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Molecular assay of Polyketide Synthase gene of Alternariol (AOH) produce by *Alternaria alternata*

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ABSTRACT: *Alternaria alternata* one of widely distribution plant pathogenic and saprophytic fungi. *A. alternata* producing more than 70 secondary metabolites, One of the important metabolites (AOH). The main effect of alternariol (AOH) lies in the generation reactive oxygen species (ROS) in rat's liver cytochromes. The aim of this work was studying the detection of polyketide synthase gene that responsible for Alternariol (AOH) production from *Alternaria alternata* by specific primer. Detection of AOH production by thin layer chromatography and the *PKSJ* gene by specific primer in PCR thermal cycle.43 sample of infected tomato by early blight disease in Karbala city collecting and cultured on PDA. For detection of mycotoxins (AOH) production. Designed this primer through the use of the complete sequence of the *PKSJ* gene (Gene bank sequence JX103645.1) from the site of Gene bank-NCBI and Primer3plus using the program to design primers and used in the PCR test. The result showed 23 out off 24 isolates were produced AOH toxin. TLC plate (Silica gel G60 20x20cm) was used for detection of AOH in comparison with OTA standard spot. 23 isolates was produced AOH toxin. TLC plate was used for detection of AOH in comparison with OTA standard spot. PKSJ primer that designed in this study was success for the detection and investigation of the gene responsible for the production of AOH, where the primer could amplify the target piece of *PKSJ* gene and produce bands by molecular weight 514 bp on agros gel for all isolates that produced the toxin according to the TLC results. Most isolates of *A.alternata* can produce AOH toxin. The specific PKSJ primer was success by PCR amplified the target gene with all isolates excepted isolate No. 2 which not produce AOH, finally the PKSJ primer is specific primer for detection of polyketide synthase gene.

KEYWORDS: *A.alternata*, AOH toxin, PCR, polyketide synthase gene, Iraq.

1 INTRODUCTION

The species *A. alternata* is one of widely distributed plant pathogenic and saprophytic fungi *A. alternata* cause plant diseases on many crops (Logrico et al., 2009), affecting the leaves, stems, contaminating fungi in wheat, sorghum and flowers, and fruits. And it is principal barley (Scott and Stoltz, 1980).

A. alternata produces more than 70 secondary metabolites, One of the important metabolites (AOH), and it common contaminants of food such as tomato fruits, fruit juices and grain crops. The main effect of alternariol (AOH) lies in the generation reactive oxygen species (ROS) in rat's liver cytochromes. Anther effects of this toxin are cytotoxic, apoptosis, senescence of cell and micronucleus formation (Schreck et al 2012).

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2 MATERIAL AND METHODS

FUNGAL ISOLATION AND IDENTIFICATION

43 sample of infected tomato by early blight disease in Karbala city collecting and cultured on PDA for 5-7 days at 25 ºC. Identification fungi according to (Ellis,1971; Booth, 1971 ; Koneman *et al*., 1997 ; Benson, 2002).

ANALYSIS OF MYCOTOXINS USING THIN LAYER CHROMATOGRAPHY (TLC)

For detection of mycotoxins (AOH) production. Three disks from each plate were extracted by shaking with 1 ml ethyl acetate for 1 hour. The solvent was vaporized in a speed vacum and the pellet resolved in 60 ml ethyl acetate. 20 µl were used for TLC with a mobile phase composed of toluol, ethylacetate and formic acid (5:4:1) on silica plates (Merck TLC silica gel 60) and visualized under UV light, 365 nm. with a standard.

PRIMER DESIGN

Designed this primer through the use of the complete sequence of the *PKSJ* gene (Gene bank sequence JX103645.1) from the site of Gene bank-NCBI and Primer3plus using the program to design primers and used in the PCR test. Primer have been equipped by the Korean company Bioneer (Table 1).

Table 1. Specific primer for detection polyketide synthase gene

Primer		Sequence	PCR product size
PKSJ	F	5´CTGCGGGAATGCTTTCGATG 3´	514 bp
gene	R	5´TGCCAATCTCGAAGGCCAAT3´	

SPECIFIC PCR AMPLIFICATION

The specificity of this primer was tested against isolates of a range of *A. alternata.* The program parameters for thermal cycling for the primer sets were as described Table (2).

Numbers and times of thermal cycles PKSJ gene

PCR Step	Repeat cycle	Temperature	Time
Initial denaturation	1	95 ºC	5 min
Denaturation	30	95 ºC	30 sec.
Annealing		58 ºC	30 sec
Extension		72 ºC	45 sec
Final extension	1	72 ºC	5 min
Hold	-	4 ºC	∞

3 RESULTS AND DISCUSSION

ISOLATION AND IDENTIFICATION

24 isolates of *A. alternata* from Karbala (Iraq) inoculated on PDA for 5-7 days at 25 °C identified macroscopically by shape and colour and microscopically depend on the shape, size and arrangement of spores and conidiophor. According to the characteristics incoming by (Booth, 1971; Koneman *et al*., 1997; Benson, 2002).

