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Full Length Research Paper

Ecological awareness of cultured colonies variation of *A. terreus* and environmental interactions in arid soils

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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 approxi mately 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 e**c**ological typi**n**g, *Aspergill**u**s terreus,* arid soils, lovas**t**atin, Iraq.

INT RODUCTION

As pergillus terreus is a co**m**mon fungus, which has an imp ortant role in desert soils (Amin et al*.,* 2010). Arid **s**oil is c omplex ecosystem with topographically distinct m**y**co floras. Its microbial communities are subj**e**cted to surf**a**ce he ting, cycles of rainfall and extre**m**e desiccation, changing water ta**b**le, CO_2 enri**c**hment and UV light supp**l**e-mentation and at**t**enuation; it is directly e**x**posed to sun light due to the abs**e**nce or abun**d**ance of vege**t**ation variation (Lipson et al., 1999).

A. terreus is a **w**idely disper**s**ed species **a**mong the soil

mycoflora; it belongs to the g enus *Asperg illus*, and of he sub -genus *Terr ei Nidulantes* (Raper and Fennell, 19 65; Va ga et al., 2 05). Many isolates of this fungus show morphological v ariation in rowth criteria (Rath et al., 19 9). Most of t he *A. terreus* isolates prod uce a variety of sec ondary metabolites that are of pharmaceutic ally sig nificance; the y are used for producing lovastatin d ug (Ca sas L´opez et al., 2003). Morpholo ical charact ers inc ude making weak classification at t e species a nd ge us levels, w hile molecular methods are powerful tools in s pecies identification (Hon g et al*.,* 2008).

I n advanced f u n g a l m olecular studies, riboso mal marker genes h ave been used, such as universal gen es found in all fun gi. Fungal communities are commonly gen otyped by th e multiple ge nes of rDNA (ITS, IGS, 18S an 25SrRNA, 25S and their introns) (Imra n and Al-Asa di, 20 4). These g enes allow for phylogenetic analysis, a nd ass ist in taxono mic classification (Chase nd Fay, 200 9).

V arious gen otypic meth ods have been used to suc cessfully do fingerprinting in several f ungi such as *A. fum igatus*, *Fusarium solan i* and *Candida* spp. (Crowhu rst et al., 1991; L oudon et al., 1993; N riasimhan a nd As okam 2010, Imran and Al-Shukry 2014). Ribotyping an lysis of the ITS region r evealed detailed information ab ut molecul r analysis and showed reproducible polymorphism i several stu dies (Chase and Fay, 2009). Ra ndom Amplification of Polymorphic DNA (RAP D) ass ay revealed polymorphic DNA patterns of *Aspergillus* spp . (Anderson et al., 1995). Furthermore, RAPD-P CR ass ay is adapt d for doing fingerprinting of fungi, a it reveals an accurate and sim ple method f or differentiation among fungal i solates (Aub n et al*.,* 199 1; Symones et al*.,* 2000; Narias imhan and Asokan, 2010) .

It is not clear whether th e variations in the char acteristics of the colony of *A. terreus* isol tes are due to en ironmental r esponse or genetic variation. As a result, there is the ne ed for a reliable molecular ecolo-gical typing of *A. terr us* isolates i n arid soils. Furthermore, he imp ortant roles played by th e se fungi in the arid regio ns inc ude biodegradation, dru g biosynthesis, recycling of materials and microbial activities and are potential sou rce of novel phar maceuticals (Amin et al*.,* 2010). Few co mparable data in the lite rature are a vailable for *A. terr eus* in the w rld (Lass-Florl et al*.,* 2007).

T he role of genetic variants on ph enotypic tra its oft en depends upon envir nmental and physiologi cal co nditions, bu t such gene- environme t interactions are poorly understood. The eneficial role of soil mycofl ora is t he focus of this study.

Se veral studies have been attempted to evaluate he ability of *A. terreus* isolates to produce lovastatin d ug (Al berts et al., 1 980; Juzlova et al*.,* 1996; Casas L´op ez et al*.,* 2003; Miyake et al*.,* 2006). The ild and mut ant str in of *A. terr eus* appears to be the most commonly producer of lov astatin, although it uses various ways to produce biologi cal and significant product (Novak et al*.,* 19 7; Hajjaj et a l*.,* 2001).

