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Ver-1 Gene Sequencing and Homology of Two Egyptian *Aspergillus* Isolates

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Sequencing of the conserved region of *ver-1* gene in each of two Egyptian *Aspergillus* sp., *A. nidulans* and *A. parasiticus* isolates, was conducted. Variation in DNA size (515 bp for *A. nidulans* and 495 bp for *A. parasiticus*) was observed with a high homology degree of 97%, however with no difference in the translated amino acid sequence. The obtained *ver-1* gene sequence of both isolates was matched, using genebank database, with that of each of a no. of strains belonging to *Aspergillus* species, protein homology with the most similar strain was then conducted. No difference in the translated amino acids was observed for *ver-1* gene of the *A. nidulans* isolate while two amino acid variations were observed in case of *A. parasiticus* isolate. The obtained sequences of the two investigated local isolates revealed no matching results with any of the *A. nidulans* strains on the gene bank database reflecting the uniqueness of the *A. nidulans* Egyptian isolate.

Keywords: *Aspergillus* sp.; Conserved region; *Ver-1* gene; Homology.

Most aspergilli that produce aflatoxin are members of *Aspergillus* section Flavi, however isolates of several *Aspergillus* species not closely related to section Flavi also have been found to produce strigmatocystin and aflatoxin[1].

However, in agricultural commodities, they are primarily produced by *Aspergillus flavus* and *Aspergillus parasiticus*. *A. flavus*, *A. pseudotamarii,* and *A. ochraceoroseus* produce only the B aflatoxins, and *Aspergillus nomius, A. bombycis,* and *A. parasiticus* produce both B and G toxins[2-11].

However, as many as 20 different aspergilli, including *A. nidulans,* and species of *Bipolaris, Chaetomium, Farrowia* and *Penicillium*, produce (intermediates in the aflatoxin pathway) sterigmatocystin (ST), a highly toxic intermediate in AFB1 biosynthetic pathway[12,13].

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Even though the AFB1 biosynthetic pathway in *A. flavus* and *A. parasiticus* and the ST biosynthetic pathway in *A nidulans* are believed to be similar, cooperative studies utilizing all three species being pursued to identify any key differences which exist in biosynthesis or regulation and shed light on the evolution and acquisition of the pathway by the aspergilli and other genera.

Aflatoxins and sterigmatocystin are synthesized by the polyketide metabolic pathway and the general accepted scheme for AF/ST biosynthesis is: polyketide precursor → norsolonic acid, NOR → averantin, AVN → hydroxyaverantin, HAVN→ averufanin, AVNN → averufin, AVF ® hydroxyversicolorin, HVN → versiconal hemiacetal acetate, VHA →versicolorin B, VER B→ versicolorin A, VER A → demethylsterigmatocystin, DMST → sterigmatocystin, ST → *O*- methylsterigmatocystin, OMST → aflatoxin B1, AFB1. A branch point in

the pathway has been established from VER B leading to different aflatoxin structural forms B1 and B2[14-20].

The regulatory gene, *aflR*, coding for the pathway regulatory factor (AFLR protein), controls the expression of the structural genes at the transcriptional level[15,21].

Sterigmatocystin, the penultimate precursor to aflatoxin, is produced by a number of non-aflatoxigenic fungi including *A. nidulans*. Brown *et al.*[22] characterized a 60 kb DNA region in *A. nidulans* that consists of a cluster of genes responsible for 25 co-regulated transcripts involved in sterigmatocystin biosynthetic pathway in this fungus.

In *A. flavus* and *A. parasiticus*, the order of the genes and their direction of transcription of the aflatoxin cluster genes are identical and there is a high degree of sequence conservation (>95%) at both the nucleotide and amino acid level. However, the order of the genes in the *A. nidulans* sterigmatocystin gene cluster is somewhat different from that of *A. parasiticus* and *A. flavus*[23].

Ver-1 encodes a 28-kDa NADPH-dependent reductase involved in conversion of versicolorin A (VA) to demethylsterigmatocystin[24-26]. Liang *et al.,*[27] confirmed that *ver-1*A of the two copies of *ver-1* (*ver-1A* and *ver-1B*) is the only functional *ver-1* gene in *A. parasiticus* SU-1 and that its gene product is involved in the conversion of versicolorin A to sterigmatocystin in AFB1 biosynthesis.

