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EFFICIENCY OF TWO MOLECULAR TOOLS BASED ON DNA USED FOR DIFFERENTIATING SOME MICROBIAL STRAINS

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ABSTRACT

In the present study, two molecular biology tools based on DNA were compared in the differentiating between some microbial strains isolated from soil. Two types (16SrRNA and 18SrRNA) of ribosomal RNA genes were used for identification of the four bacterial and three fungal isolates, respectively. The identified microbial isolates were submitted in GenBank as strains of *Escherichia coli* MSL-19 (LC455952.1); *Bacillus* sp. MSLB-1 (LC455953.1); *Bacillus* sp. MSLB2 (LC455954.1); *Bacillus* sp. MSLB3 (LC455955.1); *Penicillium* sp. MSLP1 (LC455956.1); *Aspergillus niger* MLSAs1 (LC455958.1); *Aspergillus* sp. MLSAs2 (LC455959.1). The DNA obtained from the seven microbial strains was used as templates for RAPD-PCR differentiating in the presence of eight random primers. Electrophoresis analysis was performed, and on scoring, the identity percentages between the bacterial and fungal strains were separately analyzed. A percentage of 82-83% was recorded between the *E. coli* and the three *Bacillus* strains, while, identities of 93-98% were recorded between the three *Bacillus* strains. Similar trend (90-96%) was observed between the *Penicillium* and *Aspergillus* strains. Results confirmed that identities based on the two ribosomal RNA genes (82-98%) was higher than that of RAPD-PCR (70.0-79.7%), and this is because of ribosomal RNA genes are in limited sizes (~1500-1600 bp) and specific for differentiating species, while RAPD-PCR tool depends on using some random primers could be recorded on the whole genome. The phylogenetic trees based on the two molecular tools supported the obtained results. As a conclusion, tools of RAPD-PCR and ribosomal RNA genes were successfully used to identify and detect the genetic variability of microbial strains isolated from soil.

Keywords: Efficiency, Bacteria, Fungi, Ribosomal RNA genes, RAPD-PCR.

INTRODUCTION

Bacteria and fungi are the most important factors for measuring the biological activity in the soil, where the numbers can be expressed as a measure of the amount of organic matter found in the soil and therefore, its validity for cultivation **(Atlas 1993, Acton & Gregorich 1995, Bridge and Spooner 2001, Chen et al 2003**). This is due to the ability of these microbes to analyze plant and animal residues (**Anderson 1994 and Nielsen & Winding 2002).**

The classical method for estimating the fungal diversity of soil has been number and morphology of fruiting bodies. However, the majority of fungi in soil are present either as resting stages (spores) or mycelium. Both spores and mycelium can be isolated from soil, but if a fruiting body is not formed, identification of the organisms is difficult at best, and generally impossible (**Bridge and Spooner 2001**).

It has often been based on the traditional methods in identifying the different fungi in the soil, including their numbers, morphological forms and the type of spores where most fungi are present in the form of static stage or mycelium and thus can

be isolated but if no spores or fruit body is formed, in their growth they will be isolated (**Smit et al 1999**). Recently, 18S rDNA molecular biology tool can solve these difficulties and there are now many sequences of nucleic acids of fungi species or strains in the GenBank databases, especially after the use of polymerase chain reaction technique (**Laguerre et al 1994, Kowalchuk et al 1997, Pennanen et al 2001, Nonomuraa et al 2011).**

Embong et al (2008) successfully used 18S rRNA gene for detection of fungal isolates with high sensitivity. They demonstrated that 18S rRNA-based PCR has high degrees of analytical sensitivity (100 femtogram) and specificity (100%) for the detection of a wide range of medical significant fungi.