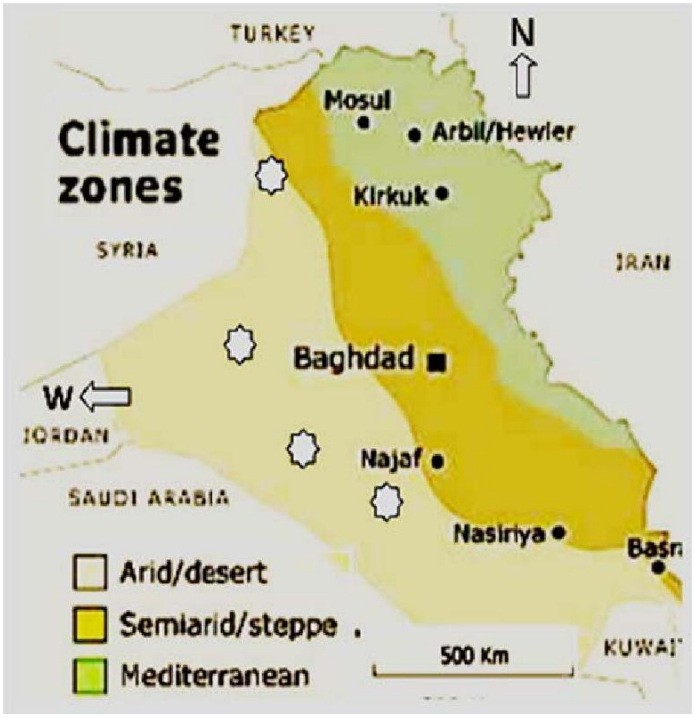

Figure 1. Climatic zo nes in the map of Iraq, ( ) sam pling sites of the arid so il for the survey of A. terreus.

Un fortunately, t here is no information on the molecular ecolo gy of fungal communities in the desert of Iraq and also there are no previous stu ies on the r le of genetic varia nts on phenotypic traits of *A. terreus* based on envir onmental changes. The aim of our study was to evalu ate the phe notypic and enotypic var iations based on IT S typing reg ion, using R APD-PCR of the environ-mental isolates of *A. terreu ;* it aimed to perform a phylo genic analy sis, explai ns their variations by mole cular ecologi cal diagnosis and how this understanding enables fungal iagnosis and screening of lovastatin prod ced by the w ild type *A. terreus* culture s.

MATE RIALS AND METHODS

Site descriptions an d samples collection

The study area is loc ated around t he geographica l coordinates f 33° 2 0' N. 44° 23' E . in Iraq. Western and south ern Iraq cover a vast d esert area of about 168.000 square km: s outh-west (Naj f and Kerbela provinc e s) and north-w est (Ramadi and South Mos l provinces) (Figure 1) . The common vegetation obs rved in the area includes: *Alhaji* , *Tam arix* and *Salso ls*. Soil pH ran ges from 6.8 to 8.8 (G uest and Al-Rawi, 1966).

A to tal of 45 soil samples (150-2 00 g each) were collected fro m October to April 2011-2012, in different localities in the desert region s. Soil temperatures were approximately around 5°C in January, 56°C in A ugust and 37°C in October at the time the collec tions were mad e. Soil sample s were taken from a depth of 5 cm and stored in poly thene 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 protinase 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-Maroof 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-Florl 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 $FeSO_4 \cdot 7H_2O$, 1 g of $MnSO_4 \cdot 4H_2O$, 200 mg of $ZnSO_4 \cdot 7H_2O$, 100 mg of $CaCl_2 \cdot 2H_2O$, 25 mg of $CuCl_2 \cdot 2H_2O$, 56 mg of H_3BO_3, and 19 mg of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ per liter of solution (Alberts et al., 1980); it was inoculated with $1 \cdot 10^7$ 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 (10^7) 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.

