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Original Article

The reliability of catechol 1,2-dioxygenase enzyme as detection factor of *Pseudomonas savastanoi* pv. *savastanoi* strains isolated from different olive growing areas in Jordan by PCR-RFLP

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Abstract

The virulence gene Catechol 1,2-dioxygenase was detected in different isolates of *Pseudomonas savastanoi* pv. *savastanoi* (Smith, 1908), through amplification of 857 bp band by Polymerase Chain Reaction, it was confirmed in all isolates that were isolated from different olive cultivars growing in different areas in Jordan. Also, digestion of the amplified PCR product of this gene for all isolates of the pathogen, using Polymerase Chain Reaction Restriction-Fragments Length Polymorphism (PCR-RFLP), it was found that the *catA* gene is highly conserved for all isolates, after digestion with *KpnI* and *BamHI* Endonucleases. Further identification was performed for all isolates; by biochemical tests and pathogenicity on olive seedlings, and detection the virulence gene *iaaL* through PCR amplification of 454bp in all isolates. Interestingly, this study revealed that detection and identification of *Pseudomonas savastanoi pv. savastanoi* by *catA* gene is reliable and certified and will give further prospects in management between olive knot through crosstalk of olive plant and their knot bacterium.

Keywords: Olive Knot, *Pseudomonas savastanoi*, Catechol dioxygenase, Virulence factor

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Introduction

Olive (*Olea europaea*) is an important fruit trees and there is an increasing interest in growing olive in many countries especially in the Mediterranean region, due to their nutritional importance of olive products; fruit and oil (Alarcón et al., 2001). Olive is the most important economical fruit trees grown in Jordan, which represent about 72% of all fruit trees. The total cultivated area of olive was about hundred thousand hectares planted with bearing and nonbearing olive trees, respectively, with total

production of about 294 thousand tons (Annual Statistical Report, 2020). One of the main challenges facing olive plantation is Olive knot disease, that have adverse effect on olive tree health and productivity; quantity and quality (Schroth et al., 1973).

Different studies were performed for isolation and description of the causal agent (Smith, 1908). Recently, *Pseudomonas savastanoi* pv. *savastanoi* is the name for the causal agent of olive knot disease (Gardan et al., 1992). Hereafter referred to as *Psv*. Infected olive trees showed symptoms of tumorous outgrowths on shoots and branches. These symptoms develop as the ability of the bacterium to produce the phytohormone indoleacetic acid (IAA) and cytokinins (CK) (Nester and Kosuge, 1981; Surico et al., 1985).

Conventionally, detection of *Psv* is based on isolation on King B medium, then identification with biochemical LOPAT tests followed by pathogenicity test on olive seedlings (Surico and Lavermicocca, 1989; Young and Triggs, 1994) or by serological techniques with monoclonal antibodies (Janse, 1991). Furthermore, different molecular methods were used for detection of *Psv* in olive plants. Enrichment-PCR assay is usually performed through the detection of 454-bp amplicon of *iaal* gene followed by *HaeIII* digestion (Penyalver et al., 2000), or by dot blot hybridization after nested PCR assay (Bertolini et al., 2003).

In Jordan, the disease has spread widely in all olive-growing areas throughout the country, it was reported to occur in different locations with different incidences (Hijazin and Khlaif, 2005; Khlaif, 2006). The disease incidence varies with location and cultivar (Khlaif, 2006).

Detection and identification of *Psv* by conventional methods are expensive and time-consuming. So Specific primers were designed for amplification of *iaaL* gene based on their published sequence (Penyalver et al., 2000). In Jordan, *Psv* was identified by PCR amplification of *iaaL* gene from DNA extracts from sap of knots formed on naturally infected olives and from bacterial cultures (Hijazin and Khlaif, 2005; Wreikat, 2021), also, study the genetic characterization for *Psv* isolates were performed using AFLP analysis (Wreikat, 2021), which revealed that there are two geographically distinct clusters; northern and middle-southern clusters for different olive knot pathogen isolates.

