International Journal of Genetics 1(2): 27-35, 2011
ISSN 2222-1301

Genotyping of *Candida albicans* and *Cryptococcus neoformans* Isolates Recovered from Human, Animals and Soil in El-Fayoum Governorate

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Abstract: In the present work RAPD-PCR was used to study the relatedness among 10 strains of both *Candida albicans* and Cryptococcus neoformans previously isolated from man, animals and soil in El-Fayoum Governorate. The profile of DNA fragments for *C. albicans* strains showed the differentiation of the genomic DNA of *C. albicanss* isolates into numbers of DNA bands, which were different in molecular weight. The dendogram analysis of RAPD pattern of *C. albicans* isolates using specific primers divided the isolates into 3 groups of high similarity (92 - 100 %) and a group of low similarity (21%). These results indicated that the strains that were isolated from the same source of samples gave a high degree of similarity between DNA fragments as in human vaginal and throat swabs. The dendogram analysis of RAPD pattern of *C. neoformans* isolates revealed a high similarity between most of the isolates that varied between 92 - 100 %. It was observed, that the strains isolated from different sites in the same species gave a high degree of similarity. On the other hand, the similarity among animal and soil isolates was low (19- 30 %), although it was 92-93 % among the vaginal isolates of buffaloes, cows and soil samples.

Key words: Genotyping % *C. albicans* % *C. neoformans* % PCR-RAPD % Molecular marker

INTRODUCTION

Random amplified polymorphic DNA (RAPD) was used to characterize the genotypic relatedness among medically important yeasts, such as *Candida* and Cryptococcus spp. by using short oligomer primers (10-mers) with arbitrarily chosen sequences in the polymerase chain reaction [1, 2]. Distinctive and reproducible sets of polymerase chain reaction products were observed for isolates of *C. albicans*, *C. lusitaniae*, *C. tropicalis* and *C. glabrata*. Minor differences in the RAPD profiles, suggestive of mutations that had occurred during the long-term maintenance of the strains [1]. RAPD-PCR was evaluated on a panel of 32°C. *C. albicans* strains, isolated from various anatomical sites of unrelated patients. Random amplified polymorphic DNA was then used in the epidemiological surveillance of the patients in the burn unit during a 9-month period [3]. The efficiency of RAPD method was evaluated and compared for the discriminatory powers of different primers used for genotyping *Candida albicans* isolates. It was concluded that, RAPD is an adequate method for studying small outbreaks in which a few number of isolates are evaluated, but it is laborious and unreliable for many number of isolates recovered in a long time period because of its poor reproducibility and difficulties in evaluating the strains generating many bands [4].

It was reported that RAPD technique can be used to construct genetic maps in a variety of species and to characterize the genotypic relatedness among medically important *Candida* spp [1]. The advantages of this technique on the one hand, that there is no need for a species data information about DNA sequence to construct specific primers as in PCR and on the other hand, there is no need for Southern blot hybridization as needed for analysis of RFLP (restriction fragment length polymorphism) results, because amplification products (amplicons) were analyzed by electrophoresis in 1.4 % agarose gels and detected by staining with ethidium bromide [5]. On the other hand, computer simulations of RAPD random patterns were used to test whether the observed degree of RAPD band pattern

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similarities could occur at random. These simulations suggested that the level of inter-specific band pattern similarities observed in the result could be obtained at random, while intraspecific pattern similarities could not. RAPD would be helpful to discriminate between isolates but not to quantitate the differences [6].

It was stated that, the taxonomic and epidemiological aspects of *C. neoformans* depend on the RAPD analysis *C. neoformans var. neoformans* and *C. neoformans var. gattii* differed in chromosomal make up and RAPD patterns [7]. The genetic relatedness of clinical and environmental *C. neoformans* strains in the Maltese Island was investigated by RAPD fingerprinting with four primers [8]. It was shown that the clinical strains D over the course of 1 year from AIDS patients had identical fingerprints and the electrophoretic patterns of the two clinical strains were also the most common patterns among the environmental strains, but the patterns among the environmental strains showed a wide variability and no correlation with the site of isolation [8].