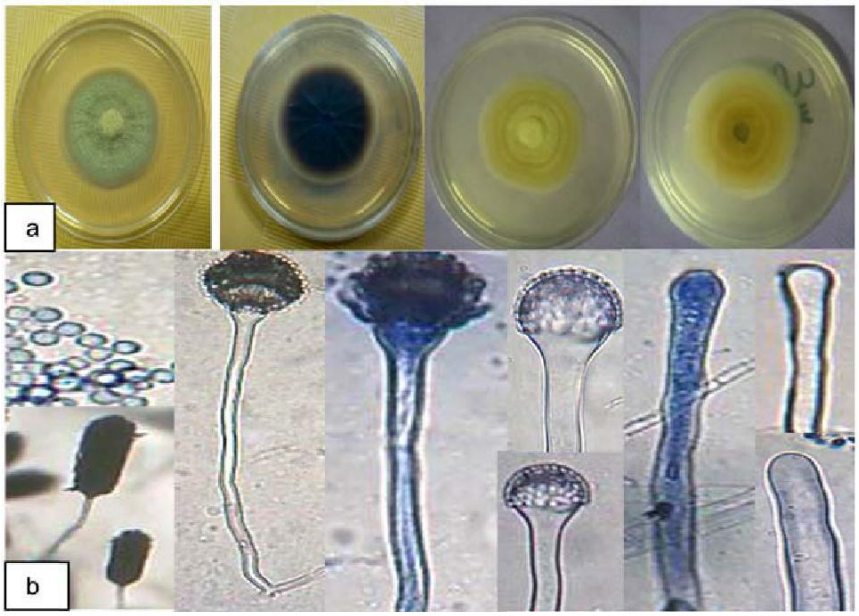

Figure 2. The appea rance of the c aracteristics of A. terreus on the PDA medi um incub ate at 28 ºC for 7 days: a-colony characters superficial and reverse view , b-microscopic characters (conidia, conidial heads, conid iophores develo pment stages).

Table 1 . Fungal species and their fr equency. Occurrence percentage and number of isolates drawn from the arid soils of Ira q.

Fungal specie	Number of isolates	O ccurrence (%)	Frequency (%)
A. nige r	15 9	13.81	24.68
A. terr eus	14 3	13.25	20.8
A. fumigates	42	9.39	6.52
A. flav us	11 5	11.04	17.31
P. chry sogenum	52	6.35	8.15
P. digitatium	53	6.36	8,15
A. alternate	5	4.41	0.7
Clados porium spp.	4	1.14	0.62
Monili spp.	16	4.97	2.48
Tricho derma spp.	10	3.31	1.55
Rhizop us spp.	28	12.7	4.34
Rhodoterla spp.	3	1.65	0.46
Candi a spp.	8	3.31	1.24
White mycelium	6	8.83	0.9
Total i solates	64 4		

Molecular ecol ogical typin

Molecular diagnosis of A. t erreus isolates

Th e specific pa ir of primers for *A. terreus* ATE1 a nd AT E2 was su ccessfully a nnealed, an d the targe ed regions of the 19 environmental isolates of *A. terre us* we re amplified. The PCR product showed monomorphic ba ds of 450 b p in length (rimer includ ed) (Figure 3a).

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

Ribotyping the I TS region fo r environm ntal isolates of A. terreus

The targeted rDN A (ITS1 -5. 8S-ITS2 region) of the 19 isolates was amplified with primer pairs: I TS1/ITS4 that