Moreover, it is important to detect one new aspect of pathological relationship between olive trees and their pathogen *Psv*; that could be beneficial for managing the olive knot disease as reported by Filiz and Bozkurt (2022), in which they studied the biological control possibilities of *Psv* by using beneficial epiphytic and endophytic bacteria isolated from the roots and shoots of healthy olive trees. Different studies through the past decades were performed to study the effectiveness of *Pseudomonas* species in recycling aromatic organic molecules (Wackett, 2003). Genomic analysis of *Psv* NCPPB3335 results in presence of 12 variable regions (VRs) (Rodríguez-Palenzuela et al., 2010), when these VRs have a role in virulence functions, it is referred to as pathogenicity islands (Gal-Mor and Finlay, 2006).

Furthermore, whole genome sequence the *Psv* NCPPB 3335 reveals the presence of 21 genes that were predicted to be putative dioxygenases-related genes, interestingly, VR8 of the sequenced *Psv* NCPPB 3335 contain four of these 21genes, these cluster of genes had a role in overcoming the plant phenolic compounds **(**Rodríguez-Palenzuela et al., 2010). Also, sequenced *P. syringae* pathovars infecting woody hosts contain VR8, whereas it is absent in all *P. syringae* strains infecting herbaceous plants (Ramos et al., 2012; Moretti et al., 2014). Also, identical orientation of the gene cluster *catBCA* encoded by VR8 of *Psv* (Nojiri et al., 2002), this region encoding *catBCA* operon, which allow *Psv* to infect and develop in woody plants through overcome the plant defense phenolic compound; the catechol (Buonaurio et al., 2015).

One of the plant aromatic compounds, Catechol which is toxic to *Psv* (Capasso et al., 1997), pathogenic *Psv*, and nonpathogenic bacterial species have the ability to degrade and detoxify this compound through their different produced enzymes (Harwood and Parales, 1996). This is a possible example of how harmless endophyte residents can aid the growth of *Psv* pathogen via metabolic complementarity (Buonaurio et al., 2015).

One of the plant natural defenses against their pathogens is the production of phenolic compounds (Agrios, 2005), these compounds are particularly present with high amount in olive tree tissues (Oi-kano et al., 2008). Furthermore, upon *Psv* attack, the production of phenolic compounds is greatly increased in olive tree knots, that could interpret the disease incidence and severity based on pathogen resistance to phenols (Cayuela et al., 2006).

Furthermore, GC/MS analysis of olive mill waste water revealed the presence of phenolic compounds; hydroxytyrosol, catechol, caffeic acid and p-coumaric acid. These polyphenols used at different concentrations *in vitro* displayed a high level of antibacterial activity against *Psv* (Krid et al., 2011).

Catechol 1,2-dioxygenase (*catA*) is a protein encoded by the sequenced genome of *Psv* NCPPB 3335, interestingly all previously sequenced *P. syringae* genomes did not had a detectable homologue for this protein, *catA* nucleotides length 930 bp and encode 310 amino acids (https://asap.ahabs.wisc.edu/asap/logon.php) (Rodríguez-Palenzuela et al., 2010).

Different endonucleases were used to characterize different strains of *P. savastanoi*. A restriction fragment length polymorphism assay was developed that allowed differentiation among *iaaL* paralogs of *Psv*, also the *Psv* genome was digested with the restriction enzymes (*BamHI* and *HindIII*) and hybridized with the *iaaL* probe (Matas et al., 2009). Also, *EcoRI* and *HindIII* endonucleases were used for *Psv* genome; differences in polymorphism was detected among strains isolated from olive plants using *EcoRI*. In addition, depend on the size of a single hybridizing band in *HindIII* analysis, apparently differentiated between *Psv* strains isolated from olive from those isolated from oleander and ash (Sisto et al., 2002).

Based on the ability of this pathogen to produce catechol 1,2-dioxygenase enzyme that degrade the defense aromatic phenolic compound catechol, which is produced by olive trees upon *Psv* attack, also RFLP analysis of this gene could give us an interpretation for the variations founded in disease incidence and severity in different olive growing areas in Jordan.

In this the study, we search about the reliability of *catechol* 1,2-*dioxygenase* gene as detection agent of *Psv*, which will support and help or as a substitute for detection the pathogen by *iaaL* gene, also to test the possible diversity of the *Psv* via RFLP analysis of *catA* gene.

