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Abstract

Olive knot disease caused by the phytopathogenic bacterium Pseudomonas savastanoiaffects olive cultivation in both quality and quantity. Endophytic bacteria are candidates for biocontrol agents according to previous research.

Keywords

Olive knot, Bacillus sp., Pseudomonas fluorescens, in vitro screening and Secondary Metabolite.RESE A RCHART I C L E

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RESEARCH ARTICLE

Biocontrol Activities of Olive Endophytic Bacteria Isolates Against *Pseudomonas savastanoi*

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ABSTRACT

Background and objectives: Olive knot disease caused by the phytopathogenic bacterium *Pseudomonas savastanoi* affects olive cultivation in both quality and quantity. Endophytic bacteria are candidates for biocontrol agents according to previous research.

Methods: In this study we collected samples (leaves and galls) randomly for pathogen and endophytic bacteria isolation from the different olive growing areas around Erbil including (Qucha Blbas, Grdasor, Sami Abdulrahman Park, and College of Agricultural Engineering Sciences) on June - October 2021.

Results: Forty-six isolates of endophytic bacteria were obtained and tested against *P. savastanoi*. The result indicated that five isolates showed growth inhibition of the pathogen among them two of the most effective isolates selected (Oq5 and Og2) with the pathogenic isolate molecularly identified using amplified 16S rDNA. The pathogenic isolate identified as *P. savastanoi* (Accession No. OP001734), isolate Oq5 *P. fluorescens* (Accession No. OP001733), and Og2 as *Bacillus sp.* (Accession No. OP001732). Also, their secondary metabolites extracted at different times of incubation and tested using the agar well diffusion method showed great inhibition of the pathogen. Both isolates showed the highest inhibition zone (25-26mm) after 10 days of incubation.

Conclusions: Our results suggest that both endophytic bacteria isolate effective biocontrol of olive knot disease.

Key Words: Olive knot; *Bacillus sp.*; *Pseudomonas fluorescens*; in vitro screening and Secondary Metabolite.

INTRODUCTION

Olive (*Olea europaea*) relates to the Oleaceae family is the second most important oil fruit tree worldwide after oil palm. It is cultivated on an area of more than eight million hectares and is traditionally and extensively cultivated in the Mediterranean region (Baldoni and Belaj 2009). The major olive producer is Syria, Iraq, Spain, Italy, Turkey, Greece, Egypt, Iran, France, and United States (Ali et al., 2014). The tree of olive faces many threats and diseases such as crown gall, wound canker, olive quick decline syndrome, and olive knot disease. Olive knot disease is caused by the Gram-negative phytopathogenic bacterium *Pseudomonas savastanoi* (Quesada et al., 2012). It is a severe disease that occurs where the wounds are caused by the damage through late winter frost and the injuries were caused by harvesting tools during wet weather or moist winds (Panagopoulos, 1993). In addition, this disease affects olive cultivation both the quality and quantity of the tree. The disease found in several countries were olive growing regions found mainly in Mediterranean countries such as Italy (Marchi et al., 2005), Australia (Hall et al., 2004), Greek (Pyrowolakis and Weltzien, 1974), Turkey (Basim et al., 2019) and Jordan (Khlaif, 2006). Galls occur in all the aerial plant parts of the olive trees mostly on the trunk and branches, sometimes, on leaves and fruits (Valverde et al., 2020). Galls and tumors development are described as overgrowth (Young, 2004) which depends on the capability of *P. savastanoi* to synthesize and secrete cytokines and indole acetic acid (IAA) (Pérez-Martinez et al., 2007).

There are many difficulties in finding a treatment for olive knot disease and the overuse of chemical pesticides to control plant diseases had harmful effects on human beings and caused soil pollution. To reduce the use of these chemicals we should find other methods to control plant pathogenic diseases, the modeling way nowadays is beneficial microorganisms as biocontrol agents or biological pesticides (Alabouvette et al., 2006).