The current study focused on sequencing the conserved region of *ver-1* gene of two Egyptian local isolates; namely *A. parasiticus* (B1, B2, G1 and G2 aflatoxin producer) and *A. nidulans* (nonaflatoxigenic), subsequent homology studies were conducted among the two investigated isolates and other matching isolates on the genebank database.

MATERIALS AND METHODS

Sequence comparison

All the homology and comparison analyses were achieved by Database BlAST nr: GenBank + EMBL+ DDBJ+ PDB. Protein similarities of the *ver*-1 conserved region BLASTp searches were done against the non- redundant protein database[28].

Fungal species

Two Egyptian fungal species namely; *Aspergillus parasiticus*, *and Aspergillus nidulans* were used in this study and kindly obtained from the culture collection of Al-Azhar University.

Fungal growth media

The fungal isolates under investigation were grown on Malt Extract Agar (MEA) (Malt extract, 20; glucose, 20; peptone, 1; agar, 20 g/L) for maintenance and Yeast Extract Sucrose (YES) (Yeast extract, 20; sucrose, 150 g/L) for DNA isolation.

DNA - based techniques

Fungal DNA extraction using Qiagen kit

The mycelial growth from 5–7 days old cultures on MEA slopes were scraped by using 2 ml of sterile distilled water. The two mls of spore suspension were used to inoculate a 100 ml YES medium in a universal 250ml flask and incubated with gentle shaking (180 rpm at 28ÚC for 48h). The mycelia from the flasks were harvested by filtration under aseptic conditions using a microcloth, washed with sterile distilled water and stored at -20 overnight in a sterile Petri dishes. The mycelia were lyophilized in a Heto lyophilizer system model Maxi Dry. The freeze-dried mycelia were ground in a mortar using a sterile pestle, and the powdery samples were placed in eppendorf tubes (1.5 ml). DNA extraction was conducted using DNeasy kit (Qiagen-Germany).

Polymerase chain reaction (PCR)

Taq PCR Master mix (purchased from Qiagene) was used to amplify the desired gene using a thermal cycler machine (gradient Robocycler 96 Stratagene, USA) by combining 50ml of the Master Mix (2.5U *Taq* DNA polymerase, 200mM of each dNTP, 1x Qiagene PCR buffer), 50 pmole of each primers, 200 ng DNA as a template in 100ml of total reaction volume. The mixture was then placed to the thermal cycler machine directly to start the appropriate PCR program including a universal denaturation cycle (5 min at 94°C), 30 cycles of annealing/extension reactions (20 sec at 94°C, 30 sec at 75°C and 60 sec at 72°C) and cycle of final extension step (5 min at 72°C) followed by soaking at 4°C. The primers sequence used in the amplification of *ver-1* gene was F direction (5'-gccgcaggccgcggagaaagtggt-3') and R direction (5'-ggggatatactcccgcgacaacagcc-3')[29].

Agarose gel electrophoresis

Agarose (2%) was added to 100 mL (1 X) of electrophoresis buffer (10X TBE, Tris-Borate EDTA, tris-base 108g/l; boric acid, 55g/L; 40 mL of 0.5M EDTA (pH8)). The gel was boiled and ethidium bromide solution (0.5mg/mL) was added at 55°C, then poured into sealed gel tray and the appropriate comb was inserted[30]. After agarose solidification, stop loading solution was added to the samples and loaded along with DNA ladder. The gel was run to the desired level of voltage and the DNA was visualized and imaged using the transilluminator of a gel documentation system (BIO-RAD, Gel Doc 2000).

Purification of PCR products from the gel

The electrophoresed PCR products were purified using a QIAEX II gel extraction kit (Qiagen).

DNA sequencing

Sequencing of amplified PCR fragments was carried out by Cy5/Cy5.5 Dye Primer Sequencing kit from Visible Genetics Inc. for use with the Open Gene automated DNA sequencing system[31,32].

RESULTS

Sequencing results of the conserved region of *ver-1* gene amplified from the two investigated isolates *A. nidulans* (nonaflatoxiginc) and *A. parasiticus* (aflatoxins B1, B2, G1 and G2 producer) are shown in Fig.(1) and Fig. (2), respectively.

Sequencing results of *ver-1* gene of the two investigated isolates indicated variations in the size and bases of the gene. Protein homology between the investigated *A. nidulans* and *A. parasiticus* isolates ranged from 90% to 100% at a coverage query of 97%. Unfortunately, the gabs between the two sequences resulted in size differences however with no change in the translated amino acids (Fig. 3).

