Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Genetic diagnosis of phytopathogenic *Agrobacterium tumefaciens* isolates from *Cydonia oblonga* trees by PCR using 16SrRNA primers

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Article info

Received 27.11.2025
Received in revised form 10.01.2025
Accepted 14.02.2025

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Khdeer, A. Q. S., & Gergees, R. N. (2025). Genetic diagnosis of phytopathogenic Agrobacterium tumefaciens isolates from Cydonia oblonga trees by PCR using 16SrRNA primers. Regulatory Mechanisms in Biosystems, 16(1), e25005. doi:10.15421/0225005

Agrobacterium is a plant pathogen causing crown disease and plant tumors in 140 or more dicotyledonous plant species. Tumor-causing agrobacteria are members of the Rhizobiaceae family of nitrogen-fixing legume symbionts, but are harmful to plants. This study's initial goal was to isolate 25 bacterial isolates from crown tumors that developed on the stems of quince trees in various areas of Mosul. The colonies were selected for tests on the basis of their size, shape and color. From these, Gram-negative bacterial isolates were chosen and subjected to the following tests ; catalase, L-tyrosine utilization, citrate utilization, utilization of carbohydrates, lactose, mannitol, H_2S production, pH range, growth at: 28, 35, 40 ºC, growth absence, ability to grow without biotine and thiamine, growth and pigmenentation in ferric ammonium citrate. Five isolates (AtCo1, AtCo2, AtCo3, AtCo4, AtCo5) demonstrated their ability to form tumors on carrot discs for periods ranging from 22 to 26 days . Growth on the following selective media for their diagnosis produced positive results ; MacConkey agar, D1 medium, Luria-Bertani medium, glucose peptone agar, potato dextrose agar, AMM, chromogenic agar medium. These five isolates provided positive diagnostic signs and were designated with the symbols AtCo1, AtCo2, AtCo3 ,AtCo4 and AtCo5, in relation to the quince plant, which is the host plant from which the bacteria were isolated. All exposures to *A. tumefaciens* formed tumors on carrot discs, according to the results of an artificial infection test used to determine the pathogenicity of the bacterial isolates. The outcomes of molecular diagnosis using agarose gel electrophoresis for the DNA of *A. tumefaciens* isolates corroborated these findings. The molecular diagnosis was achieved by amplifying the DNA using the 16S rRNA gene primer and the DNA polymerase chain reaction (sPCR) technique. This allowed the clear separation of single, shiny bands at the molecular size (1150 bp) in the five isolates, confirming their membership of the genus *Agrobacterium* . Both of the Biovar I isolates (AtCo5 and AtCo2) from quince tumors were listed in the gene bank under the accession number PQ106659.

Keywords: *Agrobacterium*; crown gall; *Cydonia oblonga*; biovar; 16SrRNA; pathogenicity.

Introduction

Agrobacterium bacteria are among the most significant pathogens and the cause of crown disease and plant tumor formation in 140 or more species of dicotyledonous plants (Islam et al., 2010). They belong to the Rhizobiaceae, a family of nitrogen-fixing legume symbionts, and unlike nitrogen-fixing symbionts, the types of *Agrobacterium* that cause tumors are harmful and do not benefit the plants (David & Tempe, 1993). According to Bivadi et al. (2014), the disease's symptoms arise from the entry of a tiny portion of the bacterial plasmid's T-DNA into the plant cell, where it integrates at a somewhat arbitrary site within the plant's genome. Plant genome engineering using agrobacteria can deliver sequences carried on a bacterial plasmid to infected cells (David & Tempe, 1993). The wide variety of plants that *Agrobacterium tumefaciens* affects makes it a source of diseases for many useful plants, such as radish, walnut trees, grapevines, stone fruits, and sugar beet. According to Zargar et al. (2010), the disease is especially detrimental to perennial crops because the tumors or galls that emerge from it are permanent. Optimal growth for *Agrobacterium* occurs at 28 °C. This bacterium is harmful due to its 200-kb tumor-inducing plasmid (pTi) (Bae et al., 2012). Mega plasimds are carried by a variety of bacterial species belonging to the genus *Agrobacterium*, including as *A. rubi*, *A. vitis*, *A. rhizogenes*, and *A. tumefaciens*. These plasmids (*Ti*, *Ri*) change plant germs that are not harmful into harmful ones (Brootharts et al., 2012) when a piece of bacterial T-DNA enters the plant cell genome due to the activity of many virulence genes located on the bacterial plasmid.