Material and Methods

Sample collection

A field survey of olive knot disease was carried out in different areas of olive plantation of different cultivars (Nabali Muhassan, Nabali Baladi, Rasei, Italian and K18) in Jordan; Northern, middle and southern. The surveyed areas included most fields, gardens and road sides planted with olive suspected to be infected with *Psv*. Random samples of olive branches showing olive knot symptoms were collected.

Bacterial isolation and identification

Isolation

Samples showing olive knot symptoms were washed under tap water for cleaning knots from soils and dusts, surface disinfected by dipping into 0.5% sodium hypochloride for one minute, rinsed three times with Sterile Distilled Water (SDW), plotted to dry on sterile filter paper. Then the samples were cut into small pieces in few drops of SDW in a sterile Petri-dish, and the resulted suspension was left for 15 minutes. A loopfull of the suspension was inoculated onto King B (KB) medium plates by sterile platinum loop. The pH of the medium was adjusted to 7.2-7.4. The inoculated plates were incubated at 25 ± 2°C till the bacterial colonies developed. Plates were checked periodically by subjecting the plates to long wave (367 nm) UV light for the detection of fluorescent colonies. Single fluorescent colony from each plate was restreaked onto new KB plates and incubated at 25 ± 2°C. The obtained fluorescent colonies were stored in1 ml of 30% glycerol tubes, and stored at 4 °C for further identification (Schaad et al., 2001).

Identification

Twenty-four hours old cultures from the obtained pure cultures were subjected to laboratory tests for characterization and identification as described by Schaad et al. (2001). These tests are LOPAT and olive pathogenicity. The same tests were run against the reference culture *Psv* (NCPPB3335). *Pseudomonas fluorescence* was used as a control.

Genomic DNA extraction from the *Psv* cultures

Genomic DNA extraction was performed according to E.Z.N.A.®Bacterial DNA Kit, Omegabiotek, USA.

Identification of the pathogenic *Psv* isolates by detection of *iaaL* gene

PCR amplification

A single oligonucleotide primer pair (IAALF (5'-GGCACCACGGGCAACATC A A -3') and IAALR (5' CGCCCTCGGAACTGCCATAC-3')) was used for the *iaaL* gene amplification (Penyalver et al., 2000).

The PCR was carried out in a total volume of 25 µL,

containing: 12.5 µL of 2X PCR Master Mix Solution (i-MAX II, iNtRON BIOTECHNOLOGY) containing per ml (i-MAX II DNA Polymerase(5U/µl), dNTPs, reaction buffer and gel loading buffer), 7.5 µL of nuclease free water, 2.5 µL of primer pair (10 pmole/µL) and 2.5 µL of template DNA.

PCR protocol

Amplification reaction was performed in a C 1000^{TM}thermal cycler (BIO-RAD, USA) in which the amplification includes the following protocol: Initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds for denaturation, annealing at 62°C for 30 seconds, and extension at 72°C for 30 seconds and then 1 cycle of additional extension at 72°C for 5 minutes. After amplification, the samples were stored at 4 °C until being electrophoresed (Penyalver et al., 2000).

Gel electrophoresis

Five microliters of the PCR product were separated by a horizontal Agarose electrophoresis in 1X TAE buffer, 1.5% (W/V) Agarose E containing 0.05% RedSafe™ (iNtRON Biotechnology) and a molecular weight marker of 100 bp (New England BioLabs, USA). Electrophoresis was performed for one hour at 120 V, and then gels were photographed under 302nm UV light.

Detection of *catA* gene from bacterial cultures by PCR

PCR protocol

A single oligonucleotide primer pair was designed using Primer 3 program based on the *catA* gene sequence available in ASAP (https://asap.ahabs.wisc.edu/asap/logon.php) (Rodríguez-Palenzuela et al., 2010). The obtained forward and reverse primer sequences were; CATAF (CGACGATGTGCAACAGTTCT) and CATAR (GTTCGTCCTCGACAGTCGTT).

The used PCR protocol and the gel electrophoresis were similar to that used for *iaaL* gene analysis.