RAPD analysis is considered a very important genetic marker for DNA and molecular biological studies, so, the designed plane of this study, was based on using of RAPD analysis for the demonstration of the polymorphism among DNAs of both *C. albicans* and *C. neoformans* strains recovered from the human, animals and soil samples.

MATERIALS AND METHODS

Yeast Strains: 10 strains of both *Candida albicans* and *Cryptococcus neoformans* previously isolated from man, animals and soil and confirmed by conventional were used in this study.

Buffers and Reagents: PCR 10X buffer, dNTP mix., Taq DNA polymerase [Fermentas, AB.Gene] [MWG, oligosynthesis-Germany], Tris – borate EDTA buffer [TBE], Blue/ orange 6x DNA loading dye & 100 bp DNA marker ladder [Promega, Madison, WI.USA], 10 mM Tris-HCl [PH $^{8.3}$], 50 mM KCl, 2.5 mM Mg Cl_2, 1.5 U AmpliTaq DNA polymerase [Perkin - Elmer], dNTP mix [deoxy nucleotide Triphosphate] [Promega, Madison, WI.USA], Tris - borate EDTA buffer [TBE], Blue/ orange 6x DNA loading dye & 100 bp DNA marker ladder [Promega, Madison, WI.USA].

Extraction of DNA: Extraction of genomic DNA from both *C. albicans* and *C. neoformans* was performed according to Bialek *et al*. [9] and Sambrook *et al*. [10]. Prepare 10% stock solution of SDS by dissolving 10 gm SDS/100 ml distilled water to obtain a final 1% working solution. 20 mg protinase K/ml distilled water stock concentration was prepared to reach a final 0.1 - 0.3 mg/ml working solution. 1% and 0.3 mg/ml final concentration of SDS and protinase K were added, respectively to each prepared sample. The mixture was kept at 37°C over night. After incubation; equal volume of saturated phenol was added and the mixture was shaken vigorously for 20 seconds. The mixture was then centrifuged at 3000 rpm for 15 minutes and transferred the aqueous phase carefully to a clean tube. An equal volume of the phenol: chloroform: isoamyle alcohol [25: 24: 1] was added to the aqueous phase, shaken and centrifuged at 3000 rpm for 15 minutes. After centrifugation, the aqueous phase was transferred carefully to a clean tube. The previous step of phenol-chloroform extraction was repeated once more. Equal amount of chloroform: isoamyle alcohol [24: 1] was added to the supernatant, then mixed and centrifuged for 15 min. After centrifugation, the aqueous phase was transferred carefully to a clean tube. The DNA was precipitated by adding 0.1 volume of 3M sodium acetate [pH $^{5.2}$] and double volume of cold absolute ethanol. After overnight incubation at -20°C, the DNA was pelleted down by centrifugation at 14000 rpm for 30 min. at 4°C. The DNA pellet was washed with 70 % ethanol, dried off and dissolved in 30 µl TE buffer. The concentration of the resulting DNA was then evaluated. Recovery yield should be between 10 and 20 ng with purity satisfactory for PCR reaction.

RAPD- PCR Assay for *C. Albicans*: Differentiation of *C. albicanss* strains by randomly amplified polymorphic DNA was done according to Munoz *et al*. [11].

For the genotyping differentiation of *C. albicanss* isolates it was used OPA-18 (5`-AGCTGACCGT-3´) primer for genotyping of that isolates by RAPD technique amplification reactions were performed in a volume of 25 µl of distilled water containing primer 5 µl (20 pM), genomic DNA 2.5 µl (1 µg/ml), one PCR bead (ready - to Go PCR bead; Pharmacia Biotech. Sweden). The RAPD - PCR amplification protocol was carried out as follows; one cycle of initial denaturation for 5 min at 94°C before 45 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min final extension at 72°C for 5 min. All reaction products were characterized by electrophoresis on 1% agrose gels in 1xTBE (Tri - borate - EDTA) buffer at 80V for 90 min and then stained in a solution of ethidium bromide at 0.5 µg/ml and determined with UV transilluminator.