Agrobacterium bacterial species that have been isolated from various host plants can be differentiated based on a variety of traits linked to the application of pathological and biochemical tests. Furthermore, a great deal of recent research has focused on employing RAPD-PCR polymerase chain reaction (PCR) to use genetic fingerprinting to diagnose bacteria and their various species in order to ascertain the degree of genetic variation among species of this genus (Procopio et al., 2009). In addition to employing the restriction fragment length polymorphism (RFLP) analysis technique to determine the degree of genetic variation among pathogenic genes, such as Vir B and Vir G, specific primers have also been used to diagnose *Agrobacterium* strains of the nopaline and octopine types in order to amplify these genes (Nesme et al., 1990).

Materials and methods

Isolation of Agrobacterium tumefaciens from crown galls on quince trees. Crown tumors formed on the stems of *Cydonia oblonga* (quince) from different areas of Mosul City (Iraq) were removed without causing any wound or cut in the tumors (Fig. 1). The crown tumors were recovered from the following six sites ; Al-Rashidiya, Yarmojah, Al-Mazra'a neighborhood, Al-Shamsiyat village, Al-Kasr village and Qabr Al-Abd village , in the period between (October–December 2024). Samples of quince tree tumors were collected in sterile boxes at a rate of 3 pieces / site and taken directly to the laboratory (Sorful et al., 2010). Then, the tumor samples were washed with running water for 2 hours to get rid of the dust particles stuck to them, and the tumors were sterilized superficially by immersing them twice in 96% ethyl alcohol for a period of time not exceeding 1–2 minutes each time. Then, they were washed with sterile distilled water 3–4 times. After that, the tumor samples were transferred to glass flasks containing 3% of NaOCl (sodium hypochlorite) and left for 15 minutes. After that, the tumor samples were washed with sterile distilled water several times in a row. The tumor samples were dried by placing them on sterile filter papers and then they were distributed superficially on glass dishes containing 20 mL of agar nutrient medium. They were kept at a temperature of 28 ± 2 °C for a period of 48–

78 hours to ensure the quality of sterilization. Then, the uncontaminated tumors were taken and cut using a sterile sieve into small pieces and then crushed using a glass rod sterilized with an alcohol flame in 70% of liquid YEM medium.

Fig. 1. A model of a quince tree infected with crown gall disease: *a* – one of the quince trees infected with crown rot disease; *b* – crown mold disease on quince branches

Isolation of A. tumefaciens from the bacterial solution and its cultivation. A series of decimal dilutions were made in order to separate *A. tumefaciens* from the bacterial solution. To obtain single and pure colonies, 0.1 mL of the final dilution was obtained and disseminated on the surface of the solid (YEM) medium (Vincent, 1970). Each colony growing on the surface of the (YEM) medium was then transferred to the surface of the solid (*Agrobacterium* mannitol M) AM selection medium (Muragesan et al., 2010) after being incubated for twenty-four hours at a temperature of 28 ± 2 ºC. They were then assigned the symbols *A. tumefaciens* AtCo1-AtCo15 in reference to the plant from which the bacteria were isolated.

Identification of A. tumefaciens isolated from crown tumors of quince trees, diagnostic tests. The bacterial colonies isolated from the crown tumors of quince plants growing on the (YEM) medium were selected on the basis of the shape, color, texture and size of the colonies by transferring the pure single colonies that had grown on the (YEM) medium to the selection medium (AM), *Agrobactrium* mannitol medium, to ensure their growth on the selection medium. The plates were incubated at a temperature of 28 ± 2 °C and bacterial smears were prepared from them and stained with Gram stain. The ability of the bacterial smear to stain was observed in addition to the shape and arrangement of the cells.

Biochemical tests for the diagnosis of Agrobacterium. Biochemical tests were performed for the bacteria isolated from the crown tumors of quince trees *C. oblonga* according to Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). The most important tests are: catalase test, carbon utilization test, citrate utilization test, carbohydrate fermentation test, triple sugar iron test (TSI), growth test at different days of temperature, growth test of different pH range, tyrosine utilization test, growth of ferric ammonium citrate test, test of the ability of bacteria to grow in the absence of some factors such as biotin or thymine (Wadhwa et al., 2018).

Testing the pathogenicity of A. tumefaciens on carrot discs. Samples of carrots (*Daucas carota*) obtained from local markets of Mosul City were used. The carrot samples were washed with running tap water to remove any dust particles and then sterilized as previously mentioned (Ali et al., 2016). The carrot samples were cut into discs and injected with *A. tumefaciens* bacteria inoculation. The experiment was observed duringr 21 days to investigate the formation of small tumors on the surfaces of carrot discs (Aysan & Sahin, 2003).