In **1990, Williams et al** described the RAPD technique as a molecular tool to test DNA samples from anonymous individuals. Subsequently, several studies using RAPD-PCR were applied using DNA templates of microorganisms (**Micheli et al 1994, Nowrouzian et al 2001 and Babalola 2003**). RAPD method was reported as a reproducible DNA fingerprinting tool (**Micheli et al 1994)**; this is because of it uses random primers which can be amplified DNA polymorphisms of any species without requiring any previous information about the nucleotide sequence of the microorganism. The DNA polymorphism products of RAPD can be used as genetic markers.

Fungal marker genes differ in length, resolution power among different fungal groups, phylogenetic power, number of publicly available sequences and available suitable primer sets (**Reich and Labes 2017**). The Internal Transcribed Spacer (ITS) region is the proposed barcode for fungi as it has species resolution for a very broad range of fungi compared to other fungal marker genes (**Schoch et al 2012**).

The present study was designed to determine the fidelity of two molecular biology tools based on DNA that could be used in the differentiating between some microbial strains isolated from soil.

PD technique was first employed by **Williams et al (1990)** to examine human DNA samples from anonymous individuals. Since then several authors have reported on the application of RAPD technique in microorganisms **(e.g. Babalola, 2002).** It uses random primers **(Williams et al 1990)** and can be applied to any species without requiring any information about the nucleotide sequence. The amplification products from this analysis exhibit polymorphism and thus can be used as genetic markers. The presence of a RAPD band, however, does not allow distinction between hetero- and homozygous states. The fragments are scored as dominantMendelian elements, and the protocols are relatively simple

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MATERIALS AND METHODS

Source of microbial strains

A number of seven purified microbial isolates [four bacterial (*E. coli* (I isolate), *Bacillus* sp. (3 isolates) and three fungal (*Penicillium* sp. (I isolate), *Aspergillus* sp.(2 isolates)] isolated from soil were kindly provided by Prof. Abdel-Fattah H.I., Department of Agricultural Microbiology, Faculty of Agriculture, Zagazig University.

Extraction of DNA

The method of **El-Domyati and Mohamed (2004)** was applied for extraction of DNA from the seven microbial isolates. DNA concentration and purity were determined as described by **Sambrook et al (1989) and Brown (1990)**, respectively.

RAPD-PCR amplification

Using eight random primers belonging to 6 Operon Kits (OPB09, OPB17, OPE04, OPF06, OPO03, OPW18, OPZ16 and OPZ17) and DNA templates of the seven bacterial and fungal strains, RAPD-PCR was conducted as reported by **El-Domyati and Mohamed (2004)** in a total volume of 50 µL. The PCR products were resolved by electrophoresis in a 1.0% agarose gel at 65 volts for 2.0 h with 1xTBE buffer. The bands were scored as presence (1.0) or absence (0.0), and

both of total amplified, monomorphic and polymorphic fragments were determined.

RAPD-PCR analysis

The formula of **Nei and Li (1979)** was applied to determine the similarity coefficient (F) between strains, and the phylogenetic trees were derived from the distance by un-weighted paired-group method (**Rohlf, 1990**).

Sequencing of 16S rRNA and 18S rRNA genes

Slants of the seven microbial strains were sent to Macrogen® (908 World Meridian Venture Center, #60-24, Gasandong, Geumchun-gu, Seoul 153-781, Korea) for determining the nucleotide sequences of 16S rRNA in the case of bacterial strains and 18S rRNA in the case of fungal strains. Four pairs for amplification of 16S rRNA and 18S rRNA were used as shown below:

Genes	Microorganisms	Sequencing (5'……3')	References
16S rRNA	*E. coli*	Fwd: aga gtt tga tcc tgg ctc ag Rev: ctt gtg cgg gcc ccc gtc aat tc	**Magray et al (2011)**
	Bacillus sp.	Fwd: aaa cty aaa kga att gac gg Rev: acg ggc ggt gtg rc	**Raza and Ameen (2016)**
18S rRNA	*Penicillium* sp.	Fwd: tcc gta ggt gaa cct gcg g Rev: tcc tcc gct tat tga tat gc	**White et al (1990)**
	Aspergillus sp.	Fwd: tcc gta ggt gaa cct gcg g Rev: tcc tcc gct tat tga tat gc	**White et al (1990)**

Sequencing analysis

DNA sequences were analyzed using BLASTN 2.2.23+software (http://www.ncbi.nlm. nih.gov/blast/) against the isolates collected from the database for genotyping. The sequence that showed the lowest e-value and maximum identity was considered as the genotype of the sample analyzed.

