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ORIGINAL ARTICLE

Occurrence and pathogenicity of *Stenotrophomonas* spp. and *Paenibacillus* spp. on tomato plants in Turkey

Sumer Horuz[1] · Mehmet Serin[1]

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Abstract

Tomato is the most produced and consumed vegetable in Turkey, and has a great importance in farmers' income. In this study, bacterial disease surveys were carried out in 130 plastic covered greenhouses in the center and eight district neighborhoods of Mersin province, Turkey from January to May, 2019 and 2020 growing periods, respectively. Suspicious tomato plants showing typical disease symptoms such as stem rot, pith necrosis and secondary root formation on the stem were collected from twelve different greenhouses. The representative bacterial strains (n = 20) were initially characterized based on pathogenicity test on tomato seedlings, phenotypic characteristics, and then identified as *Stenotrophomonas* spp., and *Paenibacillus* spp. according to their protein fingerprint patterns obtained by MALDI TOF MS system. The identification of three strains was further confirmed by sequencing of 16 S rDNA. According to BLAST analysis, the strains shared 99–100% identity with *Stenotrophomonas rhizophila, Stenotrophomonas chelatiphaga* and *Paenibacillus amylolyticus* strains deposited in GenBank. The prevalences of tomato inner pith necrosis and stem rot caused by *Paenibacillus amylolyticus* and *Paenibacillus polymxa* and pith necrosis caused by *Stenotrophomonas* spp. were 0.8% and 4.6%, respectively in the area. Representative bacterial strains were further tested for copper sensitivity in vitro, and, all strains were resistant for 1 mM copper amended KB media. This study represented the first report of newly introduced stem and core rot and pith necrosis caused by *Paenibacillus amylolyticus*, *Paenibacillus polymxa* and *Stenotrophomonas* spp., on tomato in Turkey.

Keywords Pith necrosis · Stem rot · *Solanum lycopersicum* · Emerging bacterial pathogens

Introduction

Tomato (*Solanum lycopersicum* Mill.) is an important annual vegetable species in the Solanaceae family, it is grown and consumed for commercial purposes in many countries. Turkey was ranked as the fourth in the world with 13 million tons of tomato production (4 million tons in greenhouses) all around the country (FAO 2021). Tomato can be grown throughout the whole year both in the greenhouses and fields, and it is widely grown in Antalya and Mersin provinces in Turkey. A million tons of tomato cultivation is carried out in both greenhouses and fields for table consumption in the province of Mersin, located in the Eastern Mediterranean Region, Turkey (TUIK 2022). It has been determined that approximately 200 plant pathogens infect tomato plants (Chetelat 2014; Haji Nour and Horuz 2023). The bacterial pathogens belonging to *Pseudomonas* and *Xanthomonas* genera indicated that they cause the most economic losses in tomato (Mensi et al. 2018; Tireng Karut et al. 2019). In Mersin, bacterial speck (*Pseudomonas syringae* pv. *tomato)*, bacterial canker *(Clavibacter michiganensis* subsp. *michiganensis)* (Aysan et al. 1999, Şahin et al. 2002), pith necrosis (*Pseudomonas cichorii, Pseudomonas corrugata*, *P. viridiflava, P. mediterranea, P. fluorescens*) (Sahin et al. 2005) and stem rot (*Pectobacterium caratovorum* and *Dickeya chrysanthemi*) (Aysan et al. 2005; Serin and Horuz 2022) diseases has been reported in previous studies. Tomato pith necrosis and stem rot outbreaks are occasionally widespread due to the lack of circulating air in plastic covered greenhouses and high humidity during rainy days in the production areas.