According to previous research, endophytes are microbial communities naturally present inside the tissue of the plant parts

such as (leaves, seeds, stems, and roots) (Eid et al., 2019). Several endophytic bacterial strains identified from economically important plant species include Arthrobacter spp., *Actinobacter spp.*, *Bacillus spp.* and *Pseudomonas spp.* produce several antimicrobial compounds against phytopathogens (Gray and Smith, 2005). Endophytic microbes secret specialized biologically active substances in the absence of any harm to their host tissues which increases the ability of the plant resistance to against the pathogen (Liarzi et al., 2016). Also, several enzymes produced by endophytes include glucosidases pectinases amylase, xylanases, phosphatases, and proteases which reduce the effect of the pathogens (Kannan et al., 2015). The aim of the study was to determine the biological control possibilities of *Pseudomonas savastanoi* by using endophytic bacteria isolated from the leaves of healthy olive trees.

2. Materials and Methods

2.1. Sample collection

Twenty samples were collected from the olive trees showing suspected olive knot disease symptoms including leaves and galls. In addition, eighty samples of healthy olive leaves were collected randomly from healthy trees for endophytic bacteria isolation from the different olive growing areas including (Qucha blbas, Grdasor, Sami Abdulrahman park, and College of Agricultural Engineering Sciences) Erbil/Kurdistan on June - October 2021. The collected samples were placed in paper bags and transported to Erbil Polytechnic scientific Research center laboratory.

2.2. Bacteria isolation

2.2.1. Pathogen Isolation

The pathogen isolation was carried out from infected olive plants showing typical olive knot symptoms including (leaves and rough galls). Bacteria were isolated by following the tissue plating method described by Sesma and Osbourn (2004). Approximately 5 mm^2 segments were taken from the galls, and the specimen's surface sterilized was dipped into 70% Ethanol for 3 min then rinsed in sterilized distilled water twice for one minute, and then dried on sterilized filter papers. Specimens were transferred to petri dishes containing nutrient agar (NA) medium and incubated at 25◦C for 2 days till the bacterium grew. Different bacterial colonies were observed then each of them was streaked onto semi-selective King's B medium (peptone, 20g; Glycerol,10 ml; K2HPO4, 1.5 g; MgSO4, 1.5 g; Agar,15 g; 1000 ml sterilized distilled water, pH 7.2). All components added together except MgSO4 were heated with agitation to dissolve the agar and autoclaved for 15 min at 121°C (Johnsen and Nielsen, 1999). This practice was repeated until purified bacterial cultures were achieved.

2.2.2. Endophytic bacteria isolation

Endophytic bacteria were isolated from healthy olive leaves according to Anjum and Chandra (2015) with minor modification. The fresh leaves of all collected samples were washed under slow running tap water for 1 minute followed by washing in sterilized distilled water (SD) for 2-3 minutes. Leaves were cut into small pieces using scissors and then surface sterilized with 70% ethanol for 3 minutes after that moved to sterilize double distilled water for 2 minutes two times then dried on sterilized filter papers. Specimens of sterilized samples were transferred to Petri dishes containing nutrient agar (NA) medium and incubated at 28 °C until bacteria grew. Different colonies were again streaked onto NA plates and this process was repeated until purified bacterial cultures were obtained with homogeneity colony morphology.

2.3. Pathogenicity Test

All bacterial isolates used in this study including (4 pathogen isolates and 46 endophytic bacteria isolates) from collected samples were established for their pathogenicity test using a detached leave assay according to (Imathiu et al., 2009) with minor modification. Suspension of each bacteria isolate was prepared following Rashid (2021) one loop of the (24 h) bacteria isolates was taken and transferred to 50 ml test tubes containing the sterilized distilled water and vortexes until mixed well. An optical density (OD) of 0.1 at a 600 nm wavelength (an OD of 0.1 equivalent to a concentration of 106 cfu/ml). Healthy olive leaves were surface sterilized with 70% alcohol and then, injured at the center of the upper surface using a sterile needle. The injured leaves were dipped into suspension of each bacterial isolate separately with four replications inside a Petri dish lined with sterilized filter paper while control was dipped into sterilized distilled water (SDW). The leaves were monitored daily and moisture was supplied by adding 5 µl of SDW.