RESULTS AND DISCUSSIONS

In ancient periods, the similarities and differences in morphological form of bacteria were estimated based on traditional methods, which divided microorganisms into prokaryotes and eukaryotes, and then divided them into classes, orders, families, genera and species (**Woese and Fox 1977**).

The aim of this study was to evaluate the efficiency of two tools of molecular biology based on DNA in differentiating between certain genera and microbial species, whether bacteria or fungi. To achieve the goal, three fungal isolates, initially known as *Aspergillus* sp. and *Penicillium* sp., were used and four bacterial isolates were initially defined as belonging to *Escherichia* sp. and *Bacillus* sp.

One of the most predictive genetic markers is the ribosomal genes, which are among the most common housekeeping genetic marker used to differentiate, develop and classify bacteria, where the gene of the bacteria is present in all bacteria and its nucleotide sequences is almost unchanged (**Manjul and Shirkot 2018**).

Results in **Table (1)** showed that after determining the nucleotide sequences of the 16SrRNA and 18SrRNA genes of the isolates, partial sequences of 865, 937, 902 and 892 nts of bacterial isolates were obtained and documented in the GenBank under the accession numbers LC455952.1, LC455953.1, LC455954.1 and LC455955.1. By comparing the percentage of the obtained sequences of 16S rRNA genes of the four bacterial strains, the results were logical, as the percentages ranged from 82 to 83% between *E. coli* (LC455952.1) and the strains of *Bacillus* sp. (LC455953.1, LC455954.1 and LC455955.1), while the percentages increased to 93-98% among the isolates of *Bacillus* species.

Molecular identification studies have shown that one of the most common tools to study the genetic diversity of bacteria or fungi is to use ribosomal RNA genes, whether 16S rRNA (**Liu et al 1997, Macrae 2000, Binnerup et al 2001, Duineveld et al 2001, Janssen 2006, Janda & Abbott 2007, Ntushelo 2013, Manjul & Shirkot 2018, Santosa et al 2018)** or 18S rRNA **(Kowalchuk et al 1997, Smit et al 1999, Embong et al 2008 and Banos et al 2018**), respectively. These tools show variation in the nucleotide sequences between genera as well as species, which has helped to record many databases on the sequences of 16S rDNA or 18S rRNA genes.

In the case of fungal strains, partial sequences were obtained less than that estimated in the case of the bacterial strains 460 (LC455956.1), 595 (LC455958.1) and 786 (LC455959.1) nts. Results in **Table (1)** also showed that the percentage identities were fairly high, ranging from 90% among the strains of the *Aspergillus* and *Penicillium*, to 96% for *Aspergillus* species.

Several studies were conducted on identifying bacteria and studying bacterial diversity using the 16S ribosomal RNA gene-based sequencing techniques (**Muyzer et al 1993, Wang et al 2007, Cole et al 2011, Ntushelo 2013 and Santosa et al 2018**).

Phylogenetic trees of bacterial and fungal strains based on the ribosomal RNA genes (16SrRNA and 18SrRNA) illustrated in **Figure (1)** showed that *Bacillus* strains occurred in one cluster (LC455953.1, LC455954.1, LC455955.1) while the *E. coli* strain was separated in a single cluster (LC455952.1). Similar observation was recorded in case of fungal strains, as the two *Aspergillus* (LC455958.1 and LC455959.1) strains were felled in one cluster, while, the *Penicillium* (LC455956.1) strain was found in a separate cluster.

