Pistachio (Pistacia vera L.) canker caused by Pantoea agglomerans

Milad Aeini (miladaeini@gmail.com)
Shahid Chamran University of Ahvaz
Mohamad Hamed Ghodoum Parizipour
Agricultural Sciences and Natural Resources University of Khuzestan
Mitra Omidi Nasab
Bu Ali Sina University
Maede Heidari
Shahid Chamran University of Ahvaz
Amal Fazliarab
Shahid Chamran University of Ahvaz

Research Article

Keywords: Bacterial disease, Inoculation, Molecular identification, Pistachio, Pantoea

Posted Date: May 26th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2955491/v1

Version of Record: A version of this preprint was published at European Journal of Plant Pathology on August 21st, 2023. See the published version at https://doi.org/10.1007/s10658-023-02751-7.

Abstract

Pistachio (*Pistacia vera* L.) is a perennial woody plant that produces edible seeds with a high nutritional value. Canker disease is one of the important diseases of pistachio, causing considerable loss globally. During 2019–2021, a series of symptoms, including tree weakness, brown to black lesions, canker, and exudation of black sap were observed on the trunk of pistachio trees from different regions of Lorestan province, western Iran. Bacterial isolation resulted in the development of uniform and yellow colonies on yeast-extract peptone glucose agar (YPGA) medium 24 hours post-inoculation (hpi). The colonies were then purified on YPGA and yeast-extract-dextrose-calcium-carbonate agar (YDC). Based on the analysis of morphological, biochemical, and molecular characteristics using nucleotide sequences of the gyrB, infB, atpD, and 16S rRNA, a putative pathogen was identified as *Pantoea agglomerans* (formerly *Enterobacter agglomerans*). To fulfill Koch's postulates, bacterial suspensions (10^7 CFU/ml) were inoculated to one-year-old pistachio seedlings. Thirty days after inoculation, small necrotic lesions developed on the bark in the vicinity of inoculation sites, followed by brown necrotic streaks in the inner bark. Subsequently, the bacterial strains exhibiting the characteristic colony morphology were retrieved from lesions on inoculated pistachio seedlings and verified as *P. agglomerans*. To the best of our knowledge, this is the first report on the occurrence of pistachio canker caused by *P. agglomerans*.

Introduction

Pistachio (*Pistacia vera*) is a flowering shrub species belonging to the family Anacardiaceae and originated from Central Asia and the Middle East (Ferguson et al., 2005). It is one of the most economically important nut crops globally and has been widely cultivated in the United States, Turkey, China, Syria, and Iran (Arab et al., 2021). Pistachio has been cultivated for a long time in various parts of Iran, where wild pistachio forests (*P. atlantica* and *P. khinjuk*) may be found in the northeastern region, particularly on the border between Afghanistan and Turkmenistan (Mirzaei et al., 2006). It is believed that *P. vera* has been planted in Iran for approximately 4,000 years (Sheibani, 1995). There are around 350,000 hectares of pistachio-growing areas, mainly in the central parts of the country (Esmail-Pour, 2001). In 2020, approximately 190,000 tons of pistachio nuts were produced in Iran, making it the third pistachio-producing country in the world (https://www.fao.org).

Pistachio production, however, has been adversely affected by numerous pests and diseases. The canker complex is one of the major causes of significant crop loss to pistachio orchards. To date, a large number of pathogenic fungi have been characterized as the cause of canker disease on pistachio trees globally (Armengol et al., 2008; Chen et al., 2013; Vitale et al., 2018; Aiello et al., 2019; Nouri et al., 2019; Torabi et al., 2019; Gusella et al., 2022; Ozan et al., 2022). Moreover, there are few reports on the bacterial cause of pistachio canker (dieback) in Australia (Edwards and Taylor 1997; Facelli et al., 2002; Facelli et al., 2005). Initially, a bacterial species from the genus *Xanthomonas* was characterized as the pathogen of the disease (Edwards and Taylor 1997). Further investigations showed that the causal agent is a member of *X. translucens*, which had been mostly found as a pathogenic bacterium of gramineous plants (Facelli et al., 2002). The infected pistachio trees showed typical symptoms of bacterial disease, including shoot dieback, development of lesions on trunk and limb, resin secretion, and shoot/overall death (Facelli et al., 2002). The bacterium was later identified as *X. translucens* pv. *pistachiae* (Giblot-Ducray et al., 2009). In Iran, several fungi were associated with trunk and branch canker in pistachio orchards (Alaee, 1997; Sohrabi et al., 2020). However, no bacterial agent has been isolated from pistachio trees resembling the canker disease.