2.4. In vitro Screening

To evaluate the efficacy of active endophytic bacteria against *P. savastanoi* agar plug assay was used. Prepared suspension of 24 hours cultivated colony of *P. savastanoi* spread over the surface of Petri dishes containing Mueller Hinton Agar (MHA) medium by using sterilized L-shape tool. A plug of 24 hours cultivated colony from endophytic bacteria cut aseptically through sterilized cork borer then placed onto cultures which previously inoculated with *P. savastanoi*. All plates were wrapped with parafilm and incubated at 28 °C for 24 hours then zones of inhibition were measured (Mohamad et al., 2018).

2.5. DNA extraction and molecular identification

Bacterial isolates were cultivated in a nutrient broth at 30°C for 24 hours. Quick bacteria genomic DNA extraction Kit was used to extract the total genomic DNA from all the isolates. A NanoDrop ND-100 device (Thermo Fisher Scientific, USA) was used to check the quality and quantity of the DNA. The 8F

forward primer (-5-GTGACACGTACACGT-3-) and 1492R reverse primer (-5-ATCGCACGTACACGT-3-) were used to amplify the 16S RNA gene (Turner et al., 1999). Polymerase Chain Reaction amplification (WizPure™ PCR 2X Master kit) was conducted in a 25 µL reaction containing 22 µL PCR 2XMaster which contains Taq DNA polymerase, MgCl2, dNTPs enhancer, and stabilizer. 1 µL 8F, 1 µL 1492R reverse primer, and 1 µL extracted DNA template. The amplification was conducted with the following program: The initial denaturation temperature was set at 95°C for 5 minutes, followed by 30 cycles. denaturation for 1 minute, annealing for 1 minute at 55°C, and extension at 72°C for 2 minutes. A final extension was carried out at 72°C for 5 minutes.in (XP Thermal Cycler, TC-XP – G,

BIOR TECHNOLOGY CO., LTD).

BLAST (Basic Local Alignment Search Tool) search programme from the National Institute of Biotechnology Information (http://blast.ncbi.nlm.nih.gov) was used to carry out alignment to classify and analyse homologous sequences of bacterial isolates used in this study with those species placed in the GenBank.

2.6. Phylogenetic Analysis

16S sequences of selected bacterial isolates were used for phylogenetic analysis (Neighbor-joining method with 1000 bootstrap replication) using the MEGA 11 method Neighbor-joining trees were constructed based on the total character differences and bootstrap values were calculated from 500 replications (Tamura et al., 2021).

2.7. Secondary Metabolite Test

After antagonistic activity two best bacterial isolates that showed the highest antibacterial activity from the previous experiment (Oq5, Og2) were selected. The bacterial isolates were cultivated in 100 ml of nutrient broth (NB) and incubated at 28 °C for different periods of time including (2, 4, 6, 8, and 10) days. The samples were centrifuged at 10000 rpm for 10 min (Micro 220/220R Centrifuge, Hettich) to discrete the hastens from supernatants. A vacuum filtration pump was used to filter the extracts with two layers of filter papers Whatman No.1 filter papers (ALBERTR) before drying to dryness with a rotary evaporator (HahnShin Scientific Co., Taiwan) at 40 °C for both bacterial isolates and all period of incubation. The concentrated products were regarded as crude samples and stored at 4ºC for further use. Crude extracts were dissolved in deionized distilled water (100 µg/ml) for bioassay. The well diffusion method was used to test the antibacterial activity (Rashid, 2021). 100 µl of pathogen suspension (106 cfu/ml) was spread by L shaped glass rod on NA. After the plates were dried two wells were made using a sterile cork borer (0.5 mm). Thirty µl of each culture filtrates were added to every well. The plates were left to dry then, the plates were incubated for 48 h at 30 ± 2 ◦C. The inhibition zone around the wells was measured using a ruler in millimeters (mm).

