotypes among isolates of *A. terreus* by using rDNA and RAPD data, which explored the genotypes of the isolates recovered from the four collection stations under study.

Our results show wide range of variability in phenotypic characters of all the isolates. It was clear that they all belonged to *A. terreus*, as the PCR products from the genotype confirmed the diagnosis by specific primer pairs (ATE1 and ATE2). They distinctively removed any delusion that could lead to confusing these isolates with other species, by revealing the yellow colour of the colonies. This result coincides with the results of Logotheti et al. (2009). Variations in phenotype characters are due to the concept of gene-environment correlation (Smith and Kruglyak, 2008), which can occur through multi-mechanisms. Many of these microbes live in extreme environments, for example; high temperatures,

high salt concentrations, low pH, and high radiation. Some physical factors also influence fungal growth and metabolite production (Gautam et al., 2009). The biotechnological potential of microorganisms to produce is based on their special adaptations to their environment (Gautam et al., 2010). Sunlight, salinity and soil ecology by natural selection, genetic drift and gene flow and gene mutation are representatives of the domain sources of genetic variations (Kurtzman, 1985; Carlile et al., 2001; Fe'ral, 2002; Terry et al., 2004; Lass-Florl et al., 2007; Smith and Kruglyak, 2008).

Molecular typing based on RAPD-PCR patterns used for the 19 isolates of *A. terreus* showed distinctive patterns. This result agrees with that of Lass-Florl et al. (2007) who classified clinical isolates of *A. terreus*, using RAPD-PCR patterns. These variations in the pattern may help explain the sources of variation; provide solution to several phenotypic variations in *A. terreus* colony and explain the difference in colors of reverse pigmentations. A phylogeny tree based on RAPD-PCR profile was sufficient in genotyping *A. terreus* isolates collected from the arid regions of Iraq; it showed variable degrees of similarity among the 19 isolates of *A. terreus* and divided them into many genotypes. Only two isolates showed 100% similarity coefficient values. Other isolates showed 0-80% similarity coefficient values (Figure 5). These results agree with those of Lasker (2002) whose genotyped *A. fumigatus* isolates, and also the results of Raclasky et al. (2006) and Nariasimhan and Asokan (2010).

The RAPD-PCR patterns used for *A. terreus* isolates were more effective than monomorphic ribotyping patterns used for ecological genotyping (Loudon et al., 1993; Symones et al., 2000). Finally, ecological genotyping find minor differences among isolates at the species to genus level (Birch et al., 1995).

Our results concur with that of Lewington et al. (2007), in which the wavelength of statin produced by fungal isolates ranges between 200-400 nm. On the other hand, this result conflicts with some earlier studies in which pH, medium and choice of wild type or mutant govern lovastatin production. We found that no specific pH or media induce lovastatin production. This result is in line with that of Kumar et al. (2000) who reported that lovastatin is generally produced by batch fermentation in complex media. *A. terreus* fermentations are typically carried out at 28°C and pHs of 5.8–6.3

Conclusion

This study may encourage future research of ecological genotyping of closely related environmental isolates. It showed highly discriminatory profiles of RAPD –PCR. RAPD–PCR could identify genetic diversity among closely related isolates in the same species. The molecular genotyping of *A. terreus* based on ITS region was reliable, but not as discriminating as RAPD – PCR.

These methods are useful tools in taxonomical studies, give precise, rapid results with low cost and no time consuming. This study confirmed genotyping as an important method to find solution to fungal ecological diversity problems.

Ethical approval

Both authors hereby declare that all actions have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