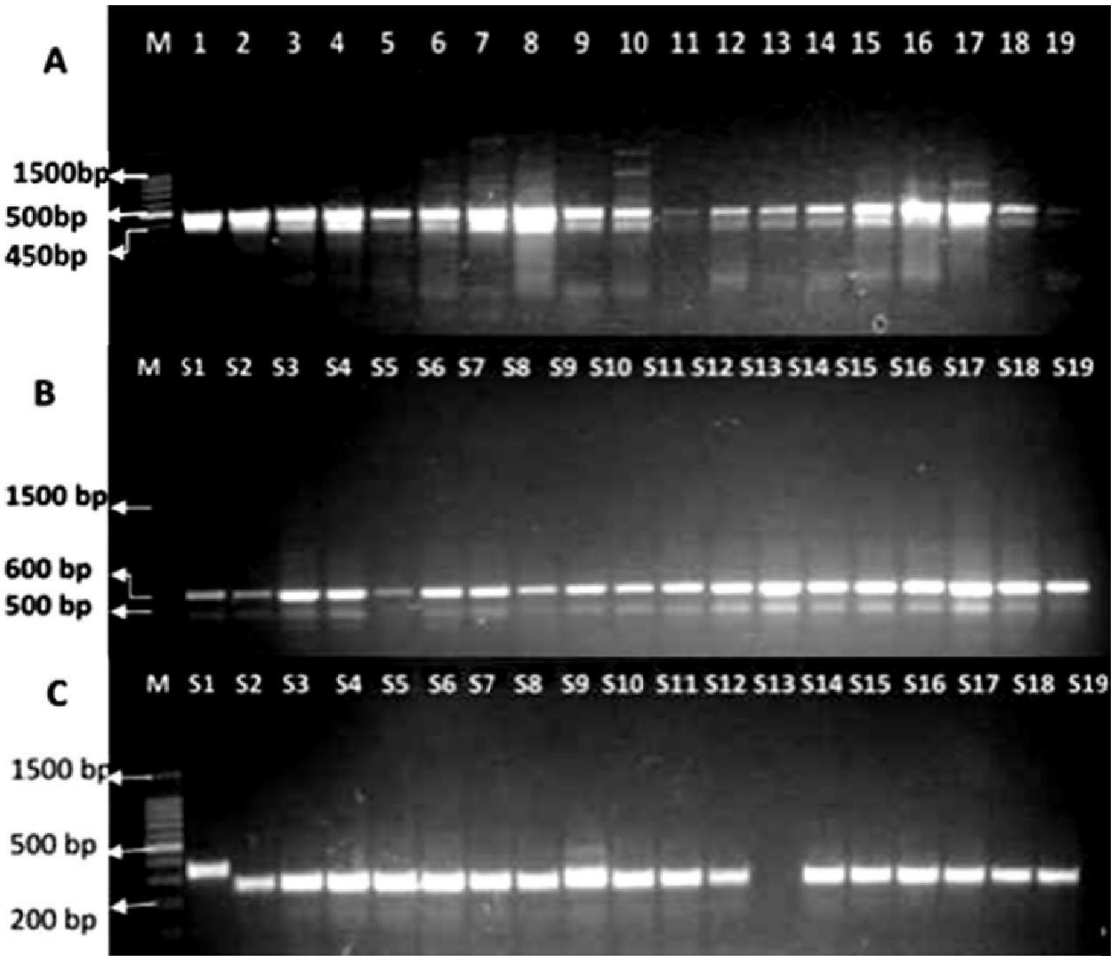

Figure 3. Agarose gel electroph oresis of the PC R products for *A. terreus* isol tes amplified by: A-pair prime rs A E1 and ATE2; B, amplified I TS1-5.8S-ITS2 region by pair primers 1TS1 - ITS4; C, am plified ITS1-ITS 2 region by pair pri mers 1TS1 -ITS2. Lane M= Molecular marker 100 bp; lanes 1- 19 *A. terreus* isolates.

produced an a plicon lengt h of approxi ately 550 -6 00 bp, which was obtained for all the tested isolates of *A. terr eus* (Figure 3b); and ITS1/ITS2 prime r that amplified the ITS1 region, which produced an amplicon length of ap roximately 280-380 bp (Figure 3c).

RAPD-PCR eco logical typi g

Se veral bands (1 to 5 ban s) in variou s sizes ranging fro m about 100 to 1000 b se pairs we re obtained by usi ng primer R108. This prim er generated by RAPD-P CR patterns can discriminate b etween very closely rela ed en ironmental i solates, but incidental si ilarities amo ng the typing of distantly related isolates may also occur. We highlighted tha t similar typing patterns of ban ds correspond to t he same eco logical genotype having he sa me locus or loci and generating similar or differ ent patterns with identical band sizes in the differ ent en ironmental is olates of *A. terreus* (Figure 4).

Phyl ogenetic tree of the *A. terreus* isolates based o n RAP D-PCR

The r esults show that there wa s a wide range of degrees of si milarity among the 19 isolates of *A. ter reus*: J and L isolates showed 100% similarity coefficient value (0 dista nce coefficient), C and D showed 80% similarity , both S, T and N, O showed 68% similarit y; H,I isolates showed 50% sim ilarity coefficient value, while the R isolate showed a distinct ecological genotype with coeff icient value f 0% similarity. Figure 5 shows all the simil rity coefficie nt values.

Scre ening of fungal cultures for lovastatin productio n

The fungal cultures were grown under subme rged fermen-tation conditions to assess their potentia l to produce lovastatin. From the results (d ata not sho n), it is clear that all the three *A. terreus* cultures were able to