3. Results and Discussion

3.1. Bacteria isolation and pathogenicity test:

Four bacteria isolates were isolated and purified from symptomatic olive trees. Forty-six morphologically different bacteria isolate isolated from healthy olive leaves (table 1). Each isolate was named based on their location isolates (Oq1 -Oq19) were obtained from Qucha Bilbas, isolates (Og1-Og5) were obtained from Grdasor, and isolates (Os1-Os17) were obtained from Sami Abdulrahman Park, and isolates (OA1- OA5) obtained from Agriculture College. One isolate was selected from among the four isolates that had disease symptoms for further experiment. All 46 endophytic bacteria isolates were tested for pathogenicity test on olive trees none of the isolates were pathogenic to olive trees.

Table (1) Endophytic bacteria isolates with their location.

NO.	Location	Isolate's name
1	Qucha Bilbas	Oq1-Oq19
2	Grdasor	Og1-Og5
3	Sami abdulrahman Park	Os1-Os17
4	Agriculture University	OA1-OA5

3.2. Antagonistic activity

All the isolated bacteria were tested against the pathogenic isolate using the agar plug method on MHA media. Among them, five isolates showed growth inhibition of the pathogen (table 2). Isolate Oq5 and Og2 showed the highest inhibition (23 mm) (fig 1) followed by isolate Os1 (15mm) then come both isolates Os15 and Os16 with (10 mm) inhibition.

Table (2) inhibition zone of active endophytic bacteria

Isolates	Inhibition zone (mm)
Oq5	23
Og2	23
Os1	15
Os15	10
Os16	10

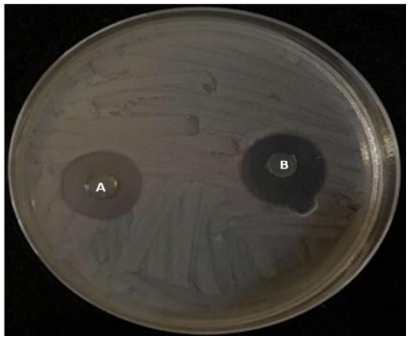

Fig 1: Growth inhibition of *P. savastanoi* by two best endophytic bacteria isolates in agar plug method on MHA after 24 h of incubation at 28˚C. A: Isolate Oq5; B: Isolate Og2.

3.3. Bacteria identification:

PCR products running in gel showed that DNA fragments of two studied active isolates and one pathogen isolate were amplified at 1400-1500bp (Fig 2). The pathogen isolate was identified as *Pseudomonas savastanoi* and endophytic isolates were identified as *P. fluorescens* (Oq5) and *Bacillus sp.* (Og2). On the basis of the results derived from the BLAST of the NCBI

(http://blast.ncbi.nlm.nih.gov/), partial 16S rDNA sequences of the Oq5 revealed the closest matches with the sequences of *P. savastanoi* strain ATCC 13522, *P. fluorescens* strain PSF1 100%, and isolate Og2 with the sequence of *Bacillus sp*. LS-057 100%.

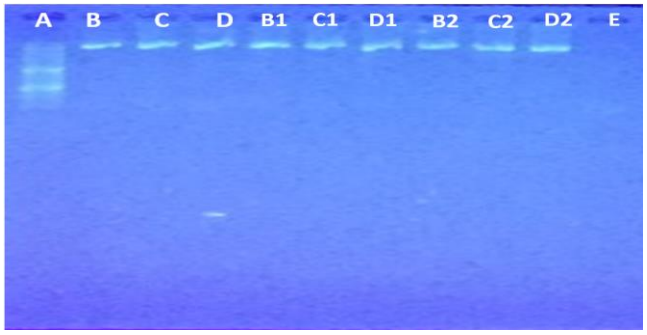

Fig 2: Amplification of 16S rDNA of pathogen and both active isolates in 1% TAE buffer fragmented at 1500 base pairs (Lane A= Molecular weight marker (1kb ladder; Lane B,B1,B2 = Pathogen; Lane C,C1,C2 = Oq5 a; Lane D,D1,D2= Og2 and E= control)

