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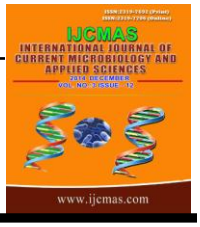

Original Research Article

Fingerprinting and assessment of genetic variability of *Subulura brumpti* recovered from domestic fowls in Taif, KSA

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A B S T R A C T

This study describes the use of ten Random Amplified Polymorphic DNA (RAPD) primers to determine the genetic variability of *Subulura brumpti* in Taif, Saudi Arabia. *Subulura brumpti* from different Saudi domestic fowls were included in this study. The results indicated that 135 bands were amplified, out of a total of 346. Of these amplified bands, 108 were polymorphic (80%) and 27 were monomorphic (20%). In addition, 57 specific positive RAPD markers for *Subulura brumpti* in KSA were identified. This is the first molecular genetic study carried out with *Subulura brumpti* in KSA. These results demonstrate the random pattern of amplification, and the heterogeneity, and polymorphism of the *Subulura brumpti* population, suggesting many distinct genotypes within Saudi Arabia. The markers obtained might have potential applications with for *Subulura brumpti* control in the poultry industry in KSA. Further detailed investigations at DNA sequence level are needed to obtain complete and accurate population structure of *Subulura brumpti* in KSA.

Keywords

Subulura brumpti, characterization, RAPD-PCR.

Introduction

Members of the genus Subulura are nematodes commonly infecting various orders of birds (Tinamiformes, Passeri formes, Strigiformes, Caprimulgiformes, Piciformes and Gruiformes) and in mammals as Primata, Marsupialia, Rodentia (Vicente *et al.*, 2000).

The common internal parasitic infections occur in poultry include cestodes, nematodes and coccidia. These worm infections may cause considerable damage and great economic loss to the poultry industry due to malnutrition, decreased feed conversion ratio, weight loss, lowered egg production and death in young birds (Puttalakshmamma *et al.*, 2008).

Understanding of genetic structure and status of genetic variation of the parasite

populations has important implications for epidemiology and effective control (Ashrafi *et al.,* 2006; Ramadan *et al.,* 2010). RAPD-PCR technique provides an effective method for obtaining genetic markers in all sorts of organisms (Tibayrenc *et al.,* 1993). It is useful genetic marker for the genetic characterization of parasite populations (Gasser *et al.,* 2006; Awad *et al.,* 2010, Ai *et al.,* 2011). This method was developed by Welsh and McClelland (1990) and Williams *et al.* (1990). This method does not require prior sequence information. RAPD –PCR is a simple and reproducible technique, which uses single oligonucleotide primers at low stringency to generate random-amplified polymorphic DNA. It has proved to be a powerful and rapid method for detecting polymorphic genetic variation. Such variation can be used in population and phylogenetic studies, in characterization of parasite strains and species and in comparison of field isolates (Gunasekar *et al.,* 2008). In situations where genetic crosses are not possible, either because the organisms belong to different species or because sexual reproduction is absent (or not feasible experimentally), these issues cannot be resolved by traditional Mendelian methods; they can be investigated only by indirect methods (Tibayrenc *et al.,* 1993).

Until now, no reports about molecular genetic fingerprinting or genetic variability at the DNA level of *S brumpti* populations in KSA have been published. In the present study RAPD PCR was used to conduct fingerprinting and investigate genetic variability among different *S brumpti* populations in KSA.

Materials and methods

Samples collection

Several nematode worms belonging to the genus *Subulura* were recovered from the caecum of domestic chicken (Balady) *Gallus gallus* domestics in Taif area Saudi Arabia.

Genomic DNA extraction

Genomic DNA was extracted from five studied isolates using Wizard® SV Genomic DNA Purification System (Promega Madison, wi, USA) following the manufacturer's instructions.

RAPD PCR

PCR amplifications were performed using ten arbitrary 10-mer RAPD primers (Operon Tech., Inc.) according to Williams *et al* (1990).The 25ul mixture contained 25-50 DNA template, 12.5 GoTaq® Green Master Mix (Promega) and 10 poml of each used primer. List of used primers are found in table 1. PCR amplifications were carried out in Eppendorf® thermal cycler, programmed for initial denaturation, 94°C for 2.5 min and 35 cycles of subsequent denaturation, 94°C for 45 s; annealing temperature, 36°C for 30 s; extension temperature, 72°C for 2 min and final extension, 72°C for 10 min. PCR products (10 ul) were electrophoresed on 10 x 14 cm 1.5% agarose gel at 100V in 1X TAE buffer with DNA ladder standard 100 bp (Jena Bioscience, Germany) for 30 min. The gels were stained with 0.5 µg/ml ethidium bromide, visualized by ultraviolet illumination and documented using a GeneSnap 4.00-Gene Genius Bio Imaging System (Syngene; Frederick, Maryland,USA).