Pantoea agglomerans (Ewing and Fife 1972) Gavini et al. 1989, formerly *Enterobacter agglomerans* Ewing and Fife 1972, is a gram-negative aerobic bacterium that is taxonomically classified into the family *Erwiniaceae* (Adeolu et al., 2006). It has been reported from various sources such as soil, animal, human, and plant (Andersson, 1999; Monier and Lindow, 2005; Feng et al., 2006; Cruz et al., 2007; Loncaric et al., 2009). *P. agglomerans* is a common epiphytic and endophytic bacterium isolated from several plant species globally (Kobayashi and Palumbo, 2000; Lindow and Brandl, 2003; Mackiewicz et al., 2016). To date, only two phytopathogenic pathovars of *P. agglomerans* have been characterized (Barash and Manulis-Sasson, 2007), which include *P. agglomerans* pv. *gypsophila* inducing hyperplasia on gypsophila (*Gypsophila paniculata* L.) and hypersensitive reaction (HR) on beet (*Beta vulgaris* L.), and *P. agglomerans* pv. *betae* causing hyperplasia on both beet and gypsophila (Beer 1991; Cooksey 1986). Additionally, the species has been found to cause symptoms in several cultivated plants (Lee et al., 2010; Yang et al., 2011; Tho et al., 2015; Fung et al., 2019; Gutiérrez-Barranquero et al., 2019; She et al., 2019; Chaohui et al., 2020). In Iran, however, *P. agglomerans* was found to be associated with citrus (*Citrus* × *paradisi* Macfad.) bacterial canker (Sherafati et al., 2014) and leaf spot on *Dieffenbachia amoena* and *Aglaonema nitidum* (Yazdani et al., 2018). Moreover, some non-pathogenic strains of *P. agglomerans* have been isolated from different plants in Iran (Vasebi et al., 2015; Rostami et al., 2018; Zarei et al., 2019). According to our knowledge, there is no report on the association of *P. agglomerans* with pistachio canker.

During 2019–2021, canker symptoms, including tree weakness, brown to black lesions on trunk and limb, and exudation of black sap, were observed on the pistachio tree in Lorestan province, western Iran. This study aims to 1) identify the causal agent of pistachio canker by bacterial isolation and pathogenicity tests on pistachio seedlings, 2) characterize the bacterial strains based on phenotypic tests, and 3) perform phylogenetic analysis based on multilocus sequence (MLSA) of the housekeeping genes.

Materials and methods

Sample collection

A total of 20 newly established pistachio (*P. vera* cv. Zarnad) planting orchards (15-30 years old) located in Lorestan province, western Iran, were surveyed during 2019-2021. Plants showing typical symptoms of bacterial canker, including gum oozing from closed buds (exudation of black sap), and bark

discoloration of branches were sampled. For this purpose, 20 symptomatic trees were selected, and a single panel containing the outer bark, xylem, and phloem tissues was isolated with sterile tools and immediately transferred to the laboratory.

Pathogen isolation

To isolate bacteria, samples were thoroughly washed with tap water and superficially disinfected by dipping into 10% (vol/vol) NaCLO for 60 s and 70% (vol/vol) C_2H_5OH for 30 s. Finally, they were rinsed thrice with sterilized distilled water (SDW) for a maximum of 3 min. Diseased tissue samples, 10 × 20 mm, from the interface between the diseased and healthy tissue, were cut with a sterilized scalpel. Tissues were macerated in SDW and incubated at 28 °C for 40 min. A loopful of suspension was spread onto YPGA (yeast-extract peptone glucose agar) plates (Merck, Germany) (Schaad et al. 2001). The Petri dishes were kept at 28 °C for 48 h. Yellow-pigmented isolates were obtained on YPGA. Single colony-purified cultures were then preserved at −80 °C in Microbank vials (RaziGlass, Iran) containing 20% (vol/vol) glycerol for further phenotypic and molecular characterizations. For the cultivation of fungi, wood fragments were plated on potato dextrose agar (PDA; HKM, Guangdong, China) plates containing streptomycin sulfate (0.5 g/L) (Al-Havi Pharmaceutical Co, Iran). Petri dishes were incubated under controlled conditions at 25°C for seven days in the dark.

Biochemical and phenotypic characteristics

Bacterial isolates (designated MO01-MO018) were subjected to the standard phenotypic and biochemical assays, including Gram reaction (*3% KOH)*, oxidase activity, growth at 37 °C, hypersensitivity response (HR) on tobacco (*Nicotiana tabacum* cv. Samsun), H_2S production from cysteine, gelatin liquefaction, nitrate reduction, arginine dihydrolase, yellow pigment on yeast extract-dextrose-calcium-carbonate agar (YDC), motility, production of 2,5 di-keto-D-gluconate, indole test, utilization of citrate, urease, gas produced from D-glucose, beta-xylosidase, beta-galactosidase, Lysine decarboxylase and carbon sources utilization (Borkar, 2017; Schaad et al., 2001). Inoculated media were incubated at 28 °C for 3–5 days. The tests were repeated three times. In all tests, the *P. agglomerans* type strain ATCC 27155 was used as a control.