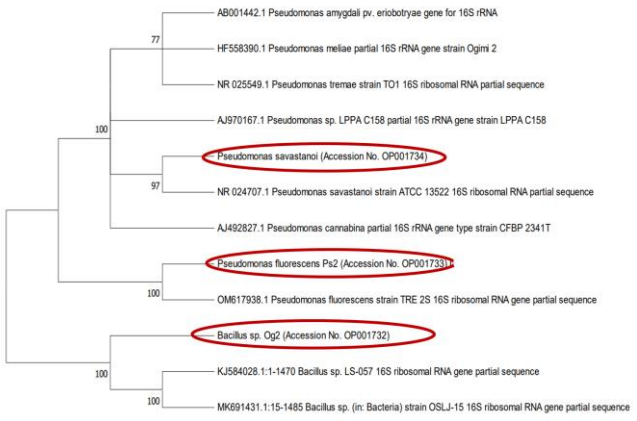

Fig 3: Phylogenetic tree constructed by the neighbour-joining method showing the phylogenetic relationships of all isolated bacteria compared with the reference sequences from gene bank

The evolutionary history was inferred using the UPGMA method (Sneath and Sokal, 1973). The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000) and are in the units of the number of base differences per site. This analysis involved 12 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1558 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

3.4. Antibacterial Activity of Secondary Metabolite

Two of the most active isolates from the agar plug method were selected and their secondary metabolites were tested against *P. savastanoi* using the well diffusion method (Table 3). Both isolates showed the highest inhibition on day 10 then comes day 8 and day 6 compared to other times of incubation which showed no inhibition.

Table (3) Zone of inhibition of endophytic bacteria secondary metabolite against *P. savastanoi* by well diffusion method

Time / Isolates	Inhibition zone (mm)					
	0 day	2 days	4 days	6 days	8 days	10 days
Oq5	0	0	0	10	12	25
Og2	0	0	0	5	13	26

4. Discussion

Pseudomonas savastanoi from olive samples were isolated, characterized, and identified. The pathogenicity tests indicated that *P. savastanoi* was highly pathogenic on olive leaves. The pathogen is reported to cause olive knot disease on different host plants including (olive, oleander, ash, broom, and Privet) (Gardan et al., 1992; Moreno-Pérez et al., 2020). Iacobellis et al. (1994) previously described *P. savastanoi* as responsible for the production of knots that depend on bacterial production of phytohormones including indole acetic acid (IAA) and cytokinin's.

Forty-six endophytic bacteria isolates have been isolated and tested against *P. savastanoi*, five isolates among them showed growth inhibition of *P. savastanoi*. Two of the most effective bacteria isolates which have a positive effect on decreasing the growth of *P. savastanoi* were molecularly identified as Bacillus sp. and Pseudomonas fluorescens. There are several studies on the antibacterial activity of *Pseudomonas sp*. isolates reported their effects on controlling plant pathogenic fungi and bacteria. Elsayed et al. (2020) mentioned in their result that *Pseudomonas spp*. is effective against a wide range of plant pathogens with

different levels of inhibition zone against various soil-borne fungal and bacterial pathogens such as *Ralstonia solanacearum*, *Rhizoctonia solani* and *Fusarium oxysporum*. Mondal et al. (2000) reported that P. fluorescens secreted minor phenolic compounds which I and IV fluorescent completely inhibited the growth of causative agent of bacterial blight disease.

P. fluorescens control crown gall disease more than 95% in peaches also, 0–60% disease control of apricot (Zhang et al., 1991). P. fluorescens significantly reduced fire blight disease which occurred in apples and pears (Stockwell et al., 2010). Khavazi et al. (2008) also mentioned in their investigation that fluorescence pseudomonas from wheat in the Iran region acts as biocontrol which showed the heights inhibition zone by causing failure in pathogen growth among others.