Data analysis

The digital image files were analyzed using Gene Tools software from Syngene. All observed individual bands were scored as present or absent (1 or 0) for each isolate.

Similarity coefficient (S) was calculated according to Nei and Li (1979).

Results and Discussion

Vicent, et al (2000) reported that species of the genus *Subulura Molin*, 1860, are commonly known to parasites birds and mammals. However they described *Subulura lacertilia* from a reptilian host. In Saudi Arabia, *Subulura suctoria* has been previously described from guinea fowl in Sarawat Mountain at Taif by Abou Znada (1993). Dehlawi (2007) reported the presence of three species of nematodes from the intestine of Balady chicken *Gallus Gallus* domestics from Jeddah namely, *Subulura brumpti, Ascaridia galli* and *Capillaria caudinflata*. Ashour & Banaja (2013) reported the presence of *Subulura brumpti* from birds in Taif. Ashour and Al-Gody (2013) using scanning electron microscopy described morphological topography of *Subulura brumpti*.

The present is the DNA analysis and the first description of this nematode from Saudi Arabia using RAPD-PCR technique, aiming to characterize this nematode. Studies on genetic variability within and between populations of *Subulura sp.* have important implications for epidemiology, control and diagnosis of *Subulura sp.* In this study, ten RAPD 10- mer primers were used and all of them were successfully reacted and generated PCR products with all isolates and provided distinct patterns of amplified genomic DNA.

Several studies have been conducted to characterize different parasites at molecular level using RAPD –PCR assay and reported several advantages of this technique on respect of parasites genetic characterization and population structure (Shalaby *et al.*, 2011; Farooq *et al.*, 2012; Taha 2012; Sahoo *et al.*, 2013).

The used primers have 346 produced bands among 135 amplified bands (Table 1). Out of which 108 bands were polymorphic bands (80%) and 27 were monomorphic bands (20%) (Table2). The highly % of polymorphism is indication of intragenotype variability within studied *Subulura brumpti* isolates. Such intraspecific variation within *Fasciola hepatica* was investigated by RAPD-PCR (Rokni *et al.*, 2010; Shahbazi *et al.*, 2011).

The ten primers produced multiple band profiles with a number of amplified DNA fragments ranging from one to 11 (Table 1). The size and number of amplified fragments were also varied with different primers. The size of amplified fragments ranged from approximately 200 bp to 3000 bp as shown in Figure (1). The maximum number (54 fragments) was amplified with primer (OP-A3). The minimum number (11 fragments) was amplified with primer (OP-A7) (Table1). This differences in size and number of amplified bands produced by used primers and studied isolates reflects the random pattern of amplification and indication of genetic heterogeneity among studied isolates (Eltoum *et al.*, 2003). Moreover it could be a result of nucleotide changes at the primer annealing site or due to addition or deletion between two priming sites (Sharma *et al.*, 2001).

As regards genetic relationships, data presented in (Table 3) showed that, the highest genetic similarity was between 220 and 221isolates (91%), while the genetic similarity between 22S and 218 isolates was the lowest one (78.7%). The genetic relationships amongst the six *Subulura brumpti* isolates are shown in Figure. 2. The six isolates were grouped into two clusters according to the genetic duistance between them. The first cluster was consisted of 4 isolates (219S, 220, 221, SB).

Table.1 List of used primers, their nucleotide sequences, total number of bands

Primer code	Prime sequence	Isolates						Total bands	Amplified bands
		219 S	220	221	218	222S	SB		
OP-A1	CAGGCCCTTC	7	6	8	7	9	1	38	18 (350-2100 bp)
OP-A2	TGCCGAGCTG	7	5	5	8	8	9	42	20 (200-1400 bp)
Op-A3	AGTCAGCCAC	11	3	6	11	13	10	54	19 (200-1400 bp)
OP-A4	AATCGGGCTG	3	4	3	3	8	5	26	12 (280-1500 bp)
OP-A7	GAAACGGGTG	3	2	2	2	0	2	11	3 (600-1400 bp)
OP-A8	GTGACGTAGG	5	5	5	4	5	6	30	8 (400-3000 bp)
OP-A9	GGGTAACGCC	4	5	5	7	4	4	29	10 (280-1850 bp)
OP-B1	GTTTCGCTCC	6	8	8	10	8	8	48	12 (350-1850 bp)
OP-B2	TGATCCCTGG	1	7	1	3	12	10	34	17 (300-2500 bp)
OP-B5	TGCGCCCTTC	6	1	8	8	4	7	34	16 (350-3000 bp)
Total		**53**	**46**	**51**	**63**	**71**	**62**	**346**	**135**