Determination of the biotype of bacteria. Tests were conducted on the pure bacterial isolates that produced positive results in simulated infection events on the examined plants. A range of biochemical tests was used to identify the biotype of several *Agrobacterium* spp. as described by Weller et al. (2009). These tests include:

– Molecular identification of *A. tumefacienes* isolates isolated from quince tumors. Molecular characterization techniques were used to confirm the phenotypic and biochemical diagnosis of the bacterial isolates isolated and purified from quince tumors after five isolates were chosen and purified and shown to belong to the genus *Agrobacterium*. PCR technology was then used to characterize the isolates to the species and strain level using diagnostic primers for the 16S rRNA gene.

– Isolation and purification of bacterial DNA. Genomic DNA samples of *A. tumefaciens* isolates from quince tumors were isolated according to the protocol of the supplier company (Gene All, Korea) for the Cell SV mini 101–106 kit, which included a set of solutions as well as a DNA binding Column tube, which were stored at 4 °C.

– Preparation of the specific PCR mix reaction. The concentration of DNA extracted from the five *A. tumefaciens* bacterial isolates AtCo1–AtCo5 was adjusted and diluted with TE buffer to 50 ng/µL/sample for specific PCR, then the Master-Mix reaction mixture and the 16S rRNA gene primer specific for the bacteria (Table 1) were prepared with the Mastermix kit provided by GeneAll, Korea as follows.

Table 1
16S rRNA primer used in diagnosing *Agrobacterium* isolates

Primer name	Primer sequence (`5–3`)
27-F	AGAGTTTGATCCTGGCTCAG
1492-R	TACCTTGTTACGACTT

Table 2
PCR amplification mixture

s	Material		Concentration		Volume µL	Total, µL
			volume	unite		
1	Amplification environment	Premix	1	X	–	
2	Initial material	Fp (infruntof) (reverse) Rp	10	Pmol	1 1	20
3	Extracted DNA	gDNA	28–25	ng/µL	6	
4	Deionized water	NFW	–	–	12	

The reaction tubes were introduced into the thermal polymerization device of the thermal cycler to control the reaction, using the specific amplification program of the acid and the thermal cycler program as shown in Table 3.

DNA extraction from gel. After electrophoresis, DNA bands were extracted according to the protocol of the supplier, using the Gene All® ExpinTM Gel SV kit 102–150 supplied by Gene All®, Korea (Ibrahim & Faisal, 2024).

Alignment of the nitrogenous base sequence of the 16S rRNA gene. The 16S rRNA gene sequence was determined by South Korea Maerogen using the Basic Local Alignment Search Tool (BLAST) algorithm in two isolates based on the DNA sequence of each isolate with the sequence of the standard isolate *A. tumefaciens* at the National Center For Bioinformatic Information (Younis & Faisal, 2024).

Table 3
Amplification program for diagnostics of *Agrobacterium* spp.

Steps			Temperature, °C	Time, min	Number of cycles
Primary fusion		initial denaturation	95	3:00	1
Fusion		denaturation	95	0:30	
Align prefixes	16S rRNA	annealing	57	0:30	40
Elongation		extension	72	0:30	
Final elongation		final extension	72	3:00	1

Results and discussion

Isolation and identification of A. tumefaciens from crown tumors of quince trees. Morphological and cultural characteristics. Twenty-five isolates from quince tumors were successfully obtained for the current investigation. Based on the colonies' size, shape, color, and texture, fifteen isolates were chosen from those growing on the YEM medium. Gram-negative bacterial isolates were chosen from these colonies after smears were made, stained with Gram stain. According to the findings of the compound light microscope analysis, they had the same phenotypic traits, which were demonstrated by the fact that they were short rods that were Gram stain negative. These findings aligned with another study's findings (Ferdous, 2021) that the bacteria recovered from tumors were Gram-negative in form.

Biochemical tests. The preliminary results were produced based on the morphological characteristics and biochemical tests for diagnosis by diagnosing seven isolates that gave initial positive indicators in diagnosis, as is clear from the results of Table 4 for the biochemical tests, the table above, and Figure 3 whose phenotypic characteristics matched those of *A. tumefaciens* bacteria. Given the conformity of the results of the biochemical tests with what was mentioned in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994), the bacteria were also diagnosed as *A. tumefaciens* due to its clear growth on nutritional media without the need for known growth factors such as biotin or thymine (Young et al., 2001), except for the two isolates AtCo6, AtCo7. According to the modifications in the nomenclature and hierarchical categorization suggested by Young et al. (2001), the results of these tests demonstrated that the isolated bacteria were either *Rhizobium radiobacter* or *A. tumefaciens*.