The obtained *ver-1* gene sequence of *A. nidulans* isolate was matched with that on the gene bank of each of 27 fungal strains (Table 1). Those 27 fungal strains belong to five *Aspergillus* species; *A. oryzae* (11 strains), *A flavus* (9 strains), *A. parasiticus* (4 strains) *A. sojae* (2 strains) and *A. nomius* (1strain). Interspecific similarity levels were observed and ranged from 91 to 100% at a query coverage range from 7% to 96%, respectively. However, 100% identities were observed with six strains (four strains of *A. oryzae*; NFRI 1133, RIB 62, SRRC 2098, SRRC 2103 and two strains of *A. flavus*; NRRL3357, NPLTX21-5) at a query coverage ranging only from 7 to 86%. Also, the results of sequencing indicated that *ver-1* DNA sequence of *A. nidulans* was highly conserved particularly among strains of *A. oryzae* and *A. flavus*.

Interestingly, no sequence matching was observed with *A. pseudotamarii, A. ochraceoroseus* (B1 producers), *A. bombycis* (B and G producers), *Bipolaris* sp., *Chaetomium* sp., *Farrowia* sp., *Penicillium* sp., (intermediate producers) and other non-producer species such as *A. niger*. Moreover, no sequence matching with any *A. nidulans* strain was observed.

On the level of protein homology, only one strain *(Aspergillus flavus* strain AF70) was chosen for homology study for being the most similar in its *ver-1* sequence to that of the investigated *A. nidulans* local isolate. (Fig. 4 and Table 1). The translated alignment between the conserved codon of the obtained sequence and that of the *A. flavus* strain AF70 (AY510453.1) revealed 99% identity at a coverage query of 96% and the substitution occurred at base 60, however on the level of protein translation no change was obtained due to substitution at the third base of the codon no. 60 (Fig. 4).

Regarding the second investigated isolate, *A. parasiticus*, its *ver-1* gene was matched with that of each of 23 fungal strains belonging to five *Aspergillus* species; *A. oryzae* (9 strains), *A. flavus* (7 strains), *A. parasiticus* (4 strains), *A. sojae* (2 strains) and *A. nomius* (1 strain). Interspecific similarity levels were observed and ranged from 94 to 99% at a query coverage range from 16% to 96%, respectively (Table 2).

Also, it should be noted that *ver-1* DNA sequence of the investigated *A. parasiticus* isolate was highly conserved among strains of *A. oryzae* and *A. flavus*, in particular.

No sequence matching was observed with *A. pseudotamarii, A. ochraceoroseus* (B1 producers), *A. bombycis* (B and G producers), *Bipolaris* sp., *Chaetomium* sp., *Farrowia* sp., *Penicillium* sp. (intermediate producers) and other non-producer species such as *A. niger*. Also, no