Table.2 Monomorphic and polymorphic bands produced by ten primers

Primer code	Amplified bands	Monomorphic bands	Homogeneity %	Polymorphic bands	Heterogeneity %
OP-A1	**18**	1	5.55	17	94.44
OP-A2	**20**	2	10	18	90
Op-A3	**19**	3	15.78	16	84.2
OP-A4	**12**	1	8.33	11	91.66
OP-A7	**3**	1	33.33	2	66.66
OP-A8	**8**	5	62.5	3	37.5
OP-A9	**10**	3	30	7	70
OP-B1	**12**	8	66.66	4	33.33
OP-B2	**17**	1	5.8	16	94.11
OP-B5	**16**	2	12.5	14	87.5
Total	**135**	27		108	

Table.3 Similarity coefficient values among the six studied Subulura Sp. Isolates

	219S	220	221	218	22S	Sb
221	1					
220	0.863	1.000				
221	0.850	0.908	1.000			
218	0.803	0.803	0.789	1.000		
22S	0.826	0.853	0.866	0.787	1.000	
Sb	0.863	0.842	0.876	0.792	0.853	1.000

Table.4 Specific markers for each isolate across RAPD-PCR analysis

Primer code	219S	220	221	218	222S	SB	Total
OP-A1	3 (900, 1250, 2100)	0	1 (1400)	3 (400, 1200, 1500)	2(350, 500)	0	9
OP-A2	0	1(280)	0	3(450, 600, 1000)	1(500)	2(1100, 1400)	7
Op-A3	0	0	0	0	4(700, 720, 750, 800, 2000)	1(500)	5
OP-A4	1(700)	0	0	1(600)	3(300, 500, 700)	0	5
OP-A7	0	0	0	0	0	0	0
OP-A8	0	0	0	0	1(350)	2(800, 1100)	3
OP-A9	0	0	1(1250)	4(1100, 1500, 1750, 1850)	0	0	5
OP-B1	2(600, 900)	0	0	4(950, 1000, 1600, 1850)	0	0	6
OP-B2	1(2000)	3(650, 750, 3000)	2(1400, 2500)	2(1100, 1200)	2(4000, 4500)	1(280)	11
OP-B5	0	0	1(1300)	7(600, 700, 720, 900, 1500, 2100, 3000)	0	0	8
Total	7	4	5	24	13	6	59

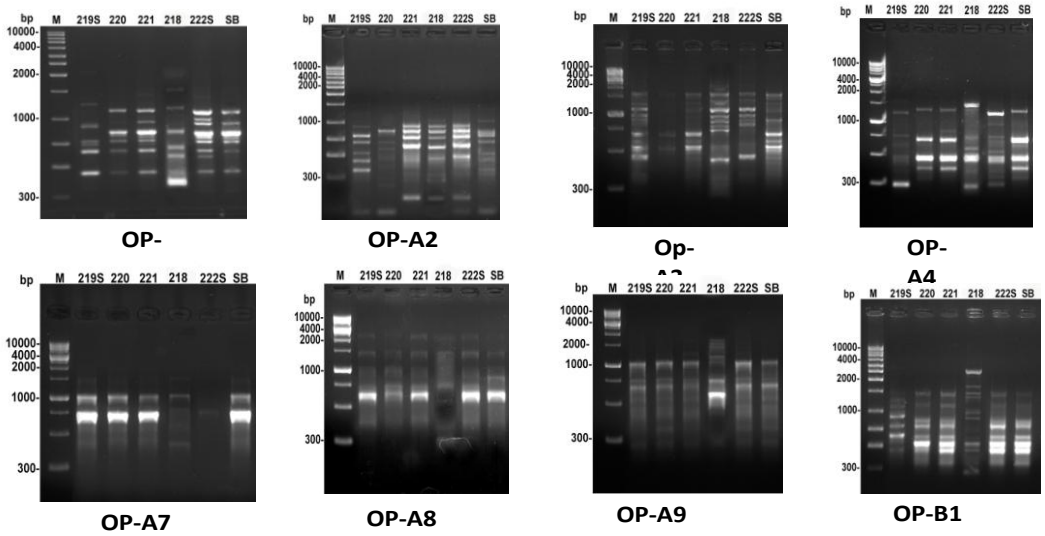

Figure 1. DNA banding pattern revealed from different RAPD primers with 6 Subulura sp. Isolates