RAPD- PCR Assay for *C. neoformans*: Differentiation of *C. neoformans* strains by randomly amplified polymorphic DNA (RAPD)was done by the primer (5`-ATTGCGTCCA-3`) according to Goodwin *et al*. [12] and Aoki *et al*. [13].

The RAPD PCR amplification protocol was carried out as follows: Initial denaturation for 4 min at 94°C (one cycle), 35 cycles of denaturation for 2 min at 94°C, annealing for 2 min at 32°C, extension for 2 min at 72°C and final extension for 10 min at 72°C (one cycle). All reaction products were characterized by electrophoresis on 1.5% agarose gels in 1xTBE (Tris-borate-EDATA) buffer at 80V for 90 min. and then stained in a solution of ethidium bromide at 0.5 µg/ml and determined with UV transilluminator [12, 13].

Statistical Analysis of the RAPD Amplification Products Software Reference Analysis: Gel-pro Analyzer software 3.1, Media Cybernatics, Inc., USA. The dendogram analysis of RAPD patterns of *C. neoformans* strains was calculated and statistical analysis by computerized software programme.

RESULTS AND DISCUSSION

Differentiation of *Candida albicans* Strains: In the recent years the number of serious opportunistic yeast infections, particularly in immuno-compromised patients has increased dramatically [14]. Among them is Candida *albicans*s, which accounts for over 80% of yeast infections. In the present work we genotyped 10 *C. albicans*s isolates recovered from clinical sources and soil in Fayoum. As shown in Figure 1 and Table 1, the profile of DNA fragments for the nine *C. albicanss* strains and the inter-species DNA fragments length polymorphisms showed a strain dependant loss or gain of DNA fragments and showed the differentiation of each genomic DNA of *C. albicanss* isolates into numbers of DNA bands which were different in molecular weight. The DNA of *C. albicanss* isolates was differentiated into 3 bands (isolates no. 1, 6, 9, 10), 5 bands (isolates no. 2, 3, 5, 8) or 6 bands (isolate no. 4). While DNA of isolates no.7 was not differentiated and remained as one band.

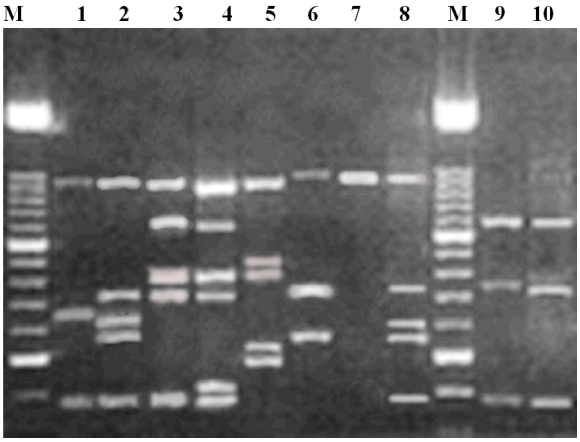

Fig. 1: RAPD-PCR assay of *C. albicans*

These results are similar to that reported by other authors [15, 16]. RAPD-PCR analysis performed in 46 clinical isolates and 2 standard strains of *C. albicanss*, revealed that the amplified numbers of bands ranged from 6 to 12 among the different strains and there were 6 identical fragments in all the amplified profiles of strains, the length of fragments varied from 300 to 2000 bp [17]. Moreover, the use of DNA polymorphisms in different species and strains of *Candida* amplifying genomic DNA with single non- specific primers was assessed [15]. The PCR method used an arbitrary primer (the 10-mer AP3), a primer derived from the intergenic spacer regions (T3B) and the microsatellite primers (GTG) 5 and (AC) 10. The numbers and sizes of the amplification products were characteristic for each species. All yeast species tested were clearly distinguished by their amplification patterns. With all primers, PCR fingerprints also displayed intraspecies variability although PCR profiles obtained from different strains of the same species were far more similar than those derived from different *Candida* species. It was also reported