Several investigations and critical reviews have demonstrated the significant impact that Bacillus species have on plant pathogens (Dimkić et al., 2017). Amin et al. (2012) in their study indicated that Bacillus species are known for the synthesis of secondary metabolites and antimicrobial effects against bacterial pathogens. Kumar et al. (2012) also mentioned that the Stilben secondary metabolite of Bacillus sp. has the antimicrobial activity to phytopathogenic bacteria and fungi. In addition, surfactin from Bacillus sp. had a protective effect on Arabidopsis roots from the phytopathogenic bacterium P. syringae through disruption of its cellular membrane (Bais et al., 2004). Also, Mora et al. (2015) from the in vitro experiment mentioned the use of Bacillus sp. against eight different bacterial pathogens such as A. tumefacien,s E. amylovora and P. syringae, X. arboricola, P. carotovorum, R. solanacearum X. axonopodis, R. radiobacter and C. michiganensis. Some genus of Bacillus possesses biocontrol properties because of their adaptive metabolism and ability to produce a range of antimicrobial compounds which can protect plants from pathogens (Pérez-García et al., 2011). The use of bacterial agents like Pseudomonas and Bacillus are excellent options to work against plant pathogens and both bacteria have important traits such as improving plant growth (Wahyudi and Astuti, 2011).

5. Conclusion:

Two isolates Pseudomonas fluorescens and Bacillus sp. bacteria from olive trees showed the highest effect on inhibition of P. savastanoi. Also, their secondary metabolites in different periods of time were tested against P. savastanoi using the well diffusion method, both isolates showed the highest inhibition on day 10 compared to other times of incubation. The current study suggested that the production of bioactive compounds for future biopesticides could improve with the exploration and discovery of new biocontrol.

Conflict of Interest

None of the authors of this paper has personal relationships or finances with other people or organizations that could inappropriately influence or bias the content of the paper.

References

Alabouvette, C., C. Olivain and C. Steinberg. 2006. Biological control of plant diseases: the European situation. Eur. J. Plant Pathol, 114(3): 329-341.

Ali, N., Chapuis, E., Tavoillot, J., and Mateille, T. 2014. Plant-parasitic nematodes associated with olive tree (Olea europaea L.) with a focus on the Mediterranean Basin: A review. Comptes Rendus Biol. 337(7-8), 423-442.

Amin, A., M. A. Khan, M. Ehsanullah, U. Haroon, S. M. F. Azam and A. Hameed. 2012. Production of peptide antibiotics by Bacillus sp: GU 057 indigenously isolated from saline soil. Braz. J. Microbiol. 43: 1340-1346.

Anjum, N. and R. Chandra. 2015. Endophytic bacteria: optimizaton of isolation procedure from various medicinal plants and their preliminary characterization. Asian J. Pharm. Clin. Res, 8(4): 233-238.

Awla, H. K., J. Kadir, R. Othman, T. S. Rashid, S. Hamid and M.-Y. Wong. 2017. Plant growth-promoting abilities and biocontrol efficacy of Streptomyces sp. UPMRS4 against Pyricularia oryzae. Biol. Control. 112: 55-63.

Baldoni, L. & A. Belaj. 2009. Olive. In Oil crops, 397-421: Springer.

Balouiri, M., M. Sadiki and S. K. Ibnsouda. 2016. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal, 6(2): 71-79.

Basim, H., E. Basim and A. Ersoy. 2019. Phenotypic and genotypic characterization of Pseudomonas savastanoi pv. savastanoi causing olive knot disease in Turkey. Appl. Ecol. Environ, 17(6): 14927-14944.

Dimkić, I., S. Stanković, M. Nišavić, M. Petković, P. Ristivojević, D. Fira and T. Berić. 2017. The profile and antimicrobial activity of Bacillus lipopeptide extracts of five potential biocontrol strains. Front. Microbiol. 8: 925.

Eid, A. M., S. S. Salim, S. E.-D. Hassan, M. A. Ismail and A. Fouda. 2019. Role of endophytes in plant health and abiotic stress management. Microbiome in Plant Health and Disease. 119-144: Springer.