PCR-RFLP analysis of *catA* gene

Ten microliters of the PCR product were digested with the following restriction endonucleases: *KpnI*, *AluI*, *HindIII*, *MseI* and *BamHI* (New England BioLabs, USA). The reactions were performed in a final volume of 25 µL with 5 U (0.5 µL of 10U/ µL) of *MseI*, *AluI* and *BamHI* enzymes, and 10 U (0.5 µL of 20 U/ µL) of the other enzymes. The assay temperature was 37 ^{o}C for all enzymes, and the incubation time was 30 minutes for *AluI*, *HindIII*, *MseI* and *BamHI*, while *KpnI* for 2 hours.

Digested products were separated by horizontal electrophoresis in 1X TAE buffer using 2% (w/v) Agarose containing 0.05% RedSafe™ (iNtRON Biotechnology). Ten microliters of the digested product were loaded in each well, also uncut PCR product of *catA* gene was loaded as a control. Molecular weight marker of 50 bp (New England BioLabs, USA) was used. Gels were run for two hours at 120 V, and photographed under 302nm UV light.

Results

Sample collection

During field inspection of the olive growing areas in Jordan, olive knot disease was found to occur in the different olive growing areas throughout Jordan including: Northern, Middle and from Southern area (Table 1).

Table-1. Collected samples, their area, location and the obtained positive isolates of *Pseudomonas savastanoi pv. savastanoi*.

Area	No of collected samples	Obtained isolates	Isolate Abbreviations
Northern	32	15	N1-N15
Middle	49	23	M1-M23
Southern	39	22	S1-S22
Total	120	60	60

Isolation of the causal agent

Weak fluorescent colonies, raised, circular and smooth were obtained on KB medium plates, when the surface of these plates was inoculated with the suspension of olive knot samples collected from naturally infected plants after subjecting them to UV light (367 nm). Sixty isolates of olive knot pathogen from naturally infected olive trees were obtained from different areas in Jordan.

Identification

The reactions of all obtained bacterial isolates that collected from infected olive trees to the different biochemical tests as follow; the reaction of the tested isolates to Levan, Oxidase, Potato soft rot and Arginine dihydrolase were negative, whereas, the hypersensitive reaction to tobacco leaves was

positive. All The reactions of the biochemical tests of the obtained isolates were identical and similar to the reactions of the same tests run against the reference culture *Psv* NCPPB3335.

Pathogenicity

The sixty biochemically identified bacterial isolates were found to be pathogenic to olive seedlings. When a bacterial mass from tested bacterial isolates were applied to the wounded sites of one-year-old olive twigs of the cultivar Nabali Muhassan, induced knots four to five weeks after inoculation. Swelling and enlargement of tissues started to appear at inoculation resulted in fleshy knot formation. These knots were similar to those induced by the reference culture (NCPPB3335).

The biochemically identified bacterial isolates used for the pathogenicity test were also used for the detection of the *iaaL* gene, *catA* gene, and *catA*-RFLP analysis from the bacterial cultures.

Identification of the pathogenic bacterial isolates by *iaaL* gene

The pathogenic bacterial isolates were identified by detection of *iaal* gene from DNA extracts of the sixty isolates and the reference strain of *Psv* cultures grown on KB medium plates forming the expected PCR product of 454 bp (Figure 1). Also, negative control was performed using NFW.

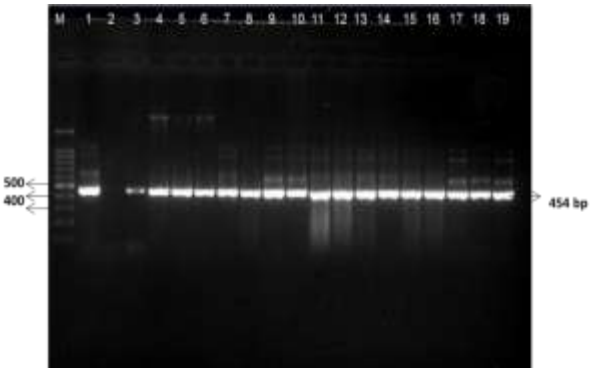

Figure-1. Gel electrophoresis (1.5 % Agarose) of *iaaL* amplicon using the primers IAALF and IAALR from selected bacterial cultures of *Psv*.

