Pak. J. Biotechnol. 14 (4) 673-675 (2017)
www.pjbt.org

ISSN Print: 1812-1837
ISSN Online: 2312-7791

MOLECULAR IDENTIFICATION OF FUNGI ASPERGILLUS FLAVUS WHICH PRODUCING TOXIN (AFB1) IN IRAQ

Muna A. Alrawi and Halima Z. Hussein

Department of plant protection, College of agriculture, Baghdad University, Iraq.
Email:Muna.abdulrahman91@yahoo.com, halimaalbahadly@yahoo.com

Article received 7.10.2017, Revised 28.11.2017, Accepted 4.12.2017

ABSTRACT

Mycotoxins are fungal toxic metabolites which naturally contaminate food and feed. Aflatoxins, when ingested, inhaled or adsorbed through the skin, have carcinogenic, hepatotoxic, teratogenic and mutagenic effects in human and animals. Genetically, Aspergillus was one of the best studied fungi and the complete genome sequence of A. flavus in addition to several strains of A. flavus group strains are available now at the National Center for Biotechnology Information (NCBI) (Rodrigues et al., 2007). Phylogenetic analysis of ITS region seems to be a useful tool to provide taxonomical information about ecological genotypes. In this study, the results showed the DNA chain reaction using primers ITS1-ITS4 located within the ribosomal gene 18S rRNA and a clear band resulting from the process of doubled. It has been used BLAST tool to find parallelism and compared nucleotide sequence for ITS region in ribosomal gene S18 with nucleotide sequence data base the other strain in the National Centre for Biotechnology Information NCBI and Gene Bank results showed 98% ratio match for isolate.

INRODUCTION

Aspergillus was one of the best studied fungi and the complete genome sequence of A. flavus in addition to several strains of *A. flavus* group strains are available now at the National Center for Biotechnology Information (Rodrigues et al., 2007, Hussein, 2017). Internal transcript spacer (ITS1 to ITS4) is a specific region located in rDNA, broadly used to differentiate *Aspergillus* species in addition to the 28S rRNA region (D1-D2) (Rodrigues et al., 2007, Mohankumar et al., 2010, Varga et al., 2011). Furthermore, other studies utilized sequencing of other target genes in the molecular identification of A. flavus, including β- tubuline, tpoisomerase II, and calmodulin genes for distantly related species because of low variability in those regions, in addition to the mitochondrial cytochrome b and aflR genes which are used to differentiate between closely related species like *A. flavus* and *A. oryzae*, and *A. parasiticus* and *A. sojae*. However, 18S rRNA stills the most variable and reliable target region for molecular identification of A. flavus (Rodrigues et al., 2007, Leema et al., 2010, Vagra et al., 2011). The aim of present study was to detect Iraqi fungal isolate by molecular technique PCR.

MATERIALS AND METHODS

Detection Fungus isolate of Aspergillus flavus by using PCR technique: After isolation development in yeast media to produce AFB1 and by using Kit to extract DNA from mycelium, extraction process by added Liquid nitrogen to the mycelium and morting the sample with Micropistle until the sample get powder, added 600µl from Nucleolysis solution. The samples were incubated at a temperature of 70°C for 30-15 minutes and then incubated at room temperature, Add 3 Micro litter of RNase and incubated at room temperature for 15 minutes, Add 400µl protein precipitation to the samples and placed it into ice for 5 minutes, Samples are placed in centrifuge speeds of 13,000rev/min for 10 minutes, Added 600µl Isopropanol and incubated for 5 minutes in ice, Samples are placed in central at medium speeds of 13,000 rev/min for two minutes and then get rid of the filtrate, Add 500 µl 70% ethanol to the samples, and placed in a centrifuge speeds of 13,000rev/min for two minutes, Aspirate the ethanol and air-dry the pellet and for 15-10 minutes, Added 80µl Rehydration Solution to the sample for one hour at 65°C.

Prepare PCR polymerase chain reaction: The PCR reaction was prepared by using Internal transcribed spacer ITS1-ITS4 in Ribosomal gene 18srRNA and the primer sequence was shown in Table 1 (Bellemain et al., 2010).the reaction mix prepared in 25µl final size and conducted the PCR reaction after set the program nucleotide sequnce have been detected in the products of polymerase chain reaction of the internal transcribed spacer ITS1-ITS4 in ribosomal gene 18S rRNA and sent to Marcogen company. The products were sequenced at Macrogen and sequences are compared with the standard sequences in the NCBI gene bank web site using the BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast-cgi).

Table: 1 primer sequence.