Table 1. Distribution and homology of *ver-1* gene of *A. nidulans* among *Aspergillus* sp.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AY510453.1	*Aspergillus flavus* isolate AF70 aflatoxin biosynthesis gene cluster, complete sequence	898	898	96%	0.0	99%
AB007804.1	*Aspergillus* oryzae ver-1 gene, partial cds, strain NFRI 1133	887	887	96%	0.0	98%
AB195804.1	*Aspergillus* oryzae ver-1, verA genes for dehydrogenase/ ketoreductase, monooxygenase, complete cds	881	881	96%	0.0	98%
AY510452.1	*Aspergillus flavus* isolate BN008 aflatoxin biosynthesis gene cluster, complete sequence	869	869	96%	0.0	98%
AB007805.1	*Aspergillus flavus* ver-1 gene, partial cds, strain RIB 1427	848	848	96%	0.0	97%
AB071288.1	*Aspergillus* oryzae genes for AFLR, AFLJ, Putative shrot-chain alchohol dehydrogenase, Putative norsolorinic acid reductase and VER1, complete cds	843	843	96%	0.0	97%
AB196490.1	*Aspergillus* oryzae DNA, aflatoxin biosynthesis gene cluster, complete sequence, strain: RIB40	843	843	96%	0.0	97%
AB076804.1	*Aspergillus* oryzae avnA, verB, avfA, omtB genes for cytochrome P450 monooxygenase, averufin dehydrogenase, O-methyltransferase B, complete cds	843	843	96%	0.0	97%
AP007159.1	*Aspergillus* oryzae RIB40 DNA, SC026	843	843	96%	0.0	97%
AB007803.1	*Aspergillus* oryzae ver-1 gene, partial cds, strain NFRI 1134	843	843	96%	0.0	97%
AY510451.1	*Aspergillus flavus* isolate AF13 aflatoxin biosynthesis gene cluster, complete sequence	769	769	95%	0.0	94%
AY371490.1	*Aspergillus* parasiticus aflatoxin pathway gene cluster, complete sequence	765	765	96%	0.0	94%
AB007808.1	*Aspergillus* sojae ver-1 gene, partial cds, strain NFRI 1148	765	765	96%	0.0	94%
AB007807.1	*Aspergillus* sojae ver-1 gene, partial cds, strain NFRI 1147	765	765	96%	0.0	94%
M91369.1	*Aspergillus* parasiticus ketoreductase (ver1) gene, complete cds	765	765	96%	0.0	94%
AY510455.1	*Aspergillus flavus* isolate AF36 aflatoxin biosynthesis gene cluster, complete sequence	763	763	95%	0.0	94%
AB007806.1	*Aspergillus flavus* ver-1 gene, partial cds, strain NFRI 1258	763	763	95%	0.0	94%
AF452809.1	*Aspergillus* parasiticus strain ATCC 56775 aflatoxin biosynthetic gene cluster, partial sequence	715	715	96%	0.0	92%
U63994.1	*Aspergillus* parasiticus truncated ketoreductase gene, complete sequence	715	715	96%	0.0	92%
AY510454.1	*Aspergillus* nomius isolate AN13137 aflatoxin biosynthesis gene cluster, complete sequence	673	673	95%	0.0	91%
XM_002379900.1	*Aspergillus flavus* NRRL3357 aflM/ ver-1/ dehydrogenase/ ketoreductase, mRNA	521	814	86%	1e-144	100%
XM_001821469.1	*Aspergillus oryzae* RIB40 hypothetical protein partial mRNA	483	764	86%	5e-133	98%
AB176961.1	*Aspergillus oryzae* DNA, aflatoxin biosynthetic pathway gene cluster, breakdown and restoration region sequence, strain: RIB 62	196	196	20%	7e-47	100%
AY987856.2	*Aspergillus flavus* isolate NPL TX13-5 Ver1 (ver1) gene, partial cds	86.1	86.1	8%	2e-13	100 %
AY987855.2	*Aspergillus flavus* isolate NPL TX21-5 Ver1 (ver1) gene, partial cds	86.1	86.1	8%	2e-13	100 %
DQ112071.1	*Aspergillus* oryzae isolate SRRC 2098 amdA gene, partial sequence; and Ver1 (ver1) gene, partial cds	69.4	69.4	7%	2e-08	100 %
DQ112070.1	*Aspergillus oryzae* isolate SRRC 2103 amdA gene, partial sequence; and Ver1 (ver1) gene, partial cds	69.4	69.4	7%	2e-08	100 %

sequence matching was detected with all *A. nidulans* strains on the genebank database, again reflecting the unique sequence of this *A. nidulans* isolate.

On the level of protein homology, only one *A. parasiticus* strain (gbAY371490.1), the most similar strain (Table 2) was chosen for homology study (Fig. 5). Regarding the translated alignment, a 98% identity was observed at a coverage query of 94%. Substitution occurred at the bases 168, 171, 177, 297, 309, 315 and 321. However, on the level of protein translation only two changes were observed at the positions 57 (serine was replaced by cysteine) and 103 amino acid (cysteine was