Gene typing based on genomic polymorphism produced by RAPD-PCR method is a recent tool which is widely used for the assessment of inter and intraspecific genetic variation by using a single short random oligonucleotide primer. In recent studies of bacterial or fungal genetics, RAPD-PCR tool generated the best DNA pattern for differentiation of microbial strains. RAPD-PCR assays have reported success among distinguish bacterial and fungal strains.

Results of RAPD-PCR analysis of the four bacterial strains are presented in **Table (2)** and **Figure (2)** which showed agarose gels electrophoresis of DNA polymorphisms produced using eight random primers. Results in **Table (2)** showed that total amplified fragments (TAF), number of monomorphic fragments (NMF), number of polymorphic fragments (NPF) and number of present unique fragments generated by RAPD-PCR of the four bacterial strains of soil using 8 random primers.

The results in **Table (2)** of the RAPD-PCR analysis showed that the bacterial strains produced 82 different molecular DNA fragments using the eight primers. The number of DNA fragments produced varied from primer to another, as Z17 and both of O03 and O04 primers leading the lowest number (04) and the highest (13) of the DNA fragments, respectively. The primers differed in the number of producing DNA fragments of 49, 44, 43 and 42 for MSLB-1, MSLB2, MSL-19 and MSLB3 strains, respectively.

The type of DNA fragments obtained between monomorphic (14 fragments) and polymorphic (68 fragments) differed. The W18 primer did not produce any monomorphic fragments, while the other seven primers produced between 1, 2 and 3 monomorphic fragments. At the level of the polymorphic fragments, the primers of Z17 and both of O03 & W18 produced 3 and 11 fragments, respectively. Regarding the present unique fragments generated by RAPD-PCR of the four bacterial strains of soil using 8 random primers. Data in **Table (2)** show that 10, 6, 10 and 3 DNA fragments were amplified using the 8 primers using the DNA templates of MSL-19, MSLB-1, MSLB2 and MSLB3 strains, respectively. The percentages of identities between the four bacterial strains (LC455952.1, LC455953.1, LC455954.1 and LC455955.1) were estimated on a RAPD-PCR analysis. The results showed that the identities were fairly low, ranging from 70.9 to 78.0% as shown in **Table (3).** This may be attributed to the failure to obtain the full length of the 16SrRNA gene. Phylogenetic trees of bacterial strains based on the RAPD-PCR illustrated in **Figure (4A)** confirmed that obtained by 16SrRNA gene analysis (**Figure 2**).

In the case of RAPD-PCR analysis of fungal strains as shown in **Table (4)** and illustrated in **Figure (3)** similar results were recorded. The DNA polymorphisms (73 DNA fragments) that amplified using 8 primers were more obvious compared to that produced using the four bacterial strains. The three fungal strains (MLSP1, MLSAs1 and MLSAs2) produced 44, 46 and 40 DNA fragments, respectively. It was observed that NMF (18 fragments) and NPF (55 fragments) were amplified, out of these fragments, 7, 15 and 10 present unique markers were obtained for the MLSP1, MLSAs1 and MLSAs2, respectively.

No DNA unique markers were obtained using primers of B09, E04, G07, and O03 were recorded for MLSAs2, MLSP1, MLSAs2, MLSP1 strains, respectively. The three fungal strains did not produce any DNA unique markers with Z16 primer. The highest number of amplified fragments (12 DNA fragments) were amplified using B17 primer followed by F06 (11), G07 (10), W18 (10), E04 (09), O03 (09), B09 (06) and Z16 (06), respectively. No monomorphic fragments were amplified using B17 primer, as 12 polymorphic fragments were recorded.

Results in **Table (5)** show percentage identities of 70.0, 70.6 and 74.2 between the fungal strains (LC455956.1, LC455958.1 and LC455959.1) based on RAPD-PCR. Phylogenetic tree (Figure 4B) of the three fungal strains based on RAPD-PCR showed that the two fungal strains (LC455958.1 and LC455959.1) well felled into the same cluster, while *Penicillium* strain was found in a separate cluster.