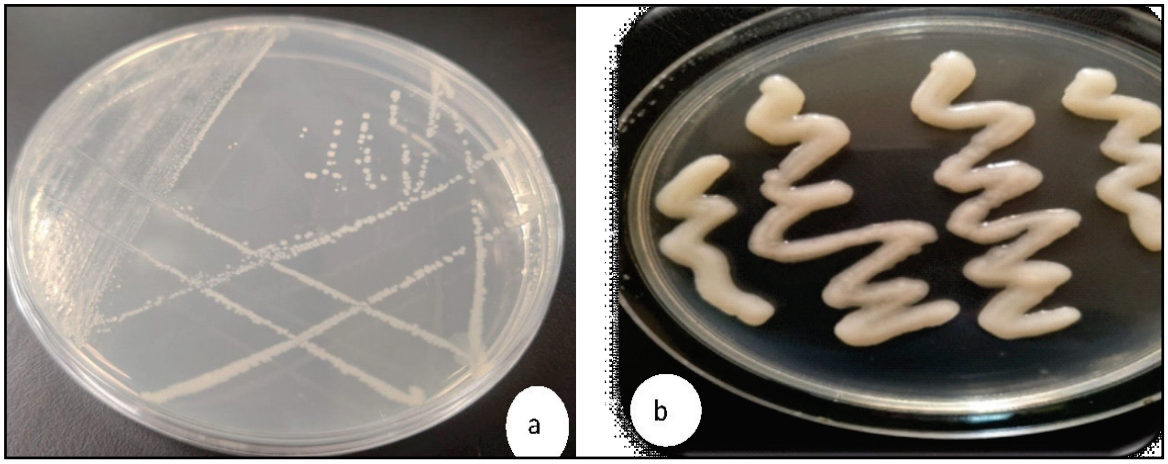

Fig. 2. Showing colonies of *A. tumefaciens*: *a* – isolation of single colonies of *A. tumefaciens* on YEM medium, *b* – diffuse growth of *A. tumefaciens* colonies on King medium

Five isolates were found to have positive diagnostic markers based on the initial results of their growth on the selective media. Following a 24-hour incubation period, the colonies of the 5 bacterial isolates growing on the MacConkey agar medium were identified by their taking on the medium's violet hue. When cultivated on different media, including Luria-Bertani medium, potato dextrose agar, and glucose peptone agar, the 5 isolates produced positive results. As can be seen in Table 5, the isolates grew on the D1 medium as well. However, when grown on the King media and tested using the UV light transilluminator, their fluorescence was negative.

Testing the pathogenicity of isolates on carrot discs. When *A. tumefaciens* was directly injected into carrot discs, the five isolates (AtCo1, AtCo2, AtCo3, AtCo4, AtCo5) demonstrated their ability to form tumors on the discs for periods ranging from 22 to 26 days after infection, as shown by the appearance of tumors on the discs' surfaces in the form of protrusions and in colors varying between green and white (Fig. 4). Additionally, no response was observed on the discs injected with sterile water (control treatment).

Table 4
Biochemical tests of bacteria isolated from crown tumors of quince trees

Testes		Response of isolated bacteria						
		A. tumefaciens (AtCo1)	*A. tumefaciens* (AtCo2)	*A. tumefaciens* (AtCo3)	*A. tumefaciens* (AtCo4)	*A. tumefaciens* (AtCo5)	*A. tumefaciens* (AtCo6)	*A. tumefaciens* (AtCo7)
Catalase		+	+	+	+	+	+	+
L-tyrosine utilization		–	–	–	–	–	–	–
Citrate utilization		–	–	–	–	–	–	–
Utilization of	+	+	+	+	+	+	+	+
carbohydrates	+	+	+	+	+	+	+	+
H_2S production		-	+	-	–	+	–	–
pH range		(4-9)	(4-9)	(4-9)	(4-9)	(4-9)	(4-9)	(4-9)
Growth at	28 °C	+	+	+	+	+	+	+
	35 °C	+	+	+	+	+	+	+
	40 °C	+	+	-	–	-	+	+
Growth absence	biotine	–	–	+	+	+	-	-
	thiamine	–	–	+	+	+	-	-
Growth and pigmenentation in ferric ammonium citrate		+	+	+	+	+	–	–