Pathogenicity assay

Pathogenicity tests were conducted using 20 certified pathogen-free two-year-old pistachio seedlings (*P. vera* cv. Zarand). The seedlings were planted in 40-cm pots (Hirad, Iran) containing loamy soil. The seedlings were maintained in a greenhouse at ambient conditions (27 ± 2 °C, 11:13 h (light: darkness) photoperiod, and 60 ± 5% relative humidity). Each representative strain was purified on nutrient broth (Merck, Germany) in 200-ml volumetric flasks and incubated in a shaking incubator at 150 rpm for 20 h. A concentration of 10^7 CFU/ml (Optical density at 600 nm wavelength [$OD_{600\ nm}$] = 0.02) was prepared for each bacterial suspension using sterile 10-mM phosphate buffer. Seedlings were inoculated by causing a superficial wound on the stem using an ethanol-sterilized scalpel, and 100 µl of inoculum was injected into the created wounds using a sterilized syringe. The inoculation wounds were fully covered by parafilm. Negative controls were obtained by inoculation of seedlings with SDW. The assayed strains were separately injected into the pistachio seedlings. The inoculated seedlings were maintained in the greenhouse for 30 days and monitored daily for any symptom development. Re-isolation was conducted on YPGA medium from seedlings exhibiting the disease symptoms. The confirmation of the re-isolated bacteria was made according to phenotypic and biochemical characteristics, and the amplification of the *atpD* gene. The pathogenicity assay was repeated two times. In addition, all obtained bacterial isolates (60) were evaluated for HR on tobacco (*Nicotiana tabacum* cv. *Samsun*) (Klement et al. 1990). A concentration of 1×10^8 (CFU/ml) in SDW was prepared using a spectrophotometer (D30, Eppendorf, Germany) ($OD_{600\ nm}$ = 0.1) and infiltrated (30 µl) into the tobacco leaves. A total of 80 tobacco leaves and plants were preserved under greenhouse conditions (50 % humidity and 20-24 °C) for 24–72 h. The HR assay was carried out three times, and SDW was used as a negative control.

DNA extraction and PCR analysis

Bacterial strains of MO05 and MO08 (as representative isolates) obtained from the cankers on pistachio trees were subjected to genotypic analysis. Their selection was according to the results of phenotypic and pathogenicity tests performed in this study. DNA was extracted using the boiling method, and bacterial cells were recovered from colonies grown on YPGA at 27 °C for 24 h, suspended in sterile vials containing 900 µl SDW (10^8 CFU/ml). One µl of 10 % KOH was added to the bacterial cell and heated to 100 °C for 10 min. Centrifugation was performed at 10,000 *g* for 10 min, and subsequently, the resultant supernatants were carefully transferred into a new sterilized microtube and utilized directly for Polymerase Chain Reaction (PCR). The quality and quantity of extracted DNA were checked by spectrophotometry (D30, Eppendorf, Germany) followed by electrophoresis on 1.5% agarose gels at 75 V for 45 min (Ausubel et al., 1992).

Partial sequence of small ribosome subunit (16S rRNA region) and three housekeeping genes (*gyrB*, *atpD*, and *infB*) were amplified using the specific primer pairs as shown in table 1 (Weisburg et al. 1991, Yamamoto and Harayama, 1995, Brady et al. 2008). PCR amplification was done in a total volume of 25 µl. The final PCR reaction mixture included 1X PCR buffer, 2.0 mM $MgCl_2$, 200 µM dNTPs (Thermo Fisher Scientific, USA), 0.2 µM primer (Inqaba Biotech, South Africa), 1.5 U of Supertherm *Taq* polymerase (JMR Holdings, London), and 10-50 ng of DNA. The mixture was subjected to an initial denaturation (10 min at 94 °C), 30 cycles of denaturation (60 s at 94 °C), annealing (60 s at 49-65 °C), extension (60 s at 72 °C), and a final extension (5 min at 72 °C). Separation of PCR products was performed on 1% agarose gels at 75 V for 45 min. The purified PCR products were dispatched to NIAGEN NOOR (Tehran, Iran) for sequencing via Sanger sequencing technology.

The resulting sequences were first subjected to base calling using BioEdit (v. 7.0.5.2) software. Their nucleotide identity was then compared to those previously deposited on GenBank using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences of 16S rRNA, *gyrB*, *atpD*, and *infB* genes from the phylogenetically related strains were retrieved from GenBank. All sequences were aligned using the multiple alignment program Clustal W (Thompson et

al., 1994). The best-fit model of nucleotide evolution for each gene was identified using MrModeltest v. 2.4 (Nylander, 2004) according to the Akaike information criterion (AIC). The Maximum Likelihood (ML) tree was constructed based on the concatenated sequences of *gyrB*, *atpD*, and *infB* genes in CLC Main Workbench software (ver. 7.6.2). The support level of each maximum likelihood was assessed by bootstrap analysis repeated 1,000 times. The representative strains were stored in the plant bacteriology laboratory at the Shahid Chamran University of Ahvaz.