- Adhikari A, Sen MM, Gupta - Bhattacharya S, Chanda S (2004). Airborne viable, non-viable and allergenic fungi in a rural agricultural area of India: a 2- year study at five outdoor sampling stations. *Sci. Total Environ.* 326:123-141.
- Alberts AW (1990). Lovastatin and Simvastatin—inhibitors of HMG CoA reductase and cholesterol-biosynthesis. *Cardiology* 77:14-21.
- Alberts AW, Chen J, Kuron G, Hunt V, Huff J, Hoffman C, Rothrock J, Lopez M, Joshua H, Harris E, Patchett A, Monaghan R, Currie S, Stapley E, Albers-Schonberg G, Hensens O, Hirshfield J, Hoogsteen K, Liesch J, Springer J (1980). Mevinolin: A Highly Potent Competitive Inhibitor Of Hydroxy Methylglutaryl Coenzyme A Reductase And A Cholesterol-Lowering Agent. *Proc. Natl. Acad. Sci. USA* 77:3957-3961.
- Amin HA, El-Menoufy HA, El-Mehalawy AA, Mostafa EE (2010). Microbial production of glycyrrhetic acid 3-O-mono-β-D-glucuronide from glycyrrhizin by *Aspergillus terreus*. *Malay. J. Microbiol.* 6 (2): 209-216.
- Anderson MJ, Gull K, Denning DW (1995). Molecular typing by Random Amplification of Polymorphic DNA and M13 Southern Hybridization of Related Paired Isolates of *Aspergillus fumigatus*. *J. Clin. Microbiol.* 34 (1): 87-93.
- Aubin JT, Collandre D, Candotti D, Ingrand C, Rouzioux, Muraux JM (1991). Several groups among human Herpes virus, six strains can be distinguished by Southern blotting and PCR. *J. Clin. Microbiol.* 29(2): 367-372.
- Aufauvre-Brown A, Cohen J, Holden DW (1992). Use of randomly amplified polymorphic DNA markers to distinguish isolates of *Aspergillus fumigatus*. *J. Clin. Microbiol.* 30:2991 -2993.
- Balajee SA, Baddley JW, Peterson SW, Nickle D, Varga J, Boey A, Lass-Flörl C, Frisvad JC, Samson RA (2009). *Aspergillus alabamensis*, a new clinically relevant species in the section Terrei. *Eukaryot. Cell* 8 (5):713-722.
- Birch M, Anderson MJ, Denning DW (1995). Molecular typing of *Aspergillus* species by random amplification of polymorphic DNA. *Eur. J. Clin. Microbiol.* 18(11): 838-841.
- Carlile MJ, Watkinson SC, Gooday GW (2001). Genetic Variation and Evolution in the fungi. 2ed Academic press, pp. 245-295.
- Casas Lpez JL, Sánchez Pérez JA, Fernández Sevilla JM, Acien Fernández FG, Molina Grima E, Chistib Y (2003). Production of ovastatin by *Aspergillus terreus*: effects of the C:N ratio and the principal nutrients on growth and metabolite production. *Enzyme Microb. Technol.* 33:270-277.
- Chase MW, Fay MF (2009). Barcoding of plants and fungi. *Science* 325:682-683.
- Crowhurst RN, Hawthorne BT, Rikkerink EHA, Templeton MD (1991). Differentiation of *Fusarium solani* f.sp. *Cucurbitae* races 1 and 2 by Random amplification of polymorphic DNA. *Curr. Genet.* 20: 391-396.
- Edwards K, Johnstone C, Thompson C (1991). A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nuclei Acids Res.* 19:1349.
- Fe'ral JP (2002). How useful are the genetic markers in attempts to understand and manage marine biodiversity? *J. Exp. Mar. Biol. Ecol.* 268: 121-145.
- Gautam SP, Bundela PS, Pandey AK, Awasthi MK, Sarsaiya S (2009). Prevalence of fungi in Municipal solid waste of Jabalpur city (M.P.). *J. Basic Appl. Mycol.* 8 (1-2): 80-81.
- Gautam SP, Bundela PS, Pandey AK, Awasthi MK, Sarsaiya S (2010). Composting of municipal solid waste of Jabalpur City. *Glob. J. Environ. Res.* 4:43-46.
- Guest E, Al Rawi A (1966). Introduction to the Flora of Iraq, Ministry of Agriculture of the Republic of Iraq Vol.1.
- Hajjaj H, Niederberger P, Duboc P (2001). Lovastatin biosynthesis by *Aspergillus terreus* in a chemically defined medium. *Appl. Environ. Microbiol.* 67:2596-602.
- Haleem RA, Abdullah SK, Jubrael JMS (2013). Occurrence and distribution of fungi associated with grapevine decline in Kurdistan region-Iraq. *Agric. Biol. J. N. Am.* 4(3):336-348.
- Hong SB, Shin HD, Nong J (2008). New taxa of Neosatorya and *Aspergillus* in *Aspergillus* section *Fumigatic.* *Antonie Van Leeuwenhoek* 93(1-2): 87-98.
- Imran ZK, Al-Asadi YF (2014). Multiple molecular markers for diagnosis of conjunctivitis caused by *Candida* spp. in Iraq. *Afr. J. Microbiol. Res.* 8(38):3482-3488.
- Imran ZK, Al-Shukry HN (2014). Molecular diagnosis of vaginal candidiasis by polymerase chain reaction (PCR) and random amplification polymorphism DNA (RAPD-PCR) in Babylon Province, Iraq. *Afr. J. Microbiol. Res.* 8(6):496-502.
- Ismail ALS, Abdullah SK (1977). Studies on the soil fungi of Iraq. *Proc. Indian Acad. Sci.* 86(3):151-154.
- Juzlova P, Martinkova L, Kren V (1996). Secondary metabolites of the fungus *Monascus*: a review. *J. Ind. Microbiol.* 16:163-170.
- Kumar MS, Jana SK, Senthil V, Shashanka V, Kumar SV, Sadhukhan AK (2000). Repeated fed-batch process for improving lovastatin production. *Process Biochem.* 36(4):363–368.
- Kurtzman CP (1985). Molecular taxonomy of the fungi. In: Bennett, I.W. and Lasuse, L.L. Eds., *Gene Manipulation in Fungi*, Academic press, Orlando. pp. 35-63.
- Lasker BA (2002). Evaluation of Performance of Four Genotypic Methods for Studying the Genetic Epidemiology of *Aspergillus fumigatus* Isolates. *J. Clin. Microbiol.* 40 (8):2886-2892.
- Lass-Flörl C, Grif K, Kontoyannis DP (2007). Molecular typing of *Aspergillus terreus* isolates in Houston, Texas, and Innsbruck, Austria: evidence of great genetic diversity. *J. Clin. Microbiol.* 45: 2686-2690.
- Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, Halsey J, Qizilbash N, Peto R, Collins R (2007). Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet* 370:9602:1829-1839.
- Lipson DA, Schmidt SK, Monson RK (1999). Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. *Ecology* 80:1623-1631.
- Logotheti M, Kotsovili-Tseloni A, Arsenis G, Legakis NI (2009). Multiplex PCR for the discrimination of *A. fumigatus*, *A. flavus*, *A. niger* and *A. terreus*. *J. Microbiol. Methods* 76:209-211.
- Loudon KW, Burnie JP, Coke AP, Matthews RC (1993). Application of polymerase chain reaction to fingerprinting *Aspergillus fumigatus* by random amplification of polymorphic DNA. *J. Clin. Microbiol.* 31:1117-1121.
- Miyake T, Uchitomi K, Zhang MY, Kono I, Nozaki N, Sammoto H, Inagaki K (2006). Effects of the principal nutrients on lovastatin production by *Monascus pilosus*. *Biosci. Biotechnol. Biochem.* 70(5):1154-1159.
- Nariasmhan B, Asokan M (2010). Genetic variability of *Aspergillus terreus* from dried grapes using RAPD-PCR. *Adv. Biosci. Biotechnol.* 1:345-353.