Table 1: The inter-species DNA fragment length polymorphism of *C. albicans* isolates with the percentages of similarity between strains

Lane 1 M.w.	Lane 2 M.w.	Lane 3 M.w.	Lane 4 M.w.	Lane 5 M.w.	Lane 6 M.w.	Lane 7 M.w.	Lane 8 M.w.	Lane 9 M.w.	Lane 10 M.w.
1448	1415	1445	1387	1418	1505	1435	1417	1141	1140
665	749	1107	1114	898	771		744	791	770
355	639	815	827	843	581		639	367	353
	582	751	747	546			580		
	359	369	406	496			361		
			353						

Table 2: Dendogram analysis of RAPD patterns of *C. albicanss* isolated strains showed the percentages of similarity between strains

Band	1	2	3	4	5	6	7	8	9	10
1	100%	32.3%	92.3%	92.1%	20.6%	95.1%	18%	51.4%	22.8%	18%
2	--	100%	44.3%	30.9%	20.8%	32.9%	18%	98.6%	22.3%	17.7%
3	--	--	100%	30.9%	20.8%	92.9%	18%	60.7%	22.3%	17.7%
4	--	--	--	100%	21.4%	30.9%	18%	28.3%	24%	20.8%
5	--	--	--	--	100%	19.9%	18%	25.5%	24.6%	23.3%
6	--	--	--	--	--	100%	18%	41.3%	22.3%	17.5%
7	--	--	--	--	--	--	100 %	18%	0.00%	0.00%
8	--	--	--	--	--	--	--	100 %	18.9%	23.2%
9	--	--	--	--	--	--	--	--	100%	100%
10	--	--	--	--	--	--	--	--		100%

Table 3: Degree of similarity of *C. albicanss* isolated strains from man, animals and soil

		Primer complementary (Pc)	
Groups	Sample source	Compared strains	Degree of similarity
Human group	8: Human vagina	2&8	98.6 %
	2: Human vagina		
	10: Human throat	9&10	100 %
	9: Human throat		
Animal group	1: Buffalo vagina	1 & 6	95.1 %
	5: Cow nasal	1 & 5	20.6 %
	6: Cow vagina	6 & 5	19.9 %
Soil group	3: Soil of cow	3 & 4	30.9 %
	4: Soil of cow		
Animal vagina& soil	1: Buffalo vagina	1&3	92.3 %
	3: Soil of cow	1&6	95.1 %
	6: Cow vagina	3&6	92.9 %
Anima nasal & soil	4: Soil of cow	4&5	21.4 %
	5: Cow nasal	4&7	-
	7: Sheep nasal	5&7	-

that 16 and 18 different patterns were identified among the *Candida* isolates by karyotyping and RAPD-PCR, respectively [19, 20].

Sequence Identity Matrix of RAPD PCR assay of *C. albicans* (Dendogram analysis of RAPD patterns of *C. albicans*).

The similarity matrix revealed cross relation between samples of human vagina (2 and 8) as the DNA fragments produced by both strains had similarity degree (98.6%) and human throat (9 and 10), where the DNA fragments produced by these strains had similarity degree of (100%), which was considered the highest similarity degree obtained among the all tested *C. albicans* isolates (Table 2). These results substantiate the opinion that the comparative studies of *C. albicanss* strains are essential for proving cross - infections in epidemiological investigations. Typing of *C. albicanss* strains is mainly based on genotypic methods, which showed that the amplification patterns of strains isolated from a given patient were identical [21].

In the animal group (Table 3), the similarity matrix revealed cross relation between animal strains (1, 6) that were isolated from the same sources as the DNA fragments produced by these strains showed a similarity degree of (95.1 %), while the similarity degrees between animal strains (5, 6) and (1, 5) which were isolated from different sources were (19.9 %) and (20.6 %), respectively. On the other hand, the DNA fragment produced by soil strains (3, 4) showed lower similarity degree (30.9 %) than human and animal isolates.