Results

Sampling and isolation

During 2019-2021, a series of symptoms, including tree weakness, brown to black lesions, canker, and exudation of black sap, were observed on the trunks of pistachio trees in Lorestan province, western Iran (Figure 1). Yellow-colored bacterial colonies mostly were isolated from canker-exhibiting pistachio trees. A total number of 20 samples of symptomatic pistachio tissues were collected, from which 80 yellow-colored bacterial isolates were recovered from Lorestan province, western Iran. No fungal isolate was recovered in this study. The bacterial isolates were subjected to further biological and molecular characterization.

Phenotypic characteristics

Bacterial colonies on YPGA were yellow-pigmented, creamy, luminous, and somehow bulged with entire margins (Figure 2a,b). The biochemical assays revealed that the gram-negative bacteria were negative for oxidase, arginine dihydrolase, taupe pigment on YDC, production of 2,5 di-keto-D-gluconate, urease, indole, gas from D-glucose, beta-xylosidase, Lysine decarboxylase and were positive for growth at 37 °C, H_2S from cysteine, gelatin liquefaction, nitrate reduction, motility, utilization of citrate and beta-galactosidase. They produced acid from arabinose, ribose, xylose, fructose, mannose, maltose, sucrose, mannitol, galactose, and tetrahalose but not from glycerol, rhamnose, meso-Inositol, sorbitol, melibiose, and sucrose (Table 2). The bacterial isolates from pistachio exhibited biochemical and phenotypic characterizations of *P. agglomerans* ATCC 27155.

Pathogenicity test

The disease symptoms were reproduced on inoculated seedlings 30 days post-inoculation (dpi). The tested strains were able to cause symptoms of bacterial cankers, such as brown discoloration of the internal tissue and gummosis (Figure 2c, d). Re-isolation of bacteria was performed from the diseased tissues. Based on morphological, biochemical, and molecular (PCR amplification with PantatpDF and PantatpDR primers) data, they were re-identified as *P. agglomerans* (Table 1). Also, no symptom was found on seedlings treated with SDW (control). The HR was developed on tobacco leaves 24-72 h post infiltration of bacterial suspensions carrying 1×10^7 CFU/ml. The SDW-inoculated plants (negative control) did not develop any visible symptoms.

Genotypic assay

16S rRNA sequence analysis

Partial sequences of the 16S rRNA gene region of approximately 1,280 bp were obtained for representative isolates of MO05 and MO08. The nucleotide sequences of the bacterial isolates demonstrated the highest identity (98.83%-99.2%) to that of *P. agglomerans* strain AMJ216. For 16S rRNA gene Maximum Likelihood (ML) trees, T92 + G + I (Tamura 3-parameter + Gamma distribution with invariantsite) (Tamura, 1992) was selected as the best substitution model. The 16S rRNA sequences of pistachio strains from Iran (Table 3) and the reference strains of *P. agglomerans* were grouped into one cluster based on ML method (Figure 3). An endophytic plant strain (NHZ-4 [MT184816]) from India together with an endophytic insect strain (AMJ216 [KY027148]) from Saudi Arabian were closely grouped with the highest bootstrap value (100 %) in the clade. Also, an airborne strain of *P. agglomerans* (6R-J-7b [EU379306]) from the USA was placed within the clade (Figure 3).

Multilocus sequence analyses of *gyrB*, *infB*, and *atpD*

Partial gene sequences of *gyrB*, *rpoB*, and *atpD* were performed for isolates MO05 and MO08, and they were deposited in GenBank under the accession numbers presented in Table 3. All sequences showed 99 %–100 % nucleotide identity to other *P. agglomerans* sequences previously deposited in GenBank. The best-fit model of nucleotide evolution for ML analysis, based on AIC, was GTR + G + I (General Time Reversible + Gamma distribution with invariant site) (Nei & Kumar, 2000) for *gyrB*, *infB*, and *atpD* genes. The pistachio isolates and three reference strains of *P. agglomerans*, including a Polish (L15 [CP034148]) and two American strains (ASB05 [CP046722], TH81 [CP031649]), formed a separate cluster with a relatively high bootstrap value (81 %) in ML tree based on concatenated nucleotide sequences of the three housekeeping genes (Figure 4). Moreover, the MO05 and MO08 isolates were closely grouped with the same bootstrap value.