PRODUCTION AND IDENTIFICATION OF AOH

The identified isolates of *A.alternata* were tested for the production of AOH by using PDA . 23 isolates was produced AOH toxin and only one isolate No. 2 was not. TLC plate (Silica gel G60 20x20cm) was used for detection of AOH in comparison with OTA standard spot. TLC plate which contain previous samples spots developed by toluene-ethyl acetate-90% formic acid

(5: 4: 1 v /v) as reported by (Debjani et al., 2012). The AOH was detected by the use of UV light (336 nm) , the AOH appeared as a blue fluorescence spot , figure (1).

This results are aggregate with confirmed Li and Liangcheng (2006) of that most isolates fungus *A. alternata* have ability to produce AOH under different conditions and on the most host (Barkai-Golan, 2008.)

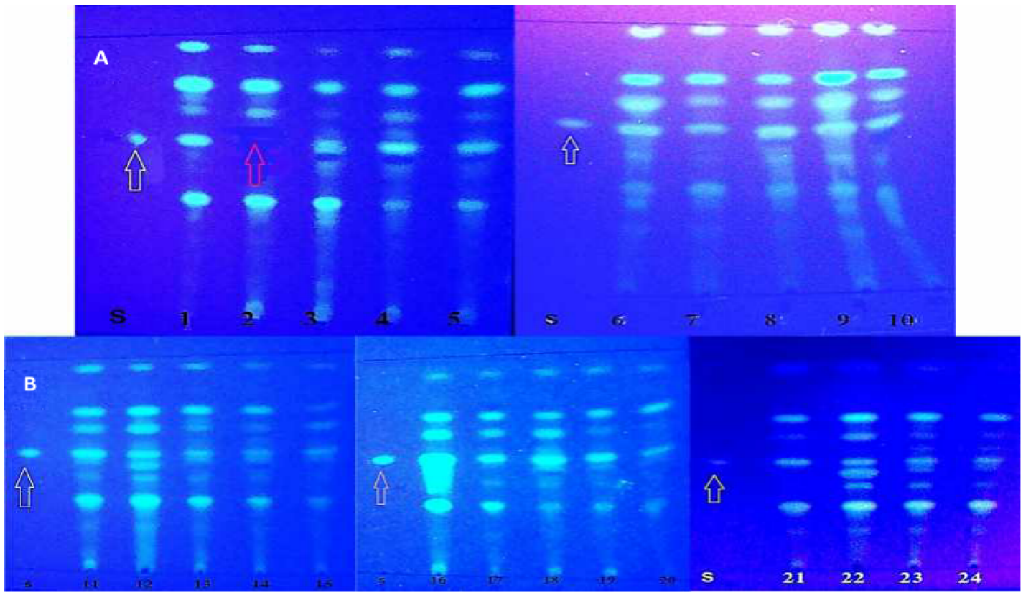

Fig. 1. TLC plate (Silica gel G60 10x5cm) under UV light (336 nm) illustrated AOH samples and standard spot by using development solution (toluene-ethyl acetate-90% formic acid (5: 4: 1 v /v).

DETECTION OF PKSJ GENE BY PCR

PKSJ Primer that designed in this study was success for the detection and investigation of the gene responsible for the production of AOH, where the primer could amplify the target piece of PKSJ gene and produce bands by molecular weight 514 bp on agros gel for all isolates that produced the toxin according to the TLC results. Primer is failure with isolate No. 2 which not have ability to produce AOH toxin.

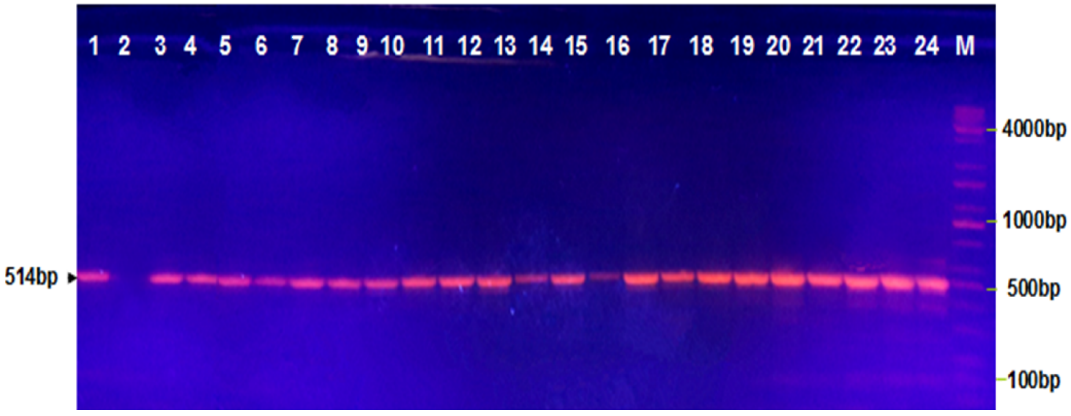

Fig. 2. Agarose gel electrophoresis for amplified PKSJ gene. Bands were fractionated by electrophoresis on a 1.5 % agarose gel (2 h., 5V/cm, 1X Tris-acetic buffer) and visualized under U.V. light after staining with ethidium bromide staining. Lane M: 4000bp ladder.

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