- Novak N, Gerdin S, Berovic M (1997). Increased lovastatin formation by *Aspergillus terreus* using repeated fed-batch process. *Biotechnol. Lett.* 19:947-948.
- Raclaský V, Trtková J, Rusková L, Buchta V, Bolehovská R, Vacková M, Hamal P (2006). Primer R108 performs best in the RAPD strain typing of three *Aspergillus* species frequently isolated from patients. *Folia Microbiol. (Praha)* 51 (2):136-140.
- Raper KB, Fennel DI (1965). *Aspergillus terreus* group. In: Raper KB, Fennel DI, eds, *The genus Aspergillus*. Baltimore, MD: The Williams & Wilkins Co., pp. 293-344.
- Rath PM, Kamphoff S, Ansorg R (1999). Value of different methods for the characterization of *Aspergillus terreus* strains. *J. Med. Microbiol.* 48: 161-166.
- Saghai-Marooof MA, Soliman KM, Jorgensen RA, Allard RW (1984). Ribosomal spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Natl. Acad. Sci. USA.* 81:8014-8019.
- Smith EN, Kruglyak L (2008). Gene-Environment Interaction in Yeast Gene Expression. *PLoS Biol.* 6(4):0810-0824.
- Sniegowski PD, Gerrish PJ, Lenski RE (1997). Evolution of high mutation rates in experimental populations of *Escherichia coli*. *Nature* 387:703-705.
- Suhail M, Irum F, Jatt T, Korejo F, Abro H (2007). *Aspergillus* Mycoflora isolated from soil of Kotri Barrage Sindh, Pakistan. *Pak. J. Bot.* 39(3): 981-984.
- Symones F, Boucher JP, Heinemann S, Norland N (2000). Molecular typing of *Aspergillus terreus* isolates by Random amplification of polymorphic DNA. *J. Hosp. Infect.* 44(4):273-280.
- Terry VC, Lars O, Yuri C, Terry C, Torben RC, Brian H, Rolf AI, Margareta J, Dyanna J, Sven J, Nadya M, Nicolai P, et al., (2004). Responses to Projected Changes in Climate and UV-B at the Species Level. *Ambio* 33:7.
- Ute M, Kim L, Peter B, Alan J (1994). Phylogenetic relationships of *Babesia divergens* as determined from comparison of small subunit ribosomal RNA gene sequences. *Mol. Biochem. Parasitol.* 68:161-165.
- Varga J, Toth B, Kocscube S, Rigo K, Teren J, Kozakiewicz Z (2005). Evolutionary relationships among *Aspergillus terreus* isolates and their relatives. *Antonie van Leeuwenhoek* 88(2):141-150.
- Wilke CO, Wang J, Ofria C, Lenski RE, Adami C (2001). Evolution of digital organisms at high mutation rate leads to survival of the flattest. *Nature* 412:331-333