By comparing the percentages obtained between the bacterial and the fungal strains by using the 16S rRNA and RAPD-PCR, it was found that differentiating *via* 16S rRNA and 18S rRNA genes between microorganisms was better than that of RAPD-PCR. **Woo et al (2008)** showed that comparison of the nucleotide sequences of 16S rRNA gene sequences are highly conserved within living microorganisms of the same genus and species. This can be attributed to the following: 1) ribosomal genes of limited length between 1500 and 1600 nts; 2) the sequences obtained were partial and not full length, 3) high sequence similarity between the species belonging to the same genus; 4) in the case of RAPD-PCR, the used random primers may exist on the genome and may be not, 5) it is not necessary to know any previous information about genome sequences in case of RAPD-PCR tools, 6) a large number of RAPD-PCR primers should be used in a trail to obtained high fidelity, and finally, the reactions of RAPD should be repeated at least three times, and the same DNA polymorphisms should be recorded. These conclusions were supported by that reported by **Cole et al (2005),** who showed that the classifier algorithm returns a confidence value with which a 16S rRNA gene sequence can be assigned to a taxon (genus and higher) that is represented by a set of sequences, based on the number of times, out of 100 trials. Also, **Patel (2001)** reported that 16S rRNA gene sequences was used to study bacterial phylogeny and taxonomy by far the most common housekeeping genetic marker used for a number of reasons.

As a conclusion, tools of RAPD-PCR and ribosomal RNA genes were successfully used to identify and detect the genetic variability of microbial strains isolated from soil. This conclusion was supported by results of **Saxena et al (2014).**

Table 1. Percentage identities between the partial sequences of 16SrRNA and 18SrRNA genes of four bacterial and three fungal strains.

Bacterial strains	Length (bp)	Accession numbers	% identities of the four bacterial strains			
			E. coli MSL-19	*Bacillus* sp. MSLB-1	*Bacillus* sp. MSLB2	*Bacillus* sp. MSLB3
			LC455952.1	LC455953.1	LC455954.1	LC455955.1
E. coli MSL-19	865	LC455952.1	100			
Bacillus sp. MSLB-1	937	LC455953.1	82	100		
Bacillus sp. MSLB2	902	LC455954.1	83	98	100	
Bacillus sp. MSLB3	892	LC455955.1	83	93	95	100
Fungal strains	Length (bp)	Accession numbers	% identities of the three fungal strains			
			Penicillium sp. MLSP1		*Aspergillusniger* MLSAs1	*Aspergillus* sp. MLSAs2
			LC455956.1		LC455958.1	LC455959.1
Penicillium sp. MLSP1	460	LC455956.1	100			
Aspergillus niger MLSAs1	595	LC455958.1	90		100	
Aspergillus sp. MLSAs2	786	LC455959.1	90		96	100

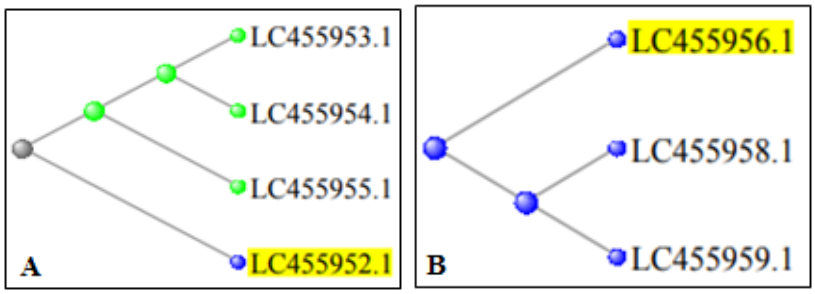

Fig. 1. Phylogenetic trees of bacterial (A) and fungal (B) strains based on the ribosomal RNA genes (16SrRNA and 18SrRNA).