Table 2. Distribution and homology of *ver-1* gene of *A. parasiticus* among genebank *Aspergillus* sp.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AY371490.1	*Aspergillus* parasiticus aflatoxin pathway gene cluster, complete sequence	845	845	94%	0.0	99%
AB007808.1	*Aspergillus* sojae ver-1 gene, partial cds, strain NFRI 1148	845	845	94%	0.0	99%
AB007807.1	*Aspergillus* sojae ver-1 gene, partial cds, strain NFRI 1147	845	845	94%	0.0	99%
M91369.1	*Aspergillus* parasiticus ketoreductase (ver1) gene, complete cds	845	845	94%	0.0	99%
AY510451.1	*Aspergillus* flavus isolate AF13 aflatoxin biosynthesis gene cluster, complete sequence	800	800	94%	0.0	97%
AY510455.1	*Aspergillus* flavus isolate AF36 aflatoxin biosynthesis gene cluster, complete sequence	795	795	94%	0.0	97%
AB007806.1	*Aspergillus* flavus ver-1 gene, partial cds, strain NFRI 1258	795	795	94%	0.0	97%
AB007805.1	*Aspergillus* flavus ver-1 gene, partial cds, strain RIB 1427	761	761	94%	0.0	95%
AB071288.1	*Aspergillus* oryzae genes for AFLR, AFLJ, Putative shrot-chain alchohol dehydrogenase, Putative norsolorinic acid reductase and VER1, complete cds	756	756	94%	0.0	95%
AB196490.1	*Aspergillus* oryzae DNA, aflatoxin biosynthesis gene cluster, complete sequence, strain: RIB40	756	756	94%	0.0	95%
AB076804.1	*Aspergillus* oryzae avnA, verB, avfA, omtB genes for cytochrome P450 monooxygenase, averufin dehydrogenase, O-methyltransferase B, complete cds	756	756	94%	0.0	95%
AP007159.1	*Aspergillus* oryzae RIB40 DNA, SC026	756	756	94%	0.0	95%
AB007803.1	*Aspergillus* oryzae ver-1 gene, partial cds, strain NFRI 1134	756	756	94%	0.0	95%
AF452809.1	*Aspergillus* parasiticus strain ATCC 56775 aflatoxin biosynthetic gene cluster, partial sequence	741	741	94%	0.0	95%
U63994.1	*Aspergillus* parasiticus truncated ketoreductase gene, complete sequence	741	741	94%	0.0	95%
AY510453.1	*Aspergillus* flavus isolate AF70 aflatoxin biosynthesis gene cluster, complete sequence	739	739	94%	0.0	95%
AY510452.1	*Aspergillus* flavus isolate BN008 aflatoxin biosynthesis gene cluster, complete sequence	728	728	94%	0.0	94%
AB007804.1	*Aspergillus* oryzae ver-1 gene, partial cds, strain NFRI 1133	728	728	94%	0.0	94%
AB195804.1	*Aspergillus* oryzae ver-1, verA genes for dehydrogenase/ ketoreductase, monooxygenase, complete cds	723	723	94%	0.0	94%
AY510454.1	*Aspergillus* nomius isolate AN13137 aflatoxin biosynthesis gene cluster, complete sequence	630	630	94%	2e-177	90 %
XM_0018 21469.1	*Aspergillus* oryzae RIB40 hypothetical protein partial mRNA	460	694	84%	2e-126	98 %
XM_002 379900.1	*Aspergillus* flavus NRRL3357 aflM/ ver-1/ dehydrogenase / ketoreductase, mRNA	444	672	84%	2e-121	97 %
AB176961.1	*Aspergillus* oryzae DNA, aflatoxin biosynthetic pathway gene cluster, breakdown and restoration region sequence, strain: RIB 62	132	132	16%	2e-27	96 %

GCCGCAGGCCGCGGAGAAAGTGGTACCGACGCTATCGCAATCCAGGCCGATGTCGGGGATCCTGAGGCAACT
GCGAAGTTAATGGCGGAGACGGTGCGCCATTTTGGCTACCTGGACATCGTGTCATCGAACGCTGGAATTGTAT
CGTTCGGTCACCTGAAAGACGTGACCCCAGAAGTATGAACCACAGATAACGCATTAAAGGCATAAGCTAAAA
AAAGTATTAGGAATTTGACCGGGTCTTCCGGGTCAACACCCGTGGCCAGTTCTTCGTGGCGCGGGAGGCCTAT
CGCCATATGCGGGAAGGAGGTCGAATTATCCTGACCAGCTCTAACACTGCTTGCGTGAAGGGGGTCCCCAAG
CATGCTGTATACTCCGGGTCCAAGGGGGCTATTGACACCTTTGTTCGCTGCATGGCAATCGACTGCGGAGACA
AGAAGATCACCGTGAATGCCGTGGCTCCTGGAGCCATTAAGACTGATATGTTTTTGGCTGTGTCGCGGGAGTA
TATCCCC

Fig. 1. Sequence of the conserved region of *ver-1* gene isolated from *A. nidulans* (515 bp).

