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First report of pathogenicity of *Pantoea* sp. in quince ree (*Cydonia oblonga* Mill.) in Iraq

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ABSTRACT

The results of isolation from infected quince trees showed pure individual colonies of bacteria, *Pantoea* sp. by microscopic and morphological characteristics for growing colonies. Biochemical tests of the diagnosis was confirmed by polymerase chain reaction (PCR). This is the first record of *Pantoea* sp. on quince tree, *Cydonia oblonga* Mill. in Karbala Province, Iraq.

Keywords: *Cydonia oblonga* Mill.، *Pantoea* sp., PCR, sequencing, Iraq.
Article type: Report.

INTRODUCTION

The *Cydonia oblonga* Mill. grows in warm and temperate regions and throughout the Middle East especially in Iran (Amiri 2008) and Turkey (Postman 2008) as well as Syria, Iraq, Afghanistan and Turkmenistan (Webster 2007) and other countries. Quince benefits from its fruits and seeds, because its fruits contain a number of vitamins, especially A and B vitamins. They also contain 64% water, 7% sugar, 0.9% protein, 0.3% fatty substances, 5% sulphur, 0.9% phosphorous, 14% calcium, 2% chlorine, 3% soda, and 0.13% potassium (Fattouh *et al.* 1999). It was found that *Pantoea* caused infection of many monocotyledonous and dicotyledonous plants, which leads to significant economic losses. Symptoms vary satisfactorily according to the host, which may appear in the form of spots on the leaves, stem, causing dieback and fruits, bulbs rot (Coutinho & Venter 2009). Because there are no studies on diseases affecting quince trees in Karbala for the purpose of minimizing damage as a result of a bacterial infection, we attempted to conduct the following experiment on quince trees, because they are exposed to infections caused by unknown bacteria, which may lead to a deterioration in production.

MATERIALS AND METHODS

Isolation and diagnosis

Samples were taken from the trunk of the infected quince trees brought from infected ones. Isolated bacteria were cultured in nutrient agar (NA) after purification for 24 h at 30 ± °C.

Diagnostic tests

Bacteriological characteristics (biochemical and physiological) of the isolates were examined using the methods of Schaad (1988), Holt *et al.* (1994), Goszczynska *et al.* (2000) and Winn *et al.* (2006) including Growth at 36°C, Levin formation, grow on 2% NaCl, growth Gram reaction, Hypersensitive Reaction test, Potato slice rot test and Catalase test.

Identification of *Pantoea* sp. bacterial by PCR technology

Bacteria were identified around the isolated parts by polymerase chain reaction technique determination of the nucleotide sequence in Asco Learning/ Centre, Baghdad, Iraq. The formed DNA (rDNA) pieces were amplified

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by employing type primers using F968 and R1401 which target 16S rDNA gene (Nübel *et al*. 1996). The PCR outcome was sequenced in Macrogen Inc. (Seoul, South Korea). The nucleotide sequencing results were compared at GenBank (NCBI) with other sequences of bacterial applying the BLAST program, Basic Local Alignment Search Tool (Zheng *et al*. 2000).

RESULTS AND DISCUSSION

Isolation and diagnosis

The results show the macroscopic characteristics of the colonies: smooth, circular, yellow pigment, regular & flat borders, 1 mm in diameter.

Fig. 1 Colonies of *Pantoea* sp. on nutrient agar (NA).

Diagnostic tests

All bacteriological characteristics (biochemical and physiological) tests were examined including growth at 36 °C, Levin formation, growth on 2% NaCl, growth Gram reaction, potato slice rot test and Catalase test (Table 1).

Table 1. Biochemical and physiological tests for *Pantoea* sp.

S.	Biochemical & physiological tests	Results
1	growth at 36 °C	-
2	Levin formation	+
3	growth on 0.02 NaCl	+
4	growth Gram reaction	-
5	Potato slice rot	-
6	Catalase test	+

Identification of *Pantoea* sp. bacterial by PCR Technique

The results of DNA extraction from *Pantoea* sp. and exposing it to polymerase chain reaction multiplication possibility (PCR) amplified products, exhibited that each size is about 1500 nitrogenous base pair (bp.). The sequence was placed in the database (Genbank) National Centre of Biotechnology Information (NBCI) Registered at the serial number of MW82531.1.

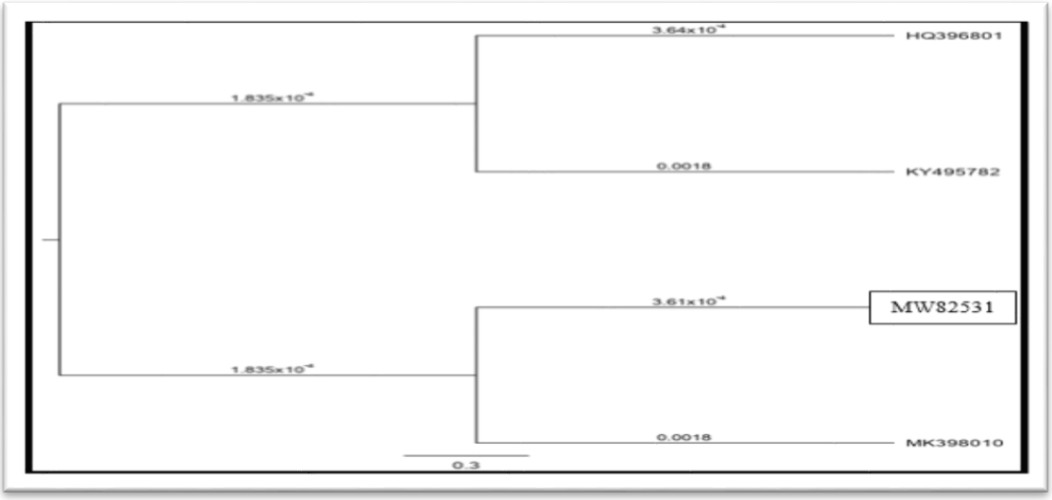

Fig. 2. Genetic tree of *Pantoea* sp.

The PCR technique (polymerase chain reaction) was used in previous experiments for its high accuracy in the diagnosis of various organisms, including bacteria, such as *Pseudomonas grimontii* and *Pseudomonas marginalis* (Sawada *et al.* 2020; Peňázová *et al.* 2020)

CONCLUSION

It is concluded from the results of the current study that it is the first report of *Pantoea* sp. isolated from *Cydonia oblonga* Mill. trees in Karbala, Iraq.

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