Discussion

In the present study, the etiology of pistachio canker disease in Lorestan province, western Iran, was investigated, leading to the identification of *P. agglomernas* as the causal agent. *P. agglomernas* is a ubiquitous bacterium associated with plants, animals, and humans (Dutkiewicz et al., 2016). This species is a frequent endophytic and epiphytic bacterium inciting various leaf, stem, or rot diseases in a range of cultivable plants, such as mango (Gutiérrez-Barranquero et al., 2019), onion (Tho et al., 2015), rice (Lee et al., 2010), walnut (Yang et al., 2011), Chinese plum (*Prunus salicina*, Chaohui et al., 2020), Chinese date (*Ziziphus jujuba*, She et al., 2019), *Citrus microcarpa* (Fung et al., 2019), *Citrus* × *paradisi* (Sherafati et al., 2014), gypsophila

(Cooksey 1986), dumb cane (*D. amoena*) and Chinese evergreen (*A. nitidum*) (Yazdani et al., 2018). However, there has been little information about the bacterial disease of pistachio. The only causal agent of bacterial canker (dieback) on pistachio trees characterized from Australia has been *X. translucens* pv. *pistachiae* (Giblot-Ducray et al., 2009). The pathogenicity of *P. agglomerans* has previously been reported as it can elicit HR in resistant and nonhost plants such as tobacco (Nizan et al., 1997) and beet (Beer, 1991). Similarly, the bacterial isolates obtained in this study were able to cause HR on tobacco leaves. Herein, two isolates of *P. agglomerans* (MO05 and MO08) were identified, which could induce canker disease in pistachio seedlings (Fig. 2c). The disease caused by the pathogenic strains of *P. agglomerans* adversely affects phloem tissue, interfering with the translocation of soluble organic compounds in pistachio trees)Zamorano et al., 2023). This might have significant economic implications for cultivated plants (Tronsmo et al., 2020).

Most canker disease on pistachio occurs in complexes as many organisms are involved in the disease (Nouri et al., 2019, Gusella et al., 2022). The fungal pathogens that pose a significant risk to pistachio production on a global scale are those belonging to the Botryosphaeriaceae (Botryosphaeriales, Ascomycota) (Gusella et al., 2022). Canker symptoms might be variable depending on the pathogen species, environmental condition, and the stage of disease development (Nouri et al., 2019, Gusella et al., 2022). In most cases, the bacterial infection causes bleeding cankers on their host plants. Plants may exudate gum at the site of infection as a defensive response against the pathogen invasion. However, bleeding canker has been generally observed during plant infection by *Phytophthora* (Cerny et al., 2009, Brown et al., 2021). *Phytophthora* infection starts from the crown developing into the upper parts of the plant (Saremi et al., 2008, Mirsoleimani et al., 2013). During bacterial disease, however, symptoms are not likely to develop on roots and crowns. In the case of fungal infection, however, fruiting bodies such as stromata, sporodochia, and acervuli are produced on dead or infected bark (Fan et al., 2018). Bacterial cankers usually seem sunken and soft, while in the fungal cankers, they may be observed as splits in the stem, blotchy outgrowth on the cells, or dead tissues within the bark (Agrios et al., 2005). Usually, the distribution of fungal cankers is more than that of bacterial cankers. One of the possible reasons is that fungi can produce spores that spread among the plants rapidly (Agrios et al., 2005). Raindrops, pathogen-infested appliances, and infected plant materials are the main sources by which bacteria are spread (West, 2014). Comparatively, these means of distribution may result in their relatively low spread among the plants.

Sequencing analysis of the 16S ribosomal RNA gene indicated that the Iranian canker-causing isolates belonged to the genus *Pantoea*. Moreover, the bacterial strains isolated from pistachio (MO05 and MO08) together with *P. agglomerans* strains (NHZ-4, AMJ216, and 6R-J-7b) formed a separate clade with the highest bootstrap value (100%) in the phylogenetic tree (Fig. 3) confirming the species identification. This is a simple and rapid method to identify bacterial strains within the genus level (Johnson et al., 2019); however, sequencing analysis of 16S rRNA reveals taxonomic discrepancies within the *Pantoea* genus. DNA sequences of housekeeping genes known as MLSA have been proposed to determine the phylogenetic relationships among *Pantoea* species (Rezzonico et al., 2009). Therefore, we used three housekeeping genes (*gyrB, infB*, and *atpD*) to classify and identify *Pantoea* isolates more precisely. This can discriminate *P. agglomerans* from other *Pantoea* spp.

Taken together, this study demonstrated that *P. agglomerans* is a new etiological agent that causes symptoms of pistachio bacterial canker. Our results suggest that this bacterial agent is a new pathogen and a latent risk to the pistachio cultivations in Iran. The findings of this study have important implications for disease surveillance and management practices. To the best of our knowledge, this is the first report of *P. agglomerans* causing canker disease on pistachio plants.