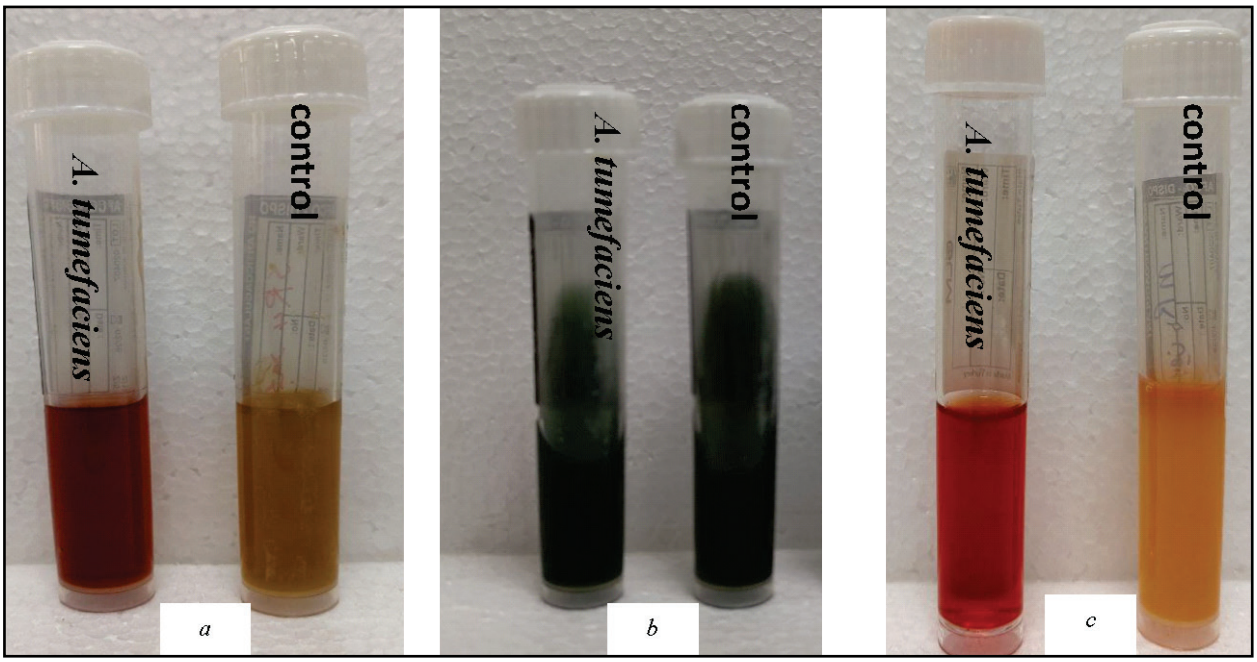

Fig. 3. Biochemical tests for the bacteria isolated from the crown tumors of quince trees: *a* – triple sugar iron test, *b* – citrate utilization test, *c* – carbohydrate fermentation test

Table 5
Growth of *A. tumefaciens* bacteria isolated on selective media

Food medium	*A. tumefaciens* (AtCo5)	*A. tumefaciens* (AtCo4)	*A. tumefaciens* (AtCo3)	*A. tumefaciens* (AtCo2)	*A. tumefaciens* (AtCo1)
MacConkey agar	+	+	+	+	+
D1 medium	+	+	+	+	+
Luria-Bertani medium	+	+	+	+	+
Fluorescent and pigmention in King B medium	–	–	–	–	–
Glucose peptone agar	+	+	+	+	+
Potato dextrose agar	+	+	+	+	+
AMM	+	+	+	+	+
Chromogenic agar medium	–	–	+	–	+

The results came as additional evidence supporting the biochemical tests that the isolated bacteria belong to the species *A. tumefaciens* which causes crown tumor disease (Islam et al., 2010).

Biochemical tests to determine the biovar. After confirming the ability of the five isolates (AtCo1, AtCo2, AtCo3, AtCo4, AtCo5) to cause pathogenesis and form tumors on carrot discs, biochemical tests were conducted to determine their biovar. The results showed that the two isolates (AtCo2, AtCo5) belonged to the first biotype Biovar I, according to the results shown in Table (6). These results were consistent with the results of the study conducted by Bouzar et al. (1993), who confirmed that 56% of the bacteria isolated from the soil belong to Biovar I and are characterized by their ability to form crown tumors on plants. Both of them showed a negative result in their acid production on PDA medium supported by calcium carbonate through the appearance of the medium in a dark color around the colonies, and they do not belong to Biovar II due to the inability of bacteria within this type to move at high pH towards basicity (Bush, 1991).

Molecular diagnosis. This reaction was performed on pure genomic DNA samples extracted from the five bacterial isolates using the primer specific to the 16S rRNA gene. After electrophoresis of the products of the double reaction using the specific primer, the results were transferred using a 1% agarose gel (Fig. 5).