Gene	Prime	Sequence
18S	TS1	5'TCCGTAGGTGAACCTGCGG3'
18S	ITS4	5'TCCTCCGCTTATTGATATGC3'

RESULTS AND DISCUSSION

Detection fungus isolate of Aspergillus flavus by using PCR technique: The results of molecular detection for isolate after electrophorese and sequence alignment showed the DNA chain reaction using primers ITS1-ITS4 located within the ribosomal gene 18S rRNA and a clear band resulting from the process of doubled these genes and primer bind to complementary sequence of the DNA template and estimate the molecule size 550~bp depending on the DNA ladder electrophorese on the same gel and under the same conditions. Internal transcribed spacer ITS within the gene rRNA considered as the most suitable areas for detection of strains also be largely province as a result of evolutionary constraints therefore be used to distinguish species and strains with high accuracy (Stoll et al., 2003; Stoll et al., 2005). The results showed nucleotide sequence for the 532nucleotide of the DNA template and carried out estimate the quality of sequence figure 1 using the Codon Code Program Aligner software figure 2. It has been used BLAST tool to find parallelism and compared nucleotide sequence for ITS region in ribosomal gene S18 with nucleotide sequence data base the other strain in the National Centre for Biotechnology Information NCBI and Gene Bank results showed 98% ratio match for isolate.

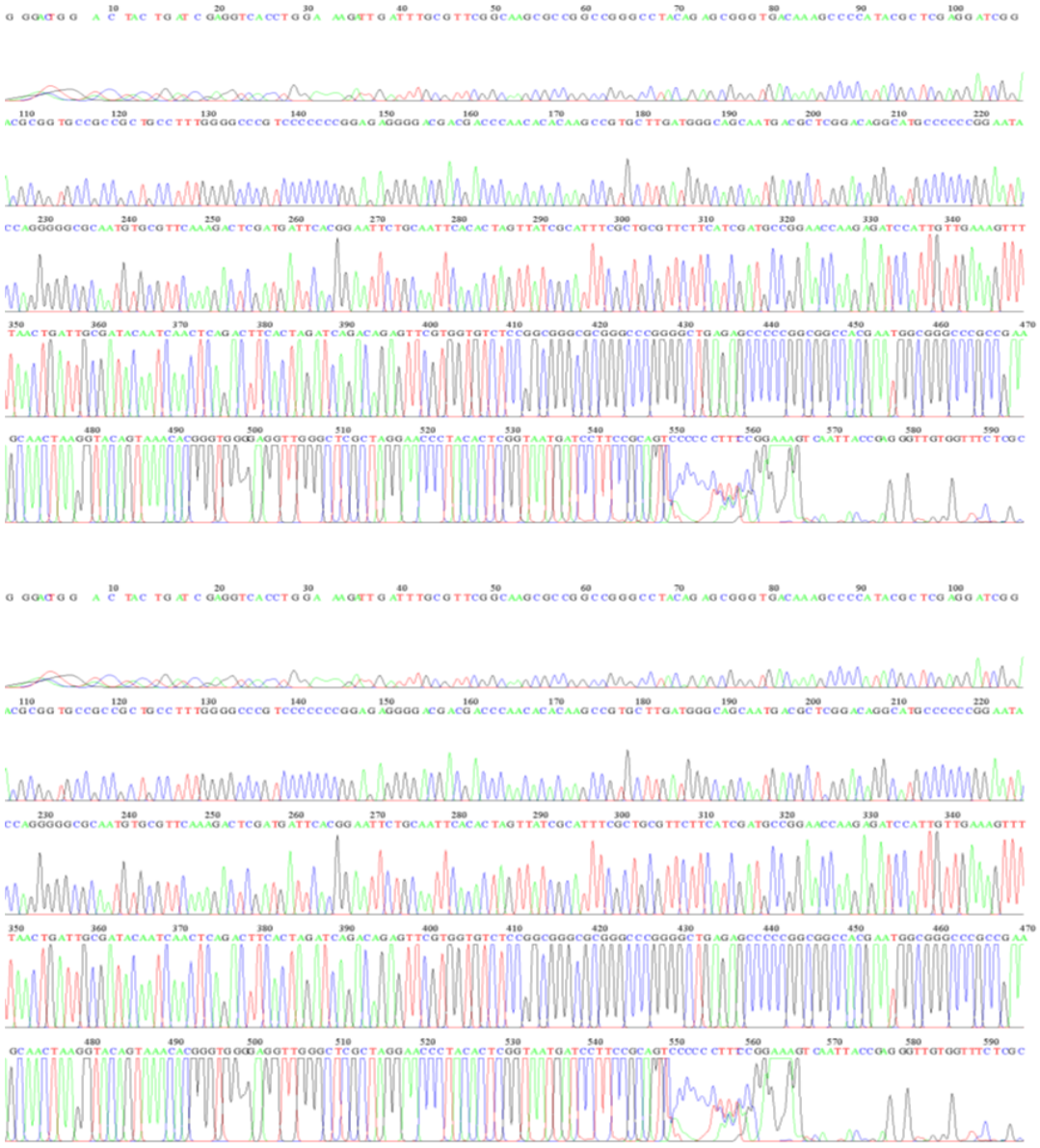

Figure 1: nucleotide concentration curve.