Declarations

Authors Contributions

MA conceived and designed the study. MO, MH, and AF carried out the experiments with assistance from MA and MHGPP. MA and MHGP performed phylogenetic analysis and MA wrote the manuscript.

Funding

The financial support of the Vice Chancellor for Research of Shahid Chamran University of Ahvaz (grant number: SCU.AP1400.33951) is hereby appreciated.

Ethical Approval

This research does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent

Informed consent was obtained from all individual participants included in this study.

Conflict of Interests

The authors declare no conflict of interest.

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Tables

Table 1. PCR primers used in this study.

Prime	5´- 3´ sequence	Ta (°C)	Amplicon (bp)	Target	Reference
fD1	AGAGTTTGATCCTGGCTCAG	63	1500	16S rRNA	Weisburg *et al*., 1991
rP2	ACGGCTACCTTGTTACGACTT				
PantatpDF	GATTGTCCAGATTATCGGCG	65	1380	*atp*D	This study
PantatpDR	TACAGTTTCTTCGCTTTTTC				
infB 01-F	ATYATGGGHCAYGTHGAYCA	49	1250	*inf*B	Brady *et al.,* 2008
infB 02-R	ACKGAGTARTAACGCAGATCCA				
UP-1	GAAGTCATCATGACCGTTCTGCA(TC)GC(TCAG)GG(TCAG)GG(TCA)AA(AG)TT(TC)GA	62	1200	*gyr*B	Yamamoto and Harayama, 1995
Up-2r	AGCAGGGTACGGATGTGCGAGCC(AG)TC(TCAG)AC(AG)TC(TCAG)GC(AG)TC(TCAG)GTCAT				

Table 2. Biochemical, physiological, and phenotypic features of *Pantoea agglomerans* isolates obtained from canker disease of pistachio (*Pistacia vera*) in Iran.

Test	Pistachio isolates	Reference strain (ATCC 27155)
Gram reaction	-	-
Oxidase	-	-
Growth at 37 °C	+	+
Tobacco hypersensitivity	+	+
H_2S from cysteine	+	+
Gelatin liquefaction	+	+
Nitrate reduction	+	+
Arginine dihydrolase	-	-
Yellow pigment on YPGA	+	+
Taupe pigment on YDC	-	-
Motility	+	+
Production of 2,5 di-keto-D-g1uconate	-	-
Indole production	-	-
Utilization of citrate	+	+
Urease	-	-
Gas produced from D-glucose	-	-
-xylosidase	-	-
-galactosidase	+	+
Lysine decarboxylase	-	-
Utilization of:		
Citrate	+	+
Malonate	+	+
Tartrate	-	-
Acid production from		
Arabinose	+	+
Ribose	+	+
Xylose	+	+
Fructose	+	+
Mannose	+	+
Glycerol	-	-
Maltose	+	+
Rhamnose	-	+
Sucrose	+	+
meso-Inositol	-	-
Mannitol	+	+
Sorbitol	-	-
Galactose	+	+
Melibiose	-	-
Sucrose	-	-
Tetrahalose	+	+

Table 3. Details of nucleotide sequences obtained from *Pantoea agglomerans* isolates in this study.

Target sequence	Nucleotide length (bp)	Isolate	GenBank accession number
16S rRNA	~1280	MO05	OP185884
		MO08	OP216723
atpD	1380	MO05	OP381059
		MO08	OP381060
gyrB	1200	MO05	OP207877
		MO08	OP207878
infB	1250	MO05	OP381057
		MO08	OP381058

Figures

Figure 1

Symptoms of canker disease observed on *Pistacia vera* cv. Zarand trees in Lorestan province, western Iran.