✉ Sumer Horuz
shoruz@erciyes.edu.tr

1 Plant Protection Department, Faculty of Agriculture, Erciyes University, Kayseri 38039, Turkey

During the years 2019 and 2020, symptoms of stem rot and pith necrosis occurred in commercial tomato producing greenhouses in Silifke district of Mersin, Turkey. Because a variety of bacterial species have been reported to cause similar symptoms on tomato, surveys in 130 individual commercial greenhouses conducted from January to May 2019 and 2020 to isolate the causative bacterial pathogens. This work was initiated to (1) identify the causal bacterial agent/s, (2) test the pathogenicity on tomato plants, phenotypic, and genotypic caharacterization of the strains by using carbon sources, MALDI-TOF MS and sequencing analyses, (3) and also reveal the copper sensitivity of bacterial strains under in vitro conditions. This study will provide a comprehensive understanding of the newly reported tomato infecting bacterial strains, and provide a principal for the prevention of tomato diseases.

Materials and methods

Sampling and bacterial isolations

During the growth season from January to May, 2019 and 2020, surveys were carried out in 130 tomato growing greenhouses located in eight neighbourhood of Silifke, district of Mersin, Turkey. The surveyed greenhouses were plastic covered and larger than 500 m square. Suspicious samples showing disease symptoms such as basal stem rot, pith necrosis and secondary root formation on the stem were collected from twelve different greenhouses that produce commercial tomatoes. The samples were immediately delivered to the laboratory within a plastic bag. A 2–3 mm piece of necrotic plant tissue from the inner of stem was cut with a sterile scalpel and disinfected by immersion in the solution of 70% ethanol for a minute. Each tissue was immediately macerated in a sterilized mortar and suspended with 2–3 ml sterile distilled saline buffer (0.85% NaCl) to release the bacteria from the inner tissues. A loopful (10 µl) suspension was streaked onto the King's Medium B (KB) (King et al. 1954) and plates were incubated at 25 °C for 48–72 h. Suspicious pure colonies were subcultured and kept on yeast extract calcium carbonate agar (YDCA) as slants at 4 °C and also stored at -20 °C in 20% aqueous glycerol until use (Lelliott and Stead 1987).

Identification of bacterial strains isolated from symptomatic tomato plants

Pathogenicity study on tomato plants

Surface disinfected tomato seeds (cv. Hazara) were sown in plastic trays containing potting mix and incubated in a plant growth cabinet (16 h light, 8 h dark) at 27 °C for three weeks. Three week-old seedlings were transplanted into 10 cm wide pots filled with sand + clay + peat mixtures (30% peat, 40% clay and 30% sand) (Coskun and Horuz 2023). Tomato plants at the 4–5 true leaf stage were used for pathogenicity tests. All representative bacterial strains were grown on KB at 25 °C for 72 h to have fresh cultures. The suspensions of each strain were prepared from those cultures in a 9 ml glass tube with sterile distilled water, and each inoculum was adjusted to an absorbance value of 0.2 (equal to 1×10^8 cfu/ml) at 600 nm with a spectrophotometer (Shimadzu, UV-1800). A suspension (50–100 µl) of each strain was injected with a sterile insulin needle (1 ml) into the tomato stems at once just 5 cm above from soil. Negative control tomato plants were injected with sterile distilled water. After inoculations, plants were kept in a greenhouse at 25 ± 2 °C, 85 ± 5% relative humidity and 16:8 h of light/dark until disease symptoms appeared in bacterial strain injected plants. Plants were watered (50 ml per plant) with tap water at two-day intervals. Disease development on tomato stem was evaluated for fifteen days after inoculations. Re-isolations were made to fulfill Koch's postulates from symptomatic plant materials. Three tomato plants were injected for each strain and pathogenicity tests were conducted twice.

Phenotypic characteristics

Twenty strains isolated from tomato plants in Mersin, Turkey were phenotypically characterized by following tests as described by Lelliot and Stead (1987): Gram staining via 3% of potassium hydroxide solution (KOH), production of fluorescent pigment on KB, mucoid growth on YDCA, Oxidative/Fermentative metabolism of glucose, Levan formation, presence of oxidase, pectolytic activity on potato slices, arginine dihydrolase activity, hypersensitive reaction (HR) on tobacco leaves (cv. Samsun), starch and aesculin hydrolysis, ability to reduce nitrates to nitrites, growth tolerance in 1%, 3%, 5%, and 10% NACI, acid production from D-mannitol, D-Sorbitol, sucrose, raffinose, L-arabinose, and melibiose. All tests were conducted in an incubator at 25 °C and were repeated twice with three replicates.