On the other hand, some of the strains that were isolated from different sources of samples gave also a high degree of similarity between DNA fragments as in animals and soil samples (1, 3 and 6), where the strains numbers (3 and 1), that were isolated from soil samples of cow and vaginal swab of buffalo had similarity degree of (92. 3 %), strains numbers (6 and 1) that were isolated from vaginal swab of cow and buffalo had similarity degree of (95. 1 %) and strains numbers (6 and 3) that were isolated from vaginal swab of cow and soil sample of cows had

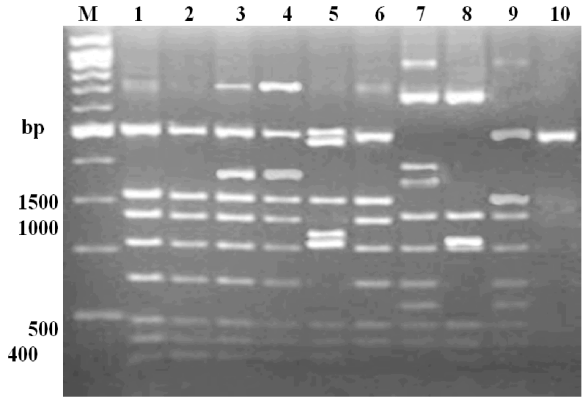

Fig. 2: RAPD-PCR assay of *C. neoformans*

similarity degree of (92.9 %). This indicates that soil here may be the reservoir of the yeast and infection in animals was transmitted from contaminated soil. This is, however not always the case, as a low degree of similarity between DNA fragments was demonstrated in strains number (4, 5) that were isolated from soil samples of cows and nasal swab of cow and had similarity degree of (21.4%).

The importance of RAPD was illustrated in an investigation of the frequency of *Candida* colonization among patients and healthy care personnel of an ICU of a teaching hospital in Kuwait. A total of 57 patients and 45 nurses were investigated. *Candida* isolates were identified to the species level by the Vitek identification system. The typing of selected isolates was performed by randomly amplified polymorphic DNA using three different arbitrary primers (CARARD1, AP3 and CT 5). While all the three primers yielded varying patterns in RAPD analysis for each *Candida* species, the results obtained by AP3 were most discriminatory. The DNA fingerprint produced by one *C. parapsilosis* blood culture isolate was similar to *C. parapsilosis* recovered from the hands of two nurses, suggesting exogenous acquisition of infection [22].

Differentation of *C. neoformans* Strains by RAPD: The RAPD pattern was used for differentiation between various strains of *C. neoformans* recovered from different sources using species-specific primers. The profile of DNA fragments for the 10 *C. neoformans* strains was observed and the inter-species DNA fragments length polymorphisms were compared, which showed a strain dependent loss or gain of DNA fragments. The genomic DNA of *C. neoformans* isolates showed DNA bands which were different in molecular weight (Figure 2 and Table 4). *C. neoformans* isolate no. 1 showed 13 bands; isolates no. 3 and 4 had 10 bands; isolates no. 7 and 9 had 9 bands and isolates no. 2, 5, 6 and 8 showed 8 bands, while DNA of isolate no. 10 was not differentiated and remained as one band.

Sequence Identity Matrix of RAPD-PCR Assay of *C. neoformans* (Dendogram Analysis of RAPD Patterns of *C. neoformans*): The result of sequence identity matrix of RAPD-PCR assay of *C. neoformans* is shown in Table 5, where the similarity among the tested strains ranged from 22 and 100%. As shown in Table 6, the sequence identity matrix of RAPD–PCR assay of *C. neoformans* divided the tested isolates into 3 groups of high similarity, where cross relation was revealed between samples of group I (1, 2, 3 and 4), as the DNA fragments produced by strains numbers (2, 1) had similarity degree of 99.6 %; strains numbers (3, 1) had

Table 4: The inter-species DNA fragment length polymorphism of C. neoformans isolates with the percentages of similarity between strains