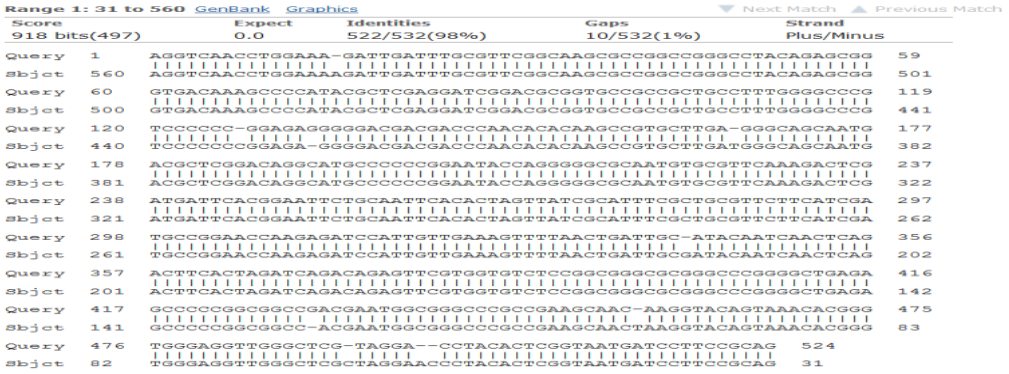

Figure 2: matching the nucleotide sequence with gene bank.

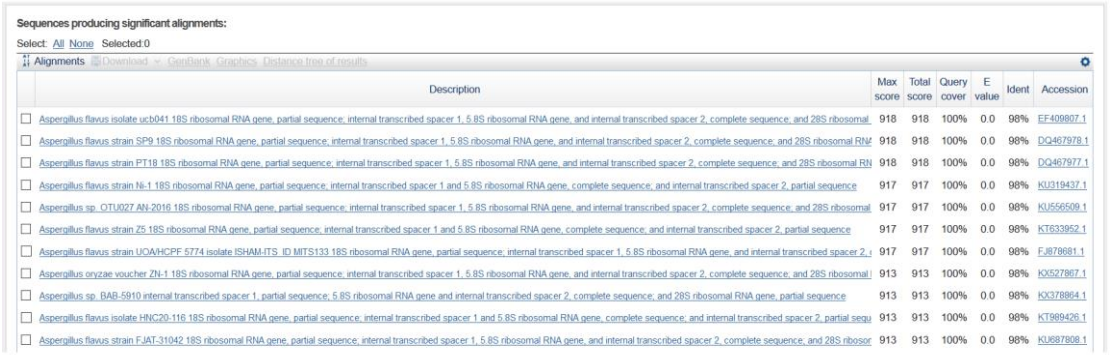

Figure 3: matching ratio between the local isolate and global isolate in gene bank

Conclusions

Through the use of PCR technology for molecular detect of isolate taken from mycotoxins laboratory /Department of Prevention of plant disease/College of Agriculture, University of Baghdad and by using ITS1-ITS4 primers in the Ribosomal gene 18S rRNA were identical ratio 98% with global isolates.

REFRENCES

Rodrigues, P., Soares, C., Kozakiewicz, Z., Paterson, R.R.M., Lima, N. and A. Venâncio, Identification and characterization of *Aspergillus flavus* and aflatoxins. Communicating Current Research and Educational Topics and Trends in Applied Microbiology A. Mendez Vilas (ED) Pp. 527-534 (2007).

Mohankumar, M., Vijayasamundeeswari, A., Karthikeyan, M., Mathiyazhagan, S., Paranidharan, V. and R. Velazhahan, Analysis of molecular variability among isolates of *Aspergillus flavus* by PCR-RFLP of the ITS regions of rDNA. Journal of Plant Protection Research 50: 446-451 (2010).

Varga, J., Frisvad, J.C. and A.R. Samson, Two new aflatoxin producing species, and an overview of *Aspergillus* section Flavi. Studies in Mycology 69: 57-80 (2011).

Leema, G., Kaliamurthy, J., Geraldine, P. and P.A. Thomas, Keratitis due to *Aspergillus flavus*: Clinical profile, molecular identification of fungal strains and detection of aflatoxin production. Molecular Vision 16: 843-854 (2010).

Bellemain, E., Carlsen, T., Brochmann, C., Coissac, E., Taberlet, P. and H. Kauserud, ITS as an environ-mental DNA barcode for fungi: an in-silico approach reveals potential PCR biases. BMC Microbiology 10: 189 (2010).

Stoll, M., Begerow,D. and F.Oberwinkler, Molecular phylogeny of Ustilago, Sporisorium, and related taxa based on combined analysis of rDNA sequences. Myco. 109: 342-356 (2005).

Stoll, M., Piepenbring, M., Begerow and F. Oberwinkler, Molecular phylogeny of *Ustilago* and *Sprosorium* spacies (Basidiomycota, *Ustlaginales*) based on internal transcribed spacer (ITS) sequences. Can. J. of Botany 81: 976-984 (2003).

Hussein, H.A., Detection of tricothecene of *Fusarium solani* Isolates by using HPLC in melon plant. Pak. J. Biotechnol. 14: 213-215 (2017).

Ali, A.M., Activity of Thuja (Thuja orientalis) Alcoholic Extract in inhibition *Aspergillus flavus* Growth and Detoxification of Aflatoxin B1 in contaminated corn seeds. Pak. J. Biotechnol. 14: 503-506 (2017).