Elsayed, T. R., S. Jacquiod, E. H. Nour, S. J. Sørensen and K. Smalla. 2020. Biocontrol of bacterial wilt disease through complex interaction between tomato plant, antagonists, the indigenous rhizosphere microbiota, and Ralstonia solanacearum. Front. Microbiol. 10: 2835.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39(4): 783-791.

Gardan, L., C. Bollet, M. A. Ghorrah, F. Grimont and P. Grimont. 1992. DNA Relatedness among the Pathovar Strains of

Pseudomonas syringae subsp. savastanoi Janse 1982 and Proposal of Pseudomonas savastanoi sp. nov. Int. J. Syst. Evol. Microbiol, 42(4): 606-612.

Gray, E. and D. Smith. 2005. Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. Soil Biol. Biochem. 37(3): 395-412.

Hall, B., E. Cother, M. Whattam, D. Noble, J. Luck and D. Cartwright. 2004. First report of olive knot caused by Pseudomonas savastanoi pv. savastanoi on olives (Olea europaea) in Australia. Australas. Plant Pathol. 33(3): 433-436.

Hall, T., I. Biosciences and C. Carlsbad. 2011. BioEdit: an important software for molecular biology. GERF Bull Biosci. 2(1): 60-61.

Iacobellis, N., A. Sisto, G. Surico, A. Evidente and E. DiMaio. 1994. Pathogenicity of Pseudomonas syringae subsp. savastanoi mutants defective in phytohormone production. J Phytopathol. 140(3): 238-248.

Imathiu, S. M., R. V. Ray, M. Back, M. C. Hare and S. G. Edwards. 2009. Fusarium langsethiae pathogenicity and aggressiveness towards oats and wheat in wounded and unwounded in vitro detached leaf assays. Eur. J. Plant Pathol. 124(1): 117-126.

Johnsen, K. and P. Nielsen. 1999. Diversity of Pseudomonas strains isolated with King's B and Gould's S1 agar determined by repetitive extragenic palindromic-polymerase chain reaction, 16S rDNA sequencing and Fourier transform infrared spectroscopy characterisation. FEMS microbiol. Let. 173(1): 155-162.

Kannan, R., T. Damodaran and S. Umamaheswari. 2015. Sodicity tolerant polyembryonic mango root stock plants: a putative role of endophytic bacteria. Afr. J. Biotechnol. 14(4): 350-359.

Khavazi, K., A. Asgharzadeh, M. Hosseini-Mazinani and R. De Mot. 2008. Biocontrol of Pseudomonas savastanoi, causative agent of olive knot disease: antagonistic potential of non-pathogenic rhizosphere isolates of fluorescent Pseudomonas. Commun. Agric. Appl. Biol. Sci. 73(1): 199-203.

Khlaif, H. 2006. Olive knot disease in Jordan. Jordan Journal of Agricultural Sciences.

Kumar, S., G. Stecher and K. Tamura. 2021. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution https://doi.org/10.1093/molbev/msab120.

Kumar, S., Stecher, G. & Tamura, K. 2012. Bioactive stilbenes from a Bacillus sp. N strain associated with a novel rhabditid entomopathogenic nematode. Lett. Appl. Microbiol. 33(7): 1870-1874.

Liarzi, O., P. Bucki, S. Braun Miyara and D. Ezra. 2016. Bioactive volatiles from an endophytic Daldinia cf. concentrica isolate affect the viability of the plant parasitic nematode Meloidogyne javanica. PloS one, 11(12): e0168437.

Marchi, G., C. Viti, L. Giovannetti and G. Surico. 2005. Spread of levan-positive populations of Pseudomonas savastanoi pv. savastanoi, the causal agent of olive knot, in central Italy. Eur. J. Plant Pathol. 112(2): 101-112.