GCCGCAGGCCGCGGAGAAAGTGGTACCGATGTCGGGGATCCTGAGGCGACAGCGAAATTAATGGCGGAGAC
GGTGCGCCATTTTGGCTACCTGGACATCGTGTCATCGAACGCTGGAATTGTATCGTTCGGTCACCTGAAAGAC
GTGACCCCAGAAGTATGAACCACAGATAACGCATTCAAGGCATATGCTAAAAAAAACACTAGGAGTTTGACA
GGGTCTTCCGGGTCAACACTCGTGGCCAGTTCTTCGTGGCGCGGGAGGCCTATCGCCATATGCGGGAAGGAG
GCCGGATTATCCTGACCAGCTCTAACACCGCTTGCGTCAAGGGGGTACCCAAACATGCTGTATACTCCGGGTC
CAAGGGGGCTATTGACACCTTTGTTCACTGCATGGCCATTGACTGCGGAGACAAGAAAATCACCGTGAATGC
GGTGGCTCCTGGAGCCATCAAGACTGATATGTTTTTGGCTGTGTCGCGGGAGGTATATCCCC

Fig. 2. Sequence of the conserved region of *ver-1* gene isolated from *A. parasiticus* (495 bp).

Alignment	Percent identity
Query 1 AAGRGESGTD 30 AAGRGESGTD Sbjct 1 AAGRGESGTD 30	100%
Query 2 PQAAEKVVP 28 PQAAEKVVP Sbjct 2 PQAAEKVVP 28	100%
Query 28 RYHFLRGLR 2 RYHFLRGLR Sbjct 28 RYHFLRGLR 2	100%
Query 504 PATQPKTYQS*WLQE 460 PATQPKTYQS*WLQE Sbjct 483 PATQPKTYQS*WLQE 439	100%
Query 29 SVPLSPRPA 3 SVPLSPRPA Sbjct 29 SVPLSPRPA 3	100%
Query 51 CRGS*GNCEVNGGDGAPFWLPGHRVIERWNCIVRSPERRDPRSMNHR*RIKGIS*KKY*E 230 CRGS*G+ E+NGGDGAPFWLPGHRVIERWNCIVRSPERRDPRSMNHR *RI+ GI *KK+*E Sbjct 30 CRGS*GDSEINGGDGAPFWLPGHRVIERWNCIVRSPERRDPRSMNHR*RIQGIC*KKH*E 209	95%
Query 231 FDRVFRVNTRGQFFVAREAYRHMREGGRIILTSSNTACVKGVPKHAVYSGSKGAIDTFVR 410 FDRVFRVNTRGQFFVAREAYRHMREGGRIILTSSNTACVKGVPKHAVYSGSKGAIDTFV Sbjct 210 FDRVFRVNTRGQFFVAREAYRHMREGGRIILTSSNTACVKGVPKHAVYSGSKGAIDTFVH 389	95%
Query 411 CMAIDCGDKKITVNAVAPGAIKTDMFLAVSRE 506 CMAIDCGDKKITVNAVAPGAIKTDMFLAVSRE Sbjct 390 CMAIDCGDKKITVNAVAPGAIKTDMFLAVSRE 485	95%
Query 514 GIYSRDTAKNISVLMAPGATAFTVIFLSPQSIAMQRTKVSIAPLDPEYTACLGTPFTQAV 335 G SRDTAKNISVLMAPGATAFTVIFLSPQS+AMQ TKVSIAPLDPEYTACLGTP TQAV Sbjct 493 GYTSRDTAKNISVLMAPGATAFTVIFLSPQSMAMQ*TKVSIAPLDPEYTACLGTPLTQAV 314	92
Query 334 LELV 323 LELV Sbjct 313 LELV 302	92
Query 274 TKNWPRVLTRKTRSNS*YFF*LMPLMRYLWFILLGSRLSGDRTIQFQRSMTRCPGSQNGA 95 TKNWPRVLTRKT SNS* FF* MP MRYLWFILLGSRLSGDRTIQFQRSMTRCPGSQNGA Sbjct 253 TKNWPRVLTRKTLSNS*CFF*HMP*MRYLWFILLGSRLSGDRTIQFQRSMTRCPGSQNGA 74	91
Query 94 PSPPLTSQLPQDPRHR 47 PSPPL S PQDPRHR Sbjct 73 PSPPLISLSPQDPRHR 26	91
Query 30 VGTTFSAACG 1 +GTTFSAACG Sbjct 30 IGTTFSAACG 1	90