Copper sensitivity of bacterial strains

The copper tolerance of the strains was determined using KB medium supplemented with copper (II) sulfate pentahydrate $CuSO_4 \cdot 5H_2O$ (Isolab, 911.023) at the doses of 0.16 mM, 0.32 mM, 0.4 mM, 0.6 mM, 0.8 mM, 1 mM, 1.2 mM, 1.4 mM, 1.6 mM, 1.8 mM, 2 mM, respectively. The suspensions of bacterial strains were prepared as described above and each inoculum was dropped (10 µl) onto KB medium.

The Petri dishes were incubated at 25 °C for 72 h. The experiment was repeated twice and three plates were used for each trial.

Rapid identification of bacteria with MALDI-TOF MS

The identity of the representative bacterial strains was confirmed by Matrix Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry (MALDI-TOF MS) (MicroFlex LT, Bruker Daltonics, Bremen, Germany) analyses as described by Pavlovic et al. (2012). Initially, the bacterial strains were freshly cultured on tryptic soy agar (TSA) for 24–36 h, and ethanol-formic acid method was used for protein isolations according to manufacturer's instructions from those pure bacterial strains. Then, the spectra obtained with the device's flex control software program (Biotyper 3.0; Microflex LT; Bruker Daltonics GmbH, Bremen, Germany) were compared with the Maldi Biotyper Real-Time Classification (RTC) software and the diagnosis process continued. As a result, the score values that exceeded the threshold of 2.0 (green color) were used for a secure species identity. The threshold between 1.7 and 1.99 (yellow color) was used for identity at genus level or probable species (Chalupová et al. 2014).

Table 1 Details of strains isolated from tomato plants in Turkey and other reference bacterial strains used in this study

Bacterial name	Strain	Location	GenBank accession numbers
Stenotrophomonas rhizophila	e-p10	Austria	NR_121739
Stenotrophomonas rhizophila	AY 2 – 1	Turkey	MZ710642
Paenibacillus amylolyticus	JCM 9906	Japan	NR_112163
Paenibacillus amylolyticus	NBRC 15,957	Japan	NR_112728
Paenibacillus amylolyticus	NRRL NRS-290	Japan	NR_025882
Paenibacillus amylolyticus	KU 3 – 1	Turkey	MZ710643
Stenotrophomonas chelatiphaga	LPM-5	Russia	NR_116366
Stenotrophomonas chelatiphaga	ST 1–1	Turkey	MZ710644
Xanthomonas euvesicatoria	DSM 19,128	Germany	NR_104773
Xanthomonas perforans	XV938	Germany	NR_104792
Xanthomonas vesicatoria	ATCC 35,937	Germany	NR_026388
Xanthomonas hortorum pv. gardneri	DSM 19,127	Germany	NR_104793
Stenotrophomonas maltophilia	LMG 958	Germany	NR_119220
Stenotrophomonas maltophilia	NBRC 14,161	Japan	NR_113648
Stenotrophomonas maltophilia	ATCC 13,637	Japan	NR_112030

DNA extraction and sequencing

Three of 20 bacterial strains were selected for sequencing and strains were freshly cultured on KB. A colony from each strain was transferred to a glass tube with 9 ml nutrient broth and shaken at 200 rpm at 25 °C for 24 h. The bacterial colonies were centrifuged at 5000 rpm for five minutes and Qiagen DNeasy Blood and Tissue Kit (QIAGEN Benelux B.V., The Netherlands) was used for the DNA isolations. The protocol was fulfilled according to the manufacturer