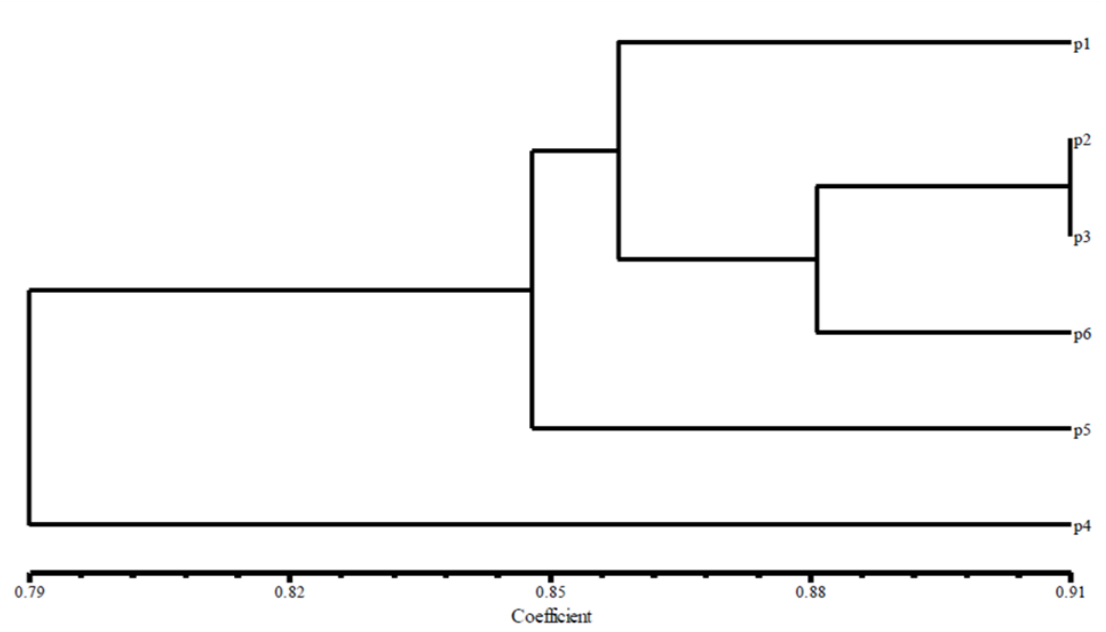

Figure2. Dendrogram demonstrating relationship between the studies 6 Subulura isolates: P1: 219S; P2: 220; P3: 221; P4: 218; P5: 222S; P6: SB.

The second cluster consisted of 2 isolates (218 and 222S). These results might be due to presence of different *Subulura brumpti* genotypes in Saudi Arabia.

Table 4 shows the specific markers obtained across RAPD-PCR analysis. Primers OP-A1 and OP-B2 generated the highest number of specific markers (9 and 11 marker) respectively. In contrast, OP-A7 primer did not produce any specific marker. On the other hand 218 and 222S isolates were the highest specific marker producers (24 and 13 specific marker) respectively. Fifty nine different specific RAPD markers were obtained across RAPD banding pattern amongst the six *Subulura brumpti* populations studied. The markers used in the present investigation therefore proved to be quite powerful in distinguishing these different *Subulura brumpti* isolates. The highest number of RAPD markers was generated using two primers (OP-A1 and OP-B2), which could be used for early detection and molecular diagnosis of *Subulura brumpti* infestation in agricultural quarantine situations. Several RAPD markers have been deduced and utilized to characterize and diagnose different parasites (Awad *et al.*, 2010; Taha 2012; Sahoo *et al.*, 2013).

In Conclusion, RAPD-PCR is a powerful technique to characterize and assessment of genetic variability in *S brumpti*. The RAPD markers obtained in the present study might have applications for agriculture quarantine purposes as well as directly benefiting the poultry industry in KSA. Detailed investigations at the DNA sequence level will, however, be needed to obtain the complete and accurate population structure of *Subulura brumpti* in KSA.

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