From the results of the migration, it can be seen that there are 5 columns representing the amplified bands of the isolates that were selected based on the biochemical and pathological tests from a specific site of the extracted genomic DNA, as a result of the specialized multiplication reaction of the primer for the 16S rRNA gene with the DNA samples, which appear in the form of five large and equal bands, as their molecular size was estimated at (1150 bp) based on their measurement with the DNA ladder size indicator. The reason for the appearance of these bands is the presence of similar or shared sequences in the sequence of nitrogenous bases present in the genomic DNA of the previously mentioned isolates. This similarity enabled them to complete the nitrogenous bases present in the specialized primer, as the specialized multiplication reaction was completed and DNA bands of large and equal sizes were produced.

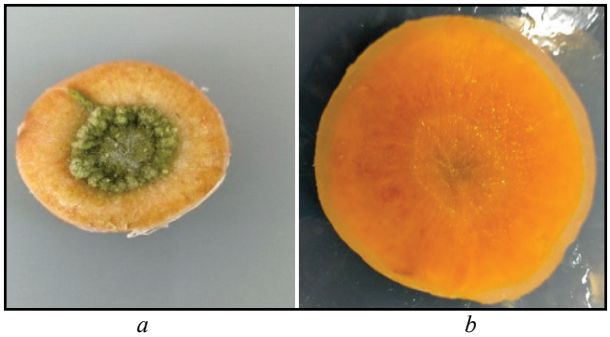

Fig. 4. Testing the pathogenicity of the bacterial isolates isolated from the crown tumors of quince trees to form tumors on carrot discs: *a* – tumor formation on carrot discs inoculated with *A. tumefaciens* (AtCo1); *b* – carrot discs inoculated with sterile distilled water

These results were in line with those of Khalid et al. (2020), who examined the phenotypic and genetic pattern of eight isolates of *Rhizobium* isolated from the nodules of the *Arachis hypogaea* plant, which belongs to the Rhizobiaceae family. Additionally, Frank et al. (2008) conducted a study in which they used the same specialized primer as used in the current study to amplify DNA between positions 27 and 1492 of the 16S rRNA genes of bacterial isolates *Escherichia*

coli for DNA analyses of isolates taken from human vaginal samples, as well as isolates of *Lactobacillus* and *Gardnerella* spp.

Table 6
Biochemical and diagnostic tests
to determine the biovar of the isolated bacteria

Biochemical test	Response of isolated bacteria	
	A. tumefaciens (AtCo2)	*A. tumefaciens* (AtCo5)
Motility at pH 7	+	+
Oxidase reaction	+	+
Growth at 35 ºC	+	+
Production acid from	+	+
Pathogenicity test	+	+
Acid-clearing in PDA-$CaCo_3$	–	–
Salt tolerance (NaCl)	+	+
3-keto lactose production	+	+

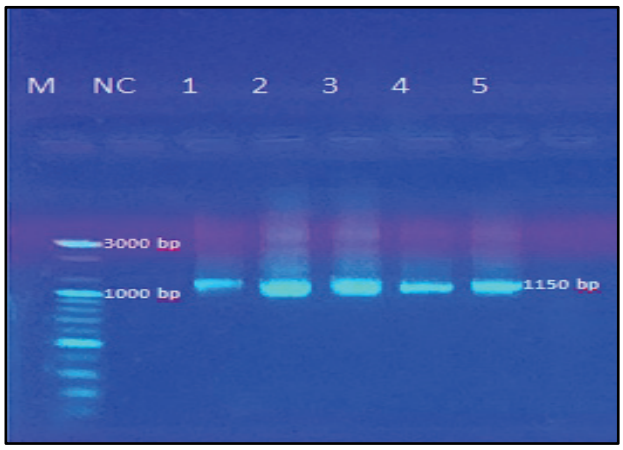

Fig. 5. Results of the specific multiplication reaction of the 5 selected bacterial isolates: M – DNA Ladder Kb, N – negative control (distil led water); columns 1–5 – amplified bands of genomic DNA from the five *A. tumefaciens* isolates isolated from quince tumors (AtCo2–AtCo5)

Study and analysis of the nitrogenous base sequence of the products of the specialized multiplication reaction of genomic DNA samples. The aim of the sequencing technique was to determine the nitrogenous base sequences of the DNA fragments obtained from the products of the 16S rRNA primer multiplication of the purified DNA samples from the local isolates isolated from the crown tumors (under study). The products of the purified DNA multiplication reaction were taken from the two isolates and the results of the comparison with the reference sequences deposited in the National Center for Biotechnology Information (NCBI) database in the GenBank appeared in their most accurate form and were as follows:

The nitrogenous base sequence of the DNA isolate (AtCo2) *Agrobacterium tumefaciens* was as follows:

aagatttatc ggggtaggat tggcccgcgt tggattagct agttggtggg gtaaaggcct accaaggcga cgatccatag ctggtctgag aggatgatca gccacattgg gactgagaca cggcccaaac tcctacggga ggcagcagtg gggaatattg gacaatgggc gcaagcctga tccagccatg ccgcgtgagt gatgaaggcc ttagggttgt aaagctcttt caccggagaa gataatgacg gtatccggag aagaagcccc ggctaacttc gtgccagcag ccgcggtaat acgaaggggg ctagcgttgt tcggaattac tgggcgtaaa gcgcacgtag gcggatattt aagtcagggg tgaaatccca gagctcaact ctggaactgc ctttgatact gggtatcttg agtatggaag aggtaagtgg aattccgagt gtagaggtga aattcgtaga tattcggagg aacaccagtg gcgaaggcgg cttactggtc cattactgac gcttgaggtg cgaaagcgtg gggagcaaac aggattagat accctggtag tccacgccgt aaacgatgaa tgttagccgt cgggcagtat actgttcggt ggcgcacgta acgcattaaa cattccgcct ggggagtacg gtcgcaagat taaaactcaa aggaattgac gggggcccgc acaagcggtg gagcatgtgg tttaattcga agcaacgcgc agaa

Fig. 6. Nucleotide sequence of the AtCo2 isolate made by the Turkish company Oligomer

These sequences were analyzed and their closeness to the current sequences registered in Gene Bank was demonstrated by entering them into the DNA BLAST tool, which may be found at https://blast.ncbi.nlm.nih.gov/Blast.cgi. According to the analysis results, these sequences and those of the *Agrobacterium tumefaciens* AGT12 strain, which is registered in the Gene Bank with the number PPS14354.1, had a high similarity of 99%.

By observing the analysis results, it was found that there is a difference in some nitrogenous bases between *Agrobacterium tumefaciens* isolates and similar strains in the gene bank in certain locations when comparing the nitrogenous base sequences of the isolates under study with the mentioned strains, as this difference is considered a point mutation that occurs in the DNA nucleotide sequences represented by replacing one nucleotide base with another in the nitrogenous base sequence or by adding or deleting a nitrogenous base from the sequence, noting that these mutations occurred automatically. Scientific research in this regard indicated that it is possible to diagnose isolates with a high degree of accuracy between bacterial species that are very close at the genetic level using the polymerase chain reaction method and analysis of DNA amplification products, which opened up broad horizons that enabled researchers to deepen scientific knowledge of the different species related to pathogenic relationships with different plants (Radomirka et al., 2007; Paudel et al., 2020).

According to numerous scientific studies, specialized primers for distinct genes, such as the pathogenic genes of *A. tumefaciens* bacteria (Ninkovic et al., 2004) or the genes encoding virulence factors (Bertolini et al., 2003), can be used in this field to diagnose different bacterial species that belong to the same genus. According to one study, many isolates from various plants that belong to the genus *Agrobacterium* were diagnosed using PCR technology (Islam et al., 2010).

According to (Ninkovic et al., 2004), the PCR method has significantly reduced the time and effort required to differentiate between various *Agrobacterium* isolates derived from various plant sources. One example given in this context is the use of particular primers for the pTi plasmid to distinguish between multiple bacterial species in order to identify distinct strains of *A. vitis* that are part of Biovar III, which causes crown gall disease on grapes (Kawaguchi et al., 2005). Because of the reduction of the interference factor that targets a particular genetic region of these organisms, it can be concluded that the small size of the bacterial (prokaryotic) genome in comparison to the eukaryotic genome facilitates genetic studies and makes them a model for genetic studies.

Genetic convergence and divergence of A.tumefaciens isolates. To clarify the relationship between local isolate and existing strains registered at the National Center for Biotechnology Information, the Phylogenetic Tree diagram showed the alignment results through comparison with a group of *A. tumefaciens* isolates under study, and this appeared clearly in the genetic relationship diagram between the isolates, while the isolates adopted in the alignment and according to the sequence of their nitrogenous bases came within the second group with convergence rates 94–98% (Fig. 8). The lowest convergence rate for isolate was with the strains CP0482322.1, CP048492.1 of *A. tumefaciens* and with a rate of 94%, while the convergence rate began to change to reach 98% with the proven strain in the National Center for Biotechnology Information, which enhances the results reached by the specialized 16S rRNA primer reaction technique, which confirms the diagnostic results of the isolate.