Lane M: molecular size marker (100 bp DNA ladder). *Lane 1*, Positive control using DNA extracted from bacterial culture of the reference *Psv* NCPPB 3335; *Lane 2*, PCR-negative control; *Lanes 3-7*, *Lanes* 8- 13 and *Lanes 14-19* are some of the obtained *Psv* isolates from northern area, middle area and *from southern area; respectively.*

Detection of *catA* gene from bacterial cultures by PCR

The *catA* gene was detected in the DNA extracts from the 60 isolates of *Psv* and of the reference strain that were grown on KB medium plates, forming the expected PCR product of 848 bp (Figure 2).

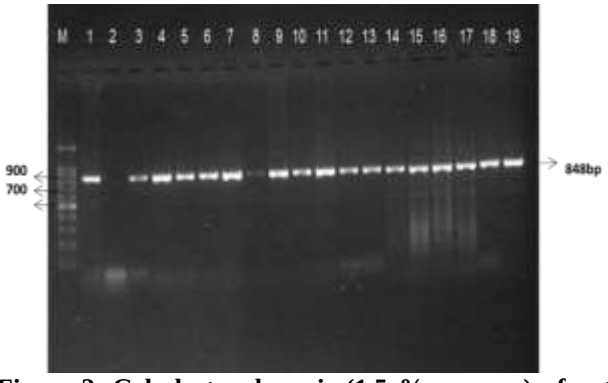

Figure-2. Gel electrophoresis (1.5 % agarose) of *catA* amplicon using the primers CATAF and CATAR from selected bacterial cultures of *Psv*.

Lane M: molecular size marker (100 bp DNA ladder). *Lane 1*, Positive control using DNA extracted from bacterial culture of the reference *Psv* NCPPB 3335; *Lane 2*, PCR-negative control; *Lanes*: 3-7; *Lane* 8-13*; Lane 14-19*; are some the obtained *Psv* isolates from northern, middle and from southern areas; respectively.

PCR-RFLP analysis of *catA* gene

Digestion of the amplified PCR product of the *catA* gene for the sixty isolates of *Psv* and of the reference strain using five different restriction endonucleases; *KpnI*, *AluI*, *HindIII*, *MseI*, *BamHI*, resulted in the presence of restriction sites in the amplicon for two enzymes only; *KpnI* and *BamHI*.

The obtained *Psv* isolates and the reference culture NCPPB3335 gave identical bands for each enzyme. The *BamHI* digested the amplified PCR product of *catA* for all isolates into 510 and 338 bp restriction fragments (Figure 3), while *KpnI* digested the PCR product of the same gene into 279and 569bp restriction fragments (Figure 4).

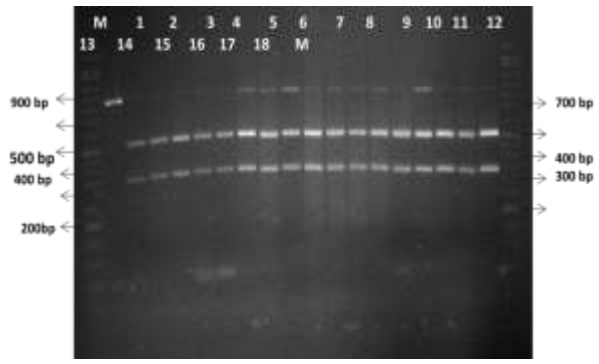

Figure-3. Gel electrophoresis (2 % Agarose) of PCR-RFLP analysis of *catA* gene after digestion with *BamHI* enzyme.

Lane M: molecular size marker (50 bp DNA ladder). *Lane 1*, uncut PCR product Positive control; *Lane 2*, Digested *catA* gene amplified from the reference culture *Psv* NCPPB 3335PCR; *Lanes 3* to *19* are some of the obtained *Psv* isolates. Lanes: 3-7; *Lane* 8-13*; Lane 14-19* are some of the obtained Psv isolates from northern, middle and from southern areas; respectively.