Mohamad, O. A., L. Li, J.-B. Ma, S. Hatab, L. Xu, J.-W. Guo, B. A. Rasulov, Y.-H. Liu, B. P. Hedlund and W.-J. Li. 2018. Evaluation of the antimicrobial activity of endophytic bacterial populations from Chinese traditional medicinal plant licorice and characterization of the bioactive secondary metabolites produced by Bacillus atrophaeus against Verticillium dahliae. Front. Microbiol. 9: 924.

Mondal, K., R. Singh, P. Dureja and J. Verma. 2000. Secondary metabolites of cotton rhizobacteria in the suppression of bacterial blight of cotton. Indian Phytopathol. 53(1): 22-27.

Mora, I., J. Cabrefiga and E. Montesinos. 2015. Cyclic lipopeptide biosynthetic genes and products, and inhibitory activity of plant-associated Bacillus against phytopathogenic bacteria. PLoS One, 10(5): e0127738.

Moreno-Pérez, A., A. Pintado, J. Murillo, E. Caballo-Ponce, S. Tegli, C. Moretti, P. Rodríguez-Palenzuela and C. Ramos. 2020. Host range determinants of Pseudomonas savastanoi pathovars of woody hosts revealed by comparative genomics and cross-pathogenicity tests. Front. Plant Sci. 11: 973.

Mouloud, G., H. Daoud, J. Bassem, I. Laribi Atef and B. Hani. 2013. New bacteriocin from Bacillus clausii strainGM17: purification, characterization, and biological activity. Appl. Biochem. Biotechnol. 171(8): 2186-2200.

Nei, M. and S. Kumar. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Panagopoulos, C.1993. Olive knot disease in Greece 1. EPPO Bulletin 23(3): 417-422.

Pérez-García, A., D. Romero and A. De Vicente. 2011. Plant protection and growth stimulation by microorganisms: biotechnological applications of Bacilli in agriculture. Curr. Opin. Biotechnol. 22(2): 187-193.

Pérez-Martínez, I., L. Rodriguez-Moreno, I. M. Matas and C. Ramos. 2007. Strain selection and improvement of gene transfer for genetic manipulation of Pseudomonas savastanoi isolated from olive knots. Res. Microbiol. 158(1): 60-69.

Pyrowolakis, E. and H. C. Weltzien. 1974. Studies on the distribution of Olive knot, induced by Pseudomonas savastanoi (Sm.) Stev. in the Greek island of Crete/Studi sulla distribuzione della rogna dell'Olivo da Pseudomonas savastanoi (Sm.) Stev. nell'isola greca di Creta. Phytopathol. Mediterr. 118-120.

Quesada, J. M., R. Penyalver and M. M. López. 2012. Epidemiology and control of plant diseases caused by phytopathogenic bacteria: the case of olive knot disease caused by Pseudomonas savastanoi pv. savastanoi. Plant Pathology, 299-326.

Rashid, T. S. 2021. Bioactive metabolites from tomato endophytic fungi with antibacterial activity against tomato

bacterial spot disease. Rhizosphere, 17: 100292.

Sesma, A. and A. E. Osbourn. 2004. The rice leaf blast pathogen undergoes developmental processes typical of root-infecting fungi. Nature, 431(7008): 582-586.

Sneath, P., H. A, and R. Sokal. 1973. Numerical Taxonomy. Freeman, San Francisco.

Stockwell, V., K. Johnson, D. Sugar and J. Loper. 2010. Control of fire blight by Pseudomonas fluorescens A506 and Pantoea vagans C9-1 applied as single strains and mixed inocula. Phytopathol. 100(12): 1330-1339.

Valverde, P., M. Zucchini, S. Polverigiani, E. Lodolini, F. J. López-Escudero and D. Neri 2020. Olive knot damages in ten olive cultivars after late-winter frost in central Italy. Sci. Hortic. 266: 109274.

Young, J. 2004 . Olive knot and its pathogens. Australas. Plant Path. 33(1): 33-39.

Zhang, C., S. Lu, C. Wei and J. Zhao. 1991. Bacterium strain 1-1-4 Pseudomonas fluorescens for biocontrol of peach crown gall disease. Acta Agric. Shanghai. 7: 65-69.