The results of this study were consistent with many studies on the possibility of finding genetic variation between the studied populations using DNA Sequencing technology for the 16S rRNA gene of *Agrobacterium* species, bacteria (Uchino et al. , 1997), where it was found through studying (Liaqat & Eltem , 2016) the genetic closeness of the isolates from peach trees that they are the closest to the standard strain *Agrobacterium tumefaciens* GQ181060 in the National Center for Biotechnology Information by a percentage of 99%. Other genes were adopted to determine the evolutionary path of the genetic pattern of *A. tumefaciens* bacteria isolates, to obtain an evolutionary tree (Pulawska & Sobiczewski, 2005). The isolates AtCo2 and AtCo5 that were taken from quince tumors and were identified by their scientific name, *A. tumefaciens*, were registered and assigned the accession number: PQ106659.

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Agrobacterium tumefaciens strain AGT12 16S ribosomal RNA gene, partial sequenc
Sequence ID: PP514354.1 Length: 1087
Range 1: 120 to 862

Score:1362 bits(737), Expect:0.0,
Identities:742/744(99%),  Gaps:1/744(0%), Strand: Plus/Plus

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            ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  120  AAGATTTATCGGGGAAGGATTGGCCCGCGTTGGATTAGCTAGTTGGTGGGGTAAAGGCCT  179

Query  61   ACCAAGGCGACGATCCATAGCTGGTCTGAGAGGATGATCAGCCACATTGGGACTGAGACA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Query  121  CGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGA  180
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Sbjct  240  CGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGA  299

Query  181  TCCAGCCATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTTCACCGGAGAA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  300  TCCAGCCATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTTCACCGGAGAA  359

Query  241  GATAATGACGGTATCCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAAT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  360  GATAATGACGGTATCCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAAT  419

Query  301  ACGAAGGGGGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGATATTT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  420  ACGAAGGGGGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGATATTT  479

Query  361  AAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAACTGCCTTTGATACTGGGTATCTTG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  480  AAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAACTGCCTTTGATACTGGGTATCTTG  539

Query  421  AGTATGGAAGAGGTAAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  540  AGTATGGAAGAGGTAAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGG  599

Query  481  AACACCAGTGGCGAAGGCGGCTTACTGGTCCATTACTGACGCTTGAGGTGCGAAAGCGTG  540
            |||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct  600  AACACCAGTGGCGAAGGCGGCTTACTGGTCCATTACTGACGC-TGAGGTGCGAAAGCGTG  658

Query  541  GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTAGCCGT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  659  GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTAGCCGT  718

Query  601  CGGGCAGTATACTGTTCGGTGGCGCACGTAACGCATTAAACATTCCGCCTGGGGAGTACG  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  719  CGGGCAGTATACTGTTCGGTGGCGCACGTAACGCATTAAACATTCCGCCTGGGGAGTACG  778

Query  661  GTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGG  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  779  GTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGG  838

Query  721  TTTAATTCGAAGCAACGCGCAGAA  744
            ||||||||||||||||||||||||
Sbjct  839  TTTAATTCGAAGCAACGCGCAGAA  862
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Fig. 7. Comparison of the sequences of the DNA fragments of the isolate (AtCo2) and the standard strain *Agrobacterium tumefaciens* AGT12 registered in GenBank

Table 7
Shows the identification of *Agrobacterium tumefaciens* isolates based on the sequences of the strain registered in GenBank

Isolate	Algin	Number of nitrogenous bases	%Similarity ratio	Standard breed	ID in gene bank	Sbjct Seq. ID
$AtCo_2$	16 SrRNA	*742*	*99%*	*Agrobacterium*	AGT12	PPS14354.1
$AtCo_5$		*726*	*99%*	*tumefaciens*	MZ571936.1	MZ571936.1

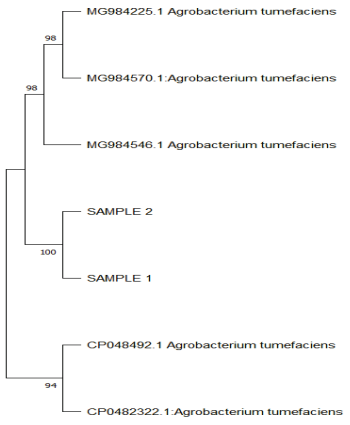

Fig. 8. Dendritic diagram of the genetic pattern of the *A. tumefaciens* isolates under study

Conclusion

The current study revealed that bacterial isolates from crown tumors formed on the stems of quince trees were diagnosed as *Agrobacterium tumefaciens* by morphological, cultural characteristics, biochemical, pathological tests and molecular diagnoses.

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