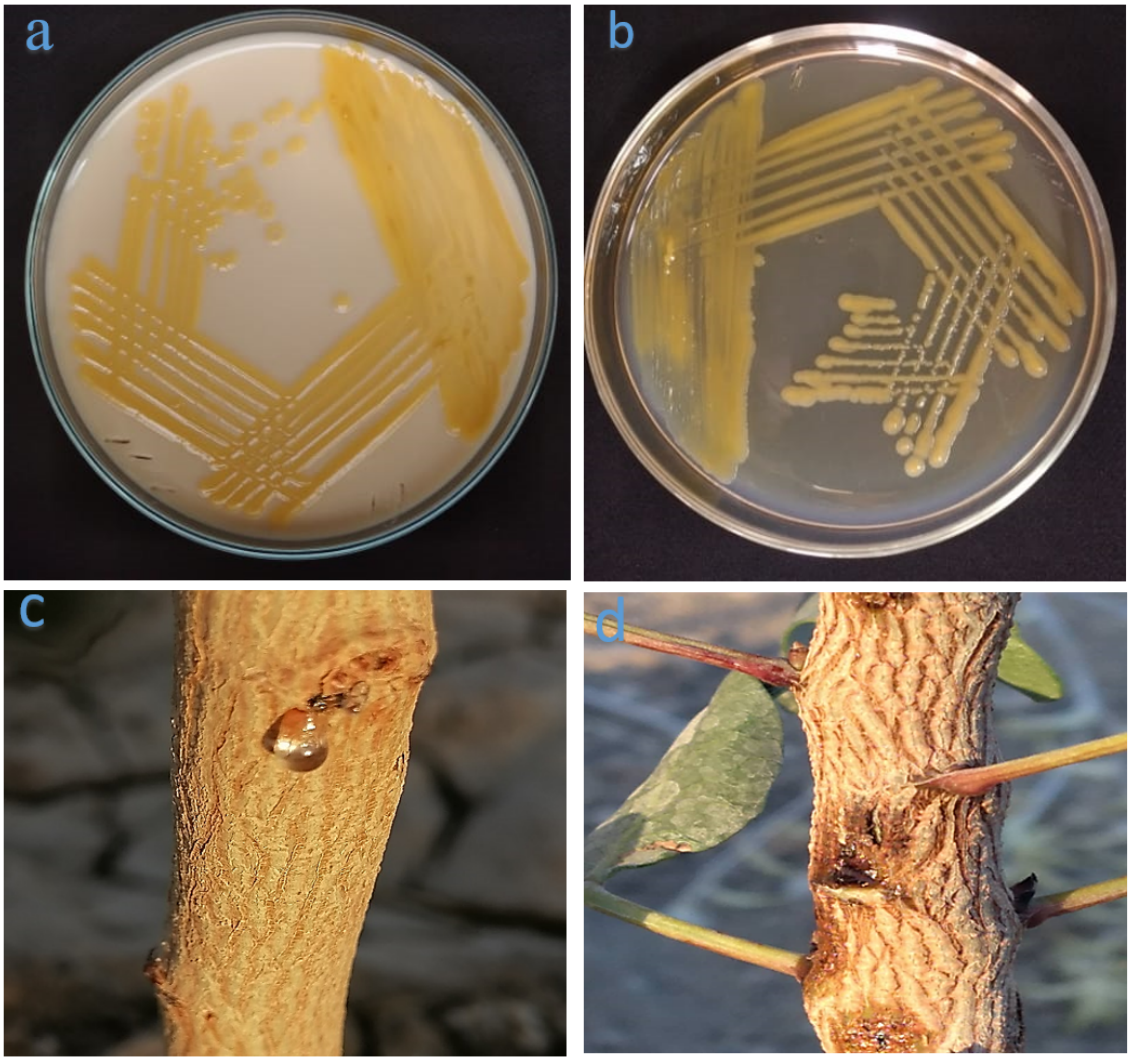

Figure 2

a: *P. agglomerans* yellow colonies on YDC medium 48 h post-incubation, b: Colony morphology of *P. agglomerans* strain MO05 isolated from the bacterial canker of pistachio on YPGA medium 48 h post-incubation, c and d: canker symptom developed on pistachio (*P. vera*cv. Zarand) seedlings inoculated with the bacterial stains.