Lane 1 M.w.	Lane 2 M.w.	Lane 3 M.w.	Lane 4 M.w.	Lane 5 M.w.	Lane 6 M.w.	Lane 7 M.w.	Lane 8 M.w.	Lane 9 M.w.	Lane10 M.w.
998	507	821	808	497	810	998	759	1009	484
915	308	499	492	481	485	812	744	489	
850	267	364	359	297	297	759	266	301	
816	209	304	300	228	257	264	213	266	
548	154	263	260	212	201	200	202	201	
499	91	206	203	85	147	147	85	147	
312	64	150	149	60	85	114	59	115	
270	38	89	86	34	60	84	32	86	
213		62	60			59		58	
157		36	36						
93									
64									
34									

Molecular weight = ranged between 30-1500 bp

Table 5: Dendogram analysis of RAPD patterns of *C. neoformans* isolated strains

Band	1	2	3	4	5	6	7	8	9	10
1	100%	99.6%	92.3%	92.3%	34.3%	34%	30.6%	30.6%	34.3%	22.8%
2	--	100%	92.7%	92.7%	30.6%	30.1%	30.7%	30.7%	34.4%	22.9%
3	--	--	100%	100%	32.3%	37%	30.7%	30.7%	33.8%	22.3%
4	--	--	--	100%	32.3%	32.3%	30.7%	30.7%	33.8%	22.3%
5	--	--	--	--	100%	92.3%	30.6%	30.6%	34.3%	22.8%
6	--	--	--	--	--	100%	30.6%	30.6%	34.3%	22.8%
7	--	--	--	--	--	--	100%	96.3%	31.8%	18.9%
8	--	--	--	--	--	--	--	100%	31.8%	18.9%
9	--	--	--	--	--	--	--	--	100%	22.1%
10	--	--	--	--	--	--	--	--	--	100%

Table 6: Degree of similarity of *C. neoformans* isolated strains from man, animals and soil

		Primer complementary	
Groups	Sample source	Compared strains	Degree of similarity
Group I (Human group)	1: Human vagina	2 & 1	99.6 %
	2: Human vagina	3 & 1	92.3 %
	3: Human throat	4 & 1	92.3 %
	4: Human throat	3 & 2	92.7 %
		4 & 2	92.7 %
		4 & 3	100 %
		1, 2, 3 & 4	94.9 %
Animal group	6: Cow vagina	6 & 7	30.6 %
	7: Buffalo nasal	7 & 9	31.8 %
	9: Buffalo vagina	6 & 9	34.3 %
		6, 7 & 9	32.2 %
Soil group	5: Soil cows	5 & 8	30.6 %
	8: Soil buffaloes		
Group III	Animal & Soil	8 & 7	96.3 %
Group II & III	Animal & Soil	6, 5, 8 & 7	94.3 %
Group II	Animal & Soil	6 & 5	92.3 %
Group I & III	Human, Animal & Soil	1, 2, 3, 4, 8 & 7	95.6 %
Group I, II & III	Human, Animal & Soil	1, 2, 3, 4, 6, 5, 8 & 7	94.6 %
Group I & II	Human, Animal & Soil	1, 2, 3, 4, 6 & 5	93.6 %

similarity degree of 92.3 %; strains number (3, 2) had similarity degree of 92.7 %; strains numbers (4, 1) had similarity degree of 92.3 %; strains number (4, 2) had similarity degree of 92.7 %; strains number (4, 3) had similarity degree of 100 %, which is considered the highest similarity degree obtained among the all tested *C. neoformans* isolates in this group and other tested groups, whereas the DNA fragments produced by strains numbers (5 and 6) of group II had similarity degree of 92.3 % and that of strains numbers (7, 8) of group III had similarity degree of 96.3 %.

On the other hand, the sequence identity matrix of RAPD-PCR assay of *C. neoformans* revealed also a group of no similarity, where the similarity matrix revealed a high distance in relation to other samples (9 and 10), as the strain No. 10 had no similarity with strain No. 9 or the other tested strains.