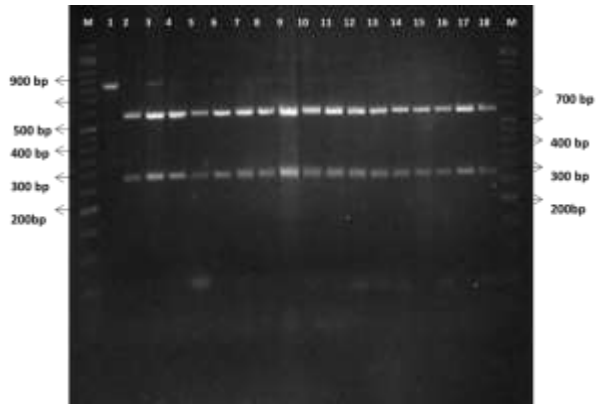

Figure-4. Gel electrophoresis (2 % Agarose) of PCR-RFLP analysis of *catA* gene after digestion with *KpnI* enzyme.

Lane M: molecular size marker (50 bp DNA ladder). *Lane 1*, uncut PCR product Positive control; *Lane 2*, Digested *catA* gene amplified from the reference culture *Psv* NCPPB 3335PCR; *Lanes 3* to *19* are some of the obtained *Psv* isolates. *Lanes*: 3-7; *Lane* 8-13 and *Lane 14-19* are some of the obtained *Psv* isolates from northern, middle and from southern areas; respectively.

Discussion

The results of our study revealed that the olive knot disease is spread in the different olive growing areas in Jordan (Khlaif, 2006; Wreikat, 2021). The sixty collected bacterial isolates of the olive knot bacterium were identified by biochemical and pathogenicity tests, and it reacted identical to the same tests run against the reference culture *Psv* NCPPB 3335, which proved that the isolated bacterium from olive knot is *Psv* (Gardan et al., 1992; Young et al., 1996).

Furthermore, *Psv* isolates were identified by PCR amplification of 454 bp amplicon, which represent the virulence factor; *iaal* gene. A 454-bp product was obtained from all collected *Psv* isolates, this result in general agreements with Hijazin and Khlaif (2005) and Wreikat (2021) who identified *Psv* isolates obtained from naturally infected olive trees in Jordan by detection of *iaaL* gene.

Moreover, detection the virulence factor; *catA* gene; from identified *Psv* isolates was performed. This gene has a role in pathogenicity (Rodríguez-Palenzuela et al., 2010) relationship between olive knot pathogen and olive plant; in which the olive trees produce aromatic compounds as a defense mechanism against pests attack (Harwood and Parales, 1996), one of these compounds is the catechcole which is secreted from olive trees as *Psv* attack (Capasso et al., 1997). In the other side, the *Psv* has ability to overcome this defense mechanism through the production of *catA* enzyme, which is responsible for catechcole degradation (Rodríguez-Palenzuela et al., 2010). PCR assay was performed to detect *catA* gene to identify *Psv* isolates, this detection was found to be useful to determine the potential *Psv* genome, in addition to *iaaL* gene, that because all isolates that possess *catA* gene, also induce knot symptoms on artificially inoculated olive seedlings.

The *catA* gene (*catA* gene, AER-0001892) is genetically encoded by *Psv* NCPPB 3335 genome, this gene was predicted to be dioxygenases and involved in degradation of phenolic compounds (Rodríguez-Palenzuela et al., 2010). To illustrate the importance of the *catA* gene as virulence factor of *Psv* on olive trees, it is reported that VR8 exists in all sequenced *P. syringae* pathovars infecting woody hosts, whereas it is absent in all *P. syringae* strains infecting herbaceous plants (Ramos et al., 2012; Moretti et al., 2014). Thus, the presence of this gene on the genome of the obtained *Psv* isolates provides the pathogen with the ability to infect the olive trees, in which overcoming one of the identified host defense; the catechol. Cayuela et al. (2006) reported that upon *Psv* attack, the production of phenolic compounds is greatly increased in olive tree knots. Also, olive tree tissues usually had ample amounts of these phenolic compounds (Oi-kano et

al., 2008).