Fig. 3. Sequence comparison of the VER protein homologs translated from GenBank database entries for *A. nidulans* sequence as a query and the sequence of *A. parasiticus* as subject

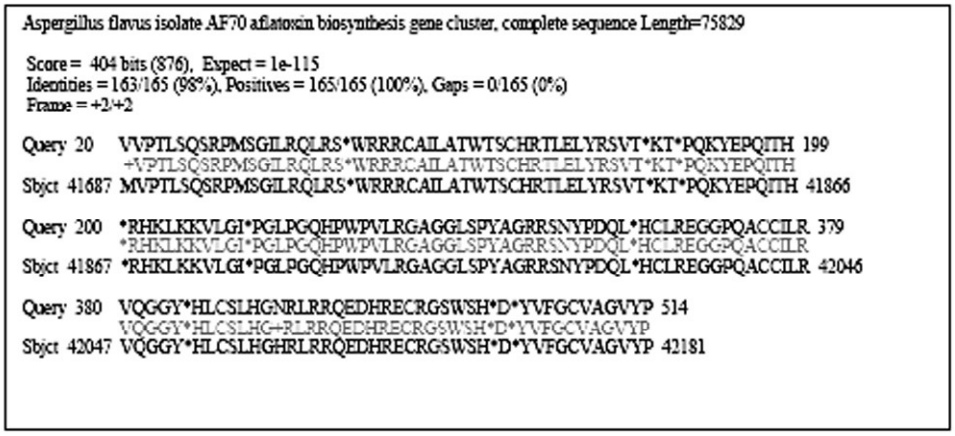

Fig. 4. Sequence comparison of the VER protein homologs translated from GenBank database entries for *A. nidulans* (local isolate) as a query and the sequences of *A. flavus* isolate AF70(AY510453.1) as a subject

Fig. 5. Sequence comparison of the VER protein homologs translated from GenBank databaseentries for *Aspergillus parasiticus* (local isolate) as a query and the sequences of *A. parasiticus* isolate (gb|AY371490.1) as a subject

replaced by serine). The rest of the substitutions resulted in no change in the amino acid type as it was in the third base of the codon (Fig. 5).

DISCUSSION

Sterigmatocystin, the penultimate precursor to aflatoxin, is produced by a number of non-aflatoxigenic fungi including *A. nidulans*. Brown *et al.*[22] characterized a 60 kb DNA region in *A. nidulans* that consists of a cluster of genes responsible for 25 co-regulated transcripts involved in sterigmatocystin biosynthetic pathway in this fungus.

The sequencing results of *ver-1* gene (conserved region) of the two Egyptian investigated isolates; *A. nidulans* (sterigmatocystin producer) and *A. parasiticus* (AFB & AFG producer), revealed the presence of variation in size (many gabs were identified) while the expected subsequent protein variation was absent as a result of the change being only in the

third position of the codons of some amino acids. This result might be attributed to the fact that *ver-1* gene is involved in the biosynthesis of both sterigmatocystin and aflatoxin. Additionally, the common environment of the two isolates (both were isolated from Aswan at Egypt) might play a role. These results are consistent with Kusumoto *et al*. (1998)[33] who compared part of the nucleotide sequence of the *ver-1* homolog in two strains of each of *A. oryzae*, *A. sojae*, and *A. flavus* with two homologs in *A. parasiticus*. The homologs in *A. oryzae* and *A. sojae* (non-aflatoxin-producers) exhibited an extremely high degree (93.8-99.8% for *A. oryzae*, and 96.0-99.5% for *A. sojae*) of sequence identity with that of *A. flavus* and *A. parasiticus*. No sequence fingerprint was found to distinguish between A. *oryzae* and *A. flavus*, or between *A. sojae* and *A. parasiticus*.

Taxonomically, *A. flavus* is highly related to *A. oryzae* as well as *A. parasiticus* to *A. sojae*[34] where each two related species represented 90% nucleotide sequence homology. Woloshuk *et al*.[35] reported that *afl-2* gene in *A. flavus* and the *apa-2* gene in *A. parasiticus* are homologs, and thus should be both designated as *aflR.* Also, Mokhtar[36] reported no DNA and no amino acid variations in the conserved region of *ver*-1 gene of two Egyptian *Aspergillus* isolates; *A. flavus* (aflatoxigenic) and *A. oryzae* (non-aflatoxigenic).