Moreover, the DNA fragments produced by strains of groups I and II showed a similarity degree of 93.6 %, whereas the DNA fragments produced by strains of group II and III had similarity degree of 94.3 %. Moreover, the strains included in groups I and III showed a similarity degree of 95.6 %. Finally, the DNA fragments produced by strains of group I, II and III showed a similarity degree of (94.6 %).

These results indicated that the strains that were isolated from the same source of samples gave a high degree of similarity between DNA fragments as in human samples in group I, where, the strains numbers (1, 2) that were isolated from vaginal swabs of human had similarity degree of 99.6% and the strains numbers (3, 4) that were isolated from throat swab of human had similarity degree of (100 %). On the other hand, it was observed that, the strains that were isolated from different sources of samples within the same species in the same group I gave a high degree of similarity between DNA fragments, as the human samples in group I included strains numbers (3,1 - 2,3- 1,4- 2,4) that were isolated from throat and vaginal swabs of human had similarity degree of 92.3% to 92.7 %.

On the other hand, the animals and soil samples in groups II and III confirmed that, the strains that were isolated from different sources of samples gave also a high degree of similarity between DNA fragments as the strains numbers (5, 6) in group II that were isolated from soil sample of cows and vaginal swab of cows, respectively had similarity degree of (92.3 %), whereas the strains numbers (7 and 8) in group III that were isolated from nasal swab of buffalo and soil sample of buffaloes, respectively had similarity degree of of (96.3 %). Moreover, the similarity matrix revealed cross relation between human strains (1, 2, 3, 4) in the human group as the DNA fragments produced by human strains showed the highest similarity degree (94.9 %), while the human strains that were isolated either from the same human source or from different human sources gave also a high degrees of similarity, as the similarity matrix revealed cross relation between human strains (2 and 1) that were isolated from vaginal swabs showed a similarity degree (99.6 %) and (3 and 4) that were isolated from throat swabs showed a similarity degree (100 %), (3 and 1) showed a similarity degree (92.3 %), (4 and 1) showed a similarity degree (92.3 %), (3 and 2) showed a similarity degree (92.7 %) and (4 and 2) showed a similarity degree (92.7 %). whereas, in the animal group, the similarity matrix revealed cross relation between animal strains (6 and 7) that were isolated from different sources as the DNA fragments produced by these strains showed a low similarity degree of (30.6 %). While the similarity degree between animal strains (6 and 9) that were isolated from the same source was low (34.3 %). Moreover, the similarity degree between animal strains (7 and 9) that were isolated from different sources was low (31.8 %) and so the similarity degree between the 3 animal strains (6, 7 and 9) in the animal group was low (32.2 %).

Variations in the pattern was also reported by others [22, 23, 24]. It was shown that three clinical isolates of *C. neoformans var. neoformans* (two from Burundi and one from Zaire) had identical pattern, but this pattern was not found in the isolates, which were recovered from the dust and around the patient's homes. Similar findings were reported in our work but with using of RAPD technique, as the human C. *neoformans* isolates in human group showed identical DNA pattern with high degrees of similarities. On the other hand, the degrees of similarities were relatively low between human strains and environmental (soil) strains and between animal strains and environmental strains.

Similar findings to our work were also reported [8]. when randomly amplified polymorphic DNA fingerprinting was used with four primers for determination of the genetic relatedness of clinical and environmental *C. neoformans* strains in the Maltese Island. It was shown that the clinical strains D over the course of 1 year from AIDS patients had identical fingerprints as in *C. neoformans* human isolates in our study and the electrophoretic patterns of the two clinical strains were also the most common patterns among the environmental strains, as in clinical and environmental isolates in our study but the patterns among the environmental strains showed a wide variability and no correlation with the site of isolation, as in soil group in our study, where the similarity degree was low (30.6 %). Moreover, they compared genetically between clinical isolates and pigeon dropping isolates of *C. neoformans* by PFGE and RAPD. They found a great resemblance between both of the *C. neoformans* isolates.

It was concluded that, RAPD-PCR could be used to determine the percentages of genetic relatedness among different strains of Candida *albicans* and *Cryptococcus neoformans* to be helpeful in the epidemiological studies.

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