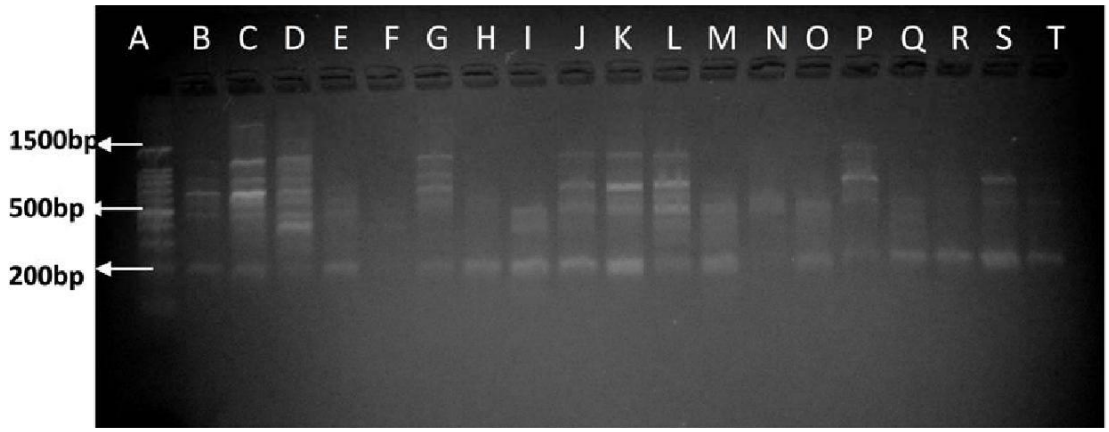

Figure 4. Agarose gel electrop horesis of the RAPD-PCR p roducts for en vironmental iso lates of *A.t rreus* by prim er (R108). The PCR products of the isolates were analys ed in 2% agarose gel. A= Molecular mark er 100bp, ecological genotype were J-R.

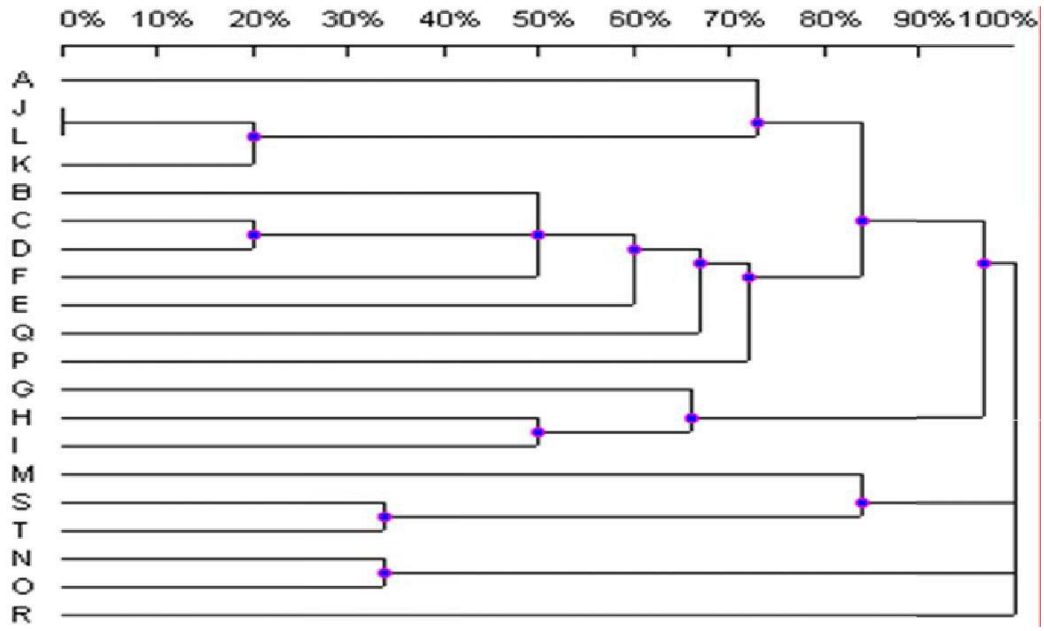

Figure 5. Phy logenetic tree dendrogram for 19 environme ntal isolates of *A. terreus* constructed using UVI band software based on the RAPD-PC R bands. A=Mo lecular marker ; J-R= isolates of *A .terreus*. Scale 0%-100 % =distance co efficient (UPG MA).

produce lovastatin. Lovastat n production was confirm ed by using thin lay er chromato graphy. It wa s observed th at, both commerci l lovastatin and the sa m ple spots h ad ap roximately same R_f valu e = 0.32, wit h light brown in both treatments and standard drug.

DIS CUSSION

Our study enhances understanding of the impact of extreme environm ental factors and shows high variation in th e characteris tics of the c olonies of the *A. terreus* isolate in each site of study. We also c oncluded tha t varia tions in the colonies in all the soil amples were gove rned by dom inant environmental cha racteristics o f the desert soil s uch as soil texture (sandy – grave l textu re), dry area s, and sites containing v aried densities and low diversity of vegetation as well as s carcity of rain (Guest and Al-R awi, 1966). The results of this study desc ribed 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 t h e 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 the19 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 biotechno-logical 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 28ºC 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-Florl 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 Lṕeza JL, SJnchez Péreza JA, FernJndez Sevillaa JM, Acién FernJndeza FG, Molina Grimaa 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 Leeuwen hoek 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-Florl C, Grif K, Kontoyiannis 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 popula-tion dynamics and nitrogen availability in an alpine ecosystem. Ecology 80:1623-1631.

Logotheti M, Kotsovili-Tseleni 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.

Nariasimhan 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-Maroof 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 mutationrates 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 Climateand 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 Leewenhoek 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