Comparing the obtained sequencing result of *ver-1* gene generated from the local Egyptian isolate *A. nidulans* to the published complete sequence obtained from the genebank revealed high identity ranging from 91 to 100% at a query coverage range from 7% to 96%, thus being consistent with the results of Chang *et al*.[37] and Watson *et al*.[38] who reported the presence of sequence variations in the three structural genes *nor-1*, *ver-1, omtA* and the regulatory gene *aflR* generated from different *Aspergillus* strains (*A. tamarii* SRRC 99, SRRC 1088; *A. oryzae* SRRC 2104, SRRC 2103, SRRC 2353, ATCC 14895, ATCC 16507; *A. sojae* SRRC 1123, ATCC 42251; *A. nomius* SRRC 362, SRRC 375; *A. parasiticus* SRRC 134, ATCC 24690, ATCC 36537, ATCC 56774, ATCC 56775; *A. niger* ATCC 9029) and different levels of DNA relatedness (39-90%).

Moreover, Mokhtar[36] matched *ver-1* gene sequences of two Egyptian *Aspergillus* isolates (*A. oryzae* and *A. flavus*) with 25 *ver-1* gene sequences of 25 strains belonging to five *Aspergillus* species; *A. oryzae* (11 strains), *A. flavus* (9 strains), *A. parasitic*us (4 strains), *A. sojae* (2 strains) and *A. nomius* (one strain) and reported identity homology results ranging from 91 to 99%.

Regarding the absence of homology between *ver-1* gene generated form *A. nidulans* isolate of the current work and that of any other of either *A. nidulans* or *A. niger*, these results agree with the data of Kozlowski and Stepien[39], who suggested that *A. niger* has diverged significantly from the other species. Also, the nature of the investigated fungi and the ecological properties may be involved in this result.

Comparing the sequencing results of *ver-1* genes generated from the local Egyptian isolate *A. parasiticus* of the present work to the published complete sequence obtained from the genebank revealed high range of identities from 94 to 99% at a query coverage range from 16% to 96%, respectively. These results are consistent with those of Chang *et al.*[37] and Watson *et al.*[38] who reported the presence of sequence variations in the three structural genes *nor-1*, *ver-1, omtA* and the regulatory gene *aflR* generated from different *Aspergillus.*

Additionally, Homologs of *aflR*, *from A. nomius, A. bombycis, A. parasiticus, A. flavus,* and *A. pseudotamarii* were studied to investigate the molecular basis for variation among aflatoxin-producing taxa in the regulation of aflatoxin production. Variability was found in putative promoter consensus elements and coding region motifs[40].

In the current study, the sequence of *ver-1* gene of *A. nidulans* local isolate possessed restricted variations only on the level of third base of codon, when it was compared with the most similar aflatoxigenic *A. flavus* AF70 strain (AY510453.1). However, in case of *ver-1* generated from *A. parasiticus* local isolate, variations in two amino acids together with changes in the third base of five codons were detected when compared with the most similar *A. parasitic* strain (gb.AY371490.1). These restricted variations between the two local Egyptian species and the published sequences might be attributed to the short region (171 amino acids for *A. nidulans* and 165 amino acids for *A. parasiticus*) used in the homology and the stability of the

conserved codons involved in the biosynthesis of aflatoxin, these results agree with Kusumoto *et al*.[31] who reported no sequence fingerprint was found to distinguish between *A. oryzae* and *A. flavus*, or between *A. sojae* and *A. parasiticus*. Also, they reported that the predicted partial amino acid sequences (181 amino acids) of the *ver-1* homologs had at most two amino acid changes relative to *A. parasiticus* SYS-4 ver-1. Also, Mokhtar[36] reported only one to two amino acid variations when compared *ver-1* gene generated from *A. oryzae* and *A. flavus* (local isolates) with the most similar aflatoxigenic *A. flavus* AF70 strain (AY510453.1).

Conclusively, a very high degree of homology in the conserved region of *ver-1* gene of the investigated Egyptian isolates was observed where, when compared to other genebank *Aspergillus* sp., only one strain was found to refrain from conservatism by possessing a two-amino acid change. The uniqueness of *Ver-1* gene of *A. nidulans* could be clearly observed for being a mismatch with all *A. nidulans* genebank strains, hence, particularly for *A. nidulans*, further studies on the characterization of the obtained sequences in the current study will be conducted.

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