Table 2. Total amplified fragments (TAF), number of monomorphic fragments (NMF), number of polymorphic fragments (NPF) and number of present unique fragments generated by RAPD-PCR of four bacterial strains of soil using 8 random primers.

RAPD Primers	TAF-P	TAF of bacterial isolates				NMF	NPF	No. of present unique markers			
		MSL-19	MSLB-1	MSLB2	MSLB3			MSL-19	MSLB-1	MSLB2	MSLB3
B09	10	10	05	04	02	1	9	4	0	0	0
B17	08	04	04	07	05	2	6	0	0	2	0
E04	13	07	08	08	08	3	10	2	1	2	0
F06	11	07	07	08	06	3	8	1	1	1	0
O03	13	07	08	07	03	2	11	2	2	2	0
W18	11	06	07	03	08	0	11	0	1	1	2
Z16	12	07	09	05	08	2	10	0	1	1	0
Z17	04	02	01	02	02	1	3	1	0	1	1
Total	82	43	49	44	42	14	68	10	6	10	3

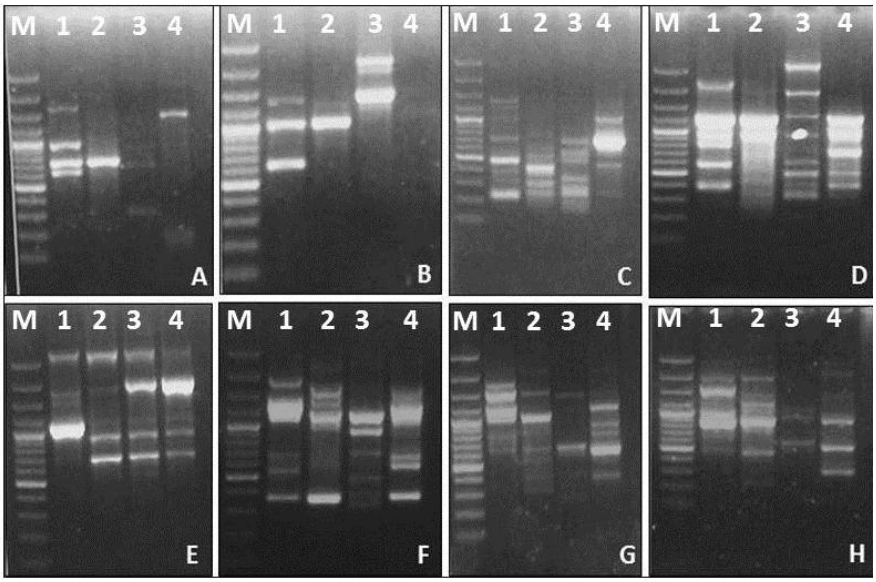

Fig. 2. Agarose gels electrophoresis of DNA polymorphisms produced by RAPD-PCR analysis of four bacterial strains (MSL-19, MSLB-1, MSLB2, MSLB3, Lanes 1, 2, 3 and 4, respectively) using eight **random primers (A (B09), B (B17), C (E04), D (F06), E (O03), F (W18), G (Z16) and H (Z17)). M, 100 bp DNA Ladder.**

Table 3. Percentages identities between the bacterial strains based on RAPD-PCR.

Bacterial strains & Accession numbers		% identities based on RAPD-PCR			
		E. coli MSL-19	*Bacillus* sp. MSLB-1	*Bacillus* sp. MSLB2	*Bacillus* sp. MSLB3
		LC455952.1	LC455953.1	LC455954.1	LC455955.1
E. coli MSL-19	LC455952.1	100			
Bacillus sp. MSLB-1	LC455953.1	72.9	100		
Bacillus sp. MSLB2	LC455954.1	71.9	78.0	100	
Bacillus sp. MSLB3	LC455955.1	70.9	74.5	79.7	100