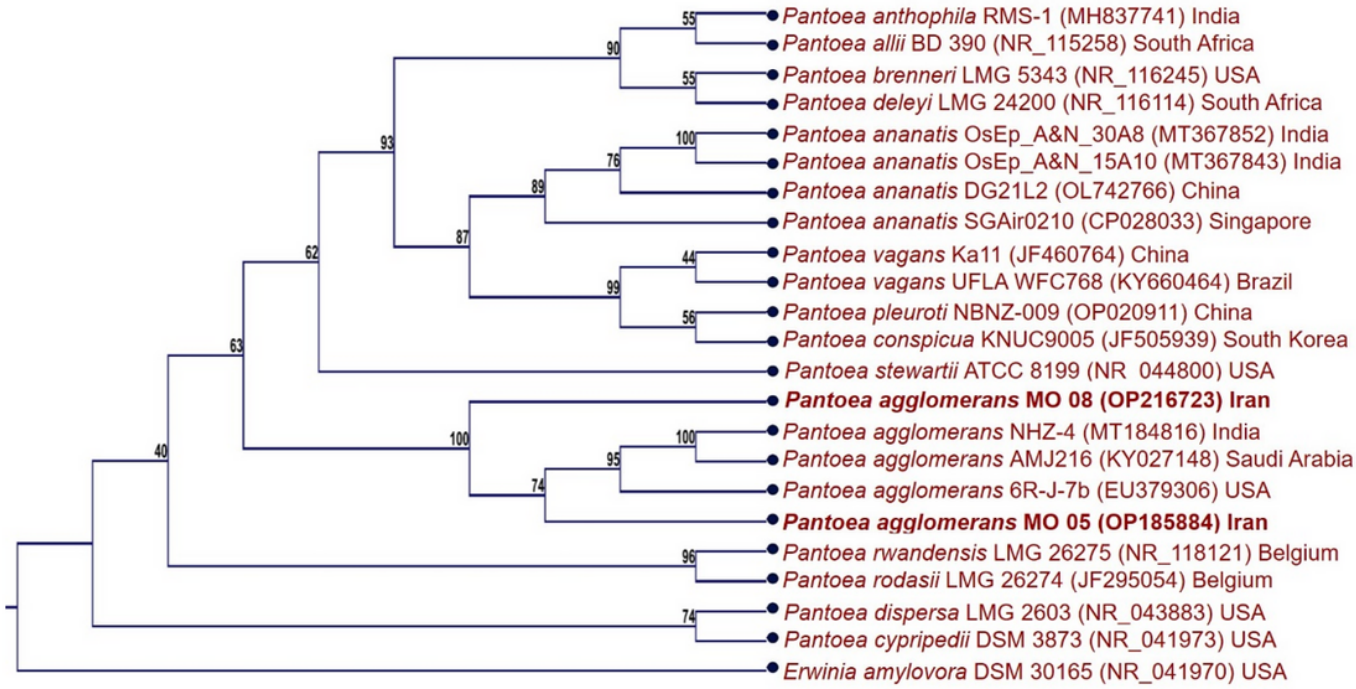

Figure 3

Maximum likelihood phylogenetic tree based on 16s rRNA gene sequences of strains MO05 and MO08 isolated from *Pistachi vera* cv. Zarand trees in Iran and other strains of *Pantoea* species retrieved from GenBank. The GenBank accession number of each strain was presented in parenthesis. Numbers at the nodes are the bootstrap values obtained for 1,000 replicates. The tree is rooted to *Erwinia amylovora* NR-041970.

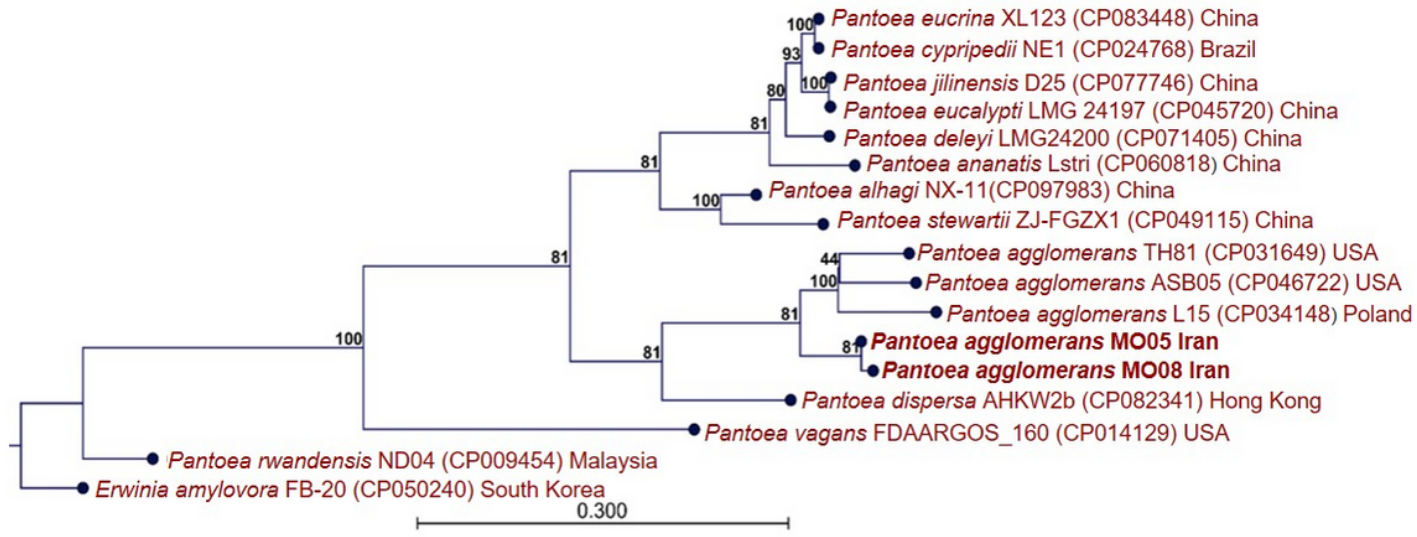

Figure 4

A phylogenetic tree inferred by using the Maximum Likelihood method and General Time Reversible model based on concatenated *atpD*, *gyrB*, and *infB* sequences of the pistachio strains MO05, and MO08 and the reference strains of *Pantoea* spp. obtained from GenBank. Numbers at the nodes are the bootstrap values obtained for 1,000 replicates. Bar, 0.300 substitutions per nucleotide position. The tree is rooted to *Erwinia amylovora* FB-20 (CP050240).

Supplementary Files

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