Furthermore, the crosstalk between olive plant and the olive knot pathogen that is illustrated in the plant production of catechol phenolic compounds and the ability of pathogen to degrade this compound through the production of *catA* enzyme (Oi-kano et al., 2008). Thus, it is of vital importance to assess the presence of genetic diversity of this gene through PCR-RFLP analysis, that could interpret the differences in disease incidence and severity, as well as the pathogen virulence in relation to different olive growing areas and cultivars.

Fortunately, the results of PCR-RFLP analysis of the *catA* gene indicated that this gene is highly conserved among the sixty different *Psv* isolates, which were obtained from three different areas of Jordan and for the reference culture NCPPB 3335; thus could ascertain the reliability of *catA* gene as detection agent of *Psv*. These results are in general agreements with Rodríguez-Palenzuela et al. (2010) who reported that this gene is found in the VR8 of *Psv* NCPPB 3335 genome that involved in degradation of phenolic compounds. So, additional work is needed such as gene sequencing of each isolate that will ascertain that this gene is highly conserved in Jordanian isolates.

Moreover, our *catA*-RFLP analysis indicated that the virulence factor *catA* found to be highly conserved of the obtained Jordanian isolates of *Psv*, in which two of the five endonucleases that were used in this study; each had certain restriction sites within gene. Thus, the variability in susceptibility/resistance of the different olive cultivars grown in Jordan as reported by Khlaif (2006) could be attributed to the presence of two *iaaL* paralogs (Zhao et al., 2002; Ma et al., 2007; Matas et al., 2009; Wreikat, 2021), or to other virulence factors encoded by *Psv* genomes of these: type III secretion system (TTSS) which could be involved in the degradation of aromatic hydrocarbons; (Sisto et al., 2004); and the exopolysaccharides (Peñaloza-Vazquez et al., 2004; Laue et al., 2006).

Thus, we can say that detection and identification of *Psv* strains based on *catA* gene is highly reliable and certified, because VR8 is absent in all sequenced *P. syringae* strains infecting herbaceous plants, and it is however present in *P. syringae* pathovars infecting woody hosts (Ramos et al., 2012; Moretti et al., 2014), whereas *iaaL* is widespread among plant-associated bacteria (Glickmann et al., 1998). Also, an overview of Genetic analysis of identified *Psv* strains, it was found that are genetically distinct mainly according to geographic regions (Sisto et al., 2007; Moretti et al., 2008; Krid et al., 2009; Basim et al., 2019; Wreikat, 2021), these findings based on the *iaal* gene and on whole *Psv* genome analysis.

Furthermore, our results revealed that there is no effect of olive cultivars on genetic variability of *Psv* based on PCR-RFLP analysis of *catA* gene. These results about the effect of cultivar in general agreements with Scortichini et al. (2004) and Basim et al. (2019), who reported that the olive plant and its pathogen did not cospeciate, also the cultivar seems not to affect the genetic diversity of *Psv* strains, whereas disagreed findings of Moretti et al. (2008), Krid et al. (2009) and Wreikat (2021) who reported that there is an effect of olive cultivars on genetic variability of *Psv*, in addition to geographical effect.

Moreover, potential management of olive knot disease via antagonistic beneficial bacteria such as *Bacillus mojavensis*, *Bacillus subtilis*, *Rahnella aquatilis and Pseudomonas koreensis* isolates could be used against Psv, the causal agent of the olive knot disease, as a biocontrol agent (Ghanney et al., 2016; Bouaichi et al., 2019; Mina et al., 2020; Filiz and Bozkurt, 2022). So, understanding the crosstalk between olive tree and *Psv* pathogen may help to enhance control management of olive knot disease, thus it is important to study an oriented management strategy toward *Psv* through degradation of the virulence factor *catA* enzyme, in which search about antagonistic bacteria against this enzyme.

Conclusion

Catechol 1,2-dioxygenase enzyme is reliable detecting virulence factor, in which, it was detected in all *Psv* cultures, obtained from naturally infected olive. Also, it could be considered a valid candidate to be studied in the management of olive knot disease, through the possibility of breeding olive trees rich with anti-*CatA* enzyme, or more researches must be done on the olive resistant cultivars and link the resistance with the natural defense compound; catechole available in the olive trees.

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