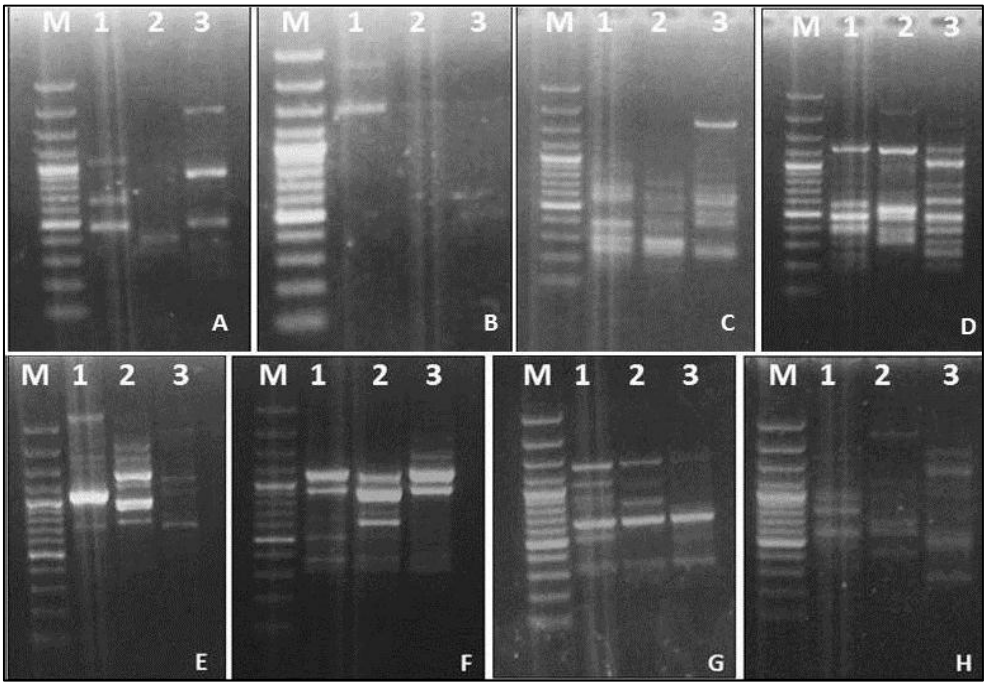

Fig. 3. Agarose gels electrophoresis of DNA polymorphisms produced by RAPD-PCR analysis of three fungal strains (MLSP1, MLSAs1, and MLSAs2, Lanes 1, 2 and 3, respectively) using eight random primers (A (B09), B (B17), C (E04), D (F06), E (O03), F (W18), G (Z16) and H (Z17)). M, 100 bp DNA Ladder.

Table 4. Total amplified fragments (TAF), number of monomorphic fragments (NMF), number of polymorphic fragments (NPF) and number of present unique fragments generated by RAPD-PCR of three fungal strains of soil using 8 random primers.

RAPD-PCR Primers	TAF-P	TAF fungal strains			NMF	NPF	No. of present unique markers		
		MLSP1	MLSAs1	MLSAs2			MLSP1	MLSAs1	MLSAs2
B09	06	05	05	02	2	4	1	1	0
B17	12	07	06	04	0	12	2	3	2
E04	09	06	07	07	5	4	0	1	2
F06	11	06	05	08	3	8	1	2	3
G07	10	05	07	03	1	9	2	4	0
O03	09	06	07	06	3	6	0	1	1
W18	10	05	04	06	1	9	1	3	2
Z16	06	06	05	04	3	3	0	0	0
Total	73	44	46	40	18	55	7	15	10

Table 5. Percentages identities between the fungal strains based on RAPD-PCR.

Bacterial strains & Accession numbers		% identities based on RAPD-PCR		
		Penicillium sp. MLSP1	*Aspergillusniger* MLSAs1	*Aspergillus* sp. MLSAs2
		LC455956.1	LC455958.1	LC455959.1
Penicillium sp. MLSP1	LC455956.1	100		
Aspergillusniger MLSAs1	LC455958.1	70.6	100	
Aspergillus sp. MLSAs2	LC455959.1	70.0	74.2	100

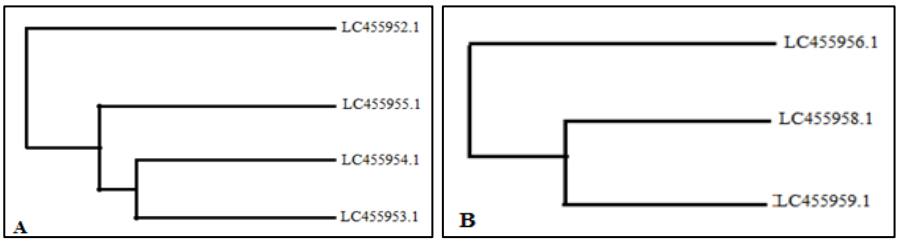

Fig. 4. Phylogenetic trees of bacterial (A) and fungal (B) strains based on RAPD-PCR analyses.

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كفاءة اثنين من الوسائل الجزيئية المعتمدة على الحمض النووي الـ د ن ا المستخدمة للتمييز بين بعض السلالات الميكروبية

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الموجـــــز

فى هذه الدراسة، تم مقارنة اثنين من وسائل البيولوجيا الجزيئية المعتمدة على الـ DNA فى التمييز بين بعض السلالات الميكروبية المعزولة من التربة. تم استخدام نوعين من جينات الريبوسومات، 18S rRNA – 16S rRNA من أجل تعريف أربع عزلات بكتيرية وثلاثة عزلات فطرية على التوالى. العزلات الميكروبية المعرفة تم توثيقها فى بنك الجينات كسلالات تابعة لبكتريا القولون *Escherichia coli* MSL–19 0(LC455952.1) *Bacillus* sp. MSLB–1 (LC455953.1); *Bacillus* sp. MSLB2 (LC455954.1); *Bacillus* sp. MSLB3 (LC455955.1); *Penicillium* sp. MLSP1 (LC455956.1); *Aspergillusniger* MLSAs1 (LC455958.1); *Aspergillus* sp. MLSAs2 .(LC455959.1). تم استخدام الحمض النووى الذى تم الحصول عليه من السلالات الميكروبية السبعة كقالب لـ RAPD–PCR للتمييز بينهما باستخدام ثمانية بادئات عشوائية. تم إجراء التفريد الكهربائى، وعمل تسجيل للحزم، وتم تحليل نسب التمييز بين السلالات البكتيرية والفطرية بشكل منفصل. تم تسجيل نسبة 82–83 ٪ بين *E. coli* وسلالات *Bacillus* الثلاثة، فى حين تم تسجيل نسب تمييز 93–98 ٪ بين سلالات *Bacillus* الثلاثة. ولوحظ اتجاه مماثل (90–96 ٪) بين سلالات *Penicillium* و*Aspergillus*. أكدت النتائج أن نسب التمييز المعتمدة على جينى الحمض النووى الريبوزى الريبوسومى (82–98٪) كانت أعلى من تلك الناتجة عند استخدام RAPD–PCR (70.0–79.7)٪، وهذا قد يعزى إلى أن جينات RNA الريبوسومية ذات أحجام محدودة (~ 1500–1600 نيوتيدة) ومميزه للأنواع الميكروبية ، فى حين تعتمد أداة RAPD–PCR على استخدام بعض البادئات العشوائية التى يمكن تواجدها على الجينوم بأكمله. دعمت نتائج أشجار التقارب الوراثى المستندة إلى اثنين من الأدوات الجزيئية النتائج التى تم الحصول عليها. وكخلاصة تم استخدام كل من RAPD–PCR وجينات الريبوسومات لتتعريف وتحديد التباين الجينى للسلالات الميكروبية المعزولة من التربة.

الكلمات الدالة: الكفاءة، البكتريا، الفطريات، جين الريبوسومي، تفاعل البلمرة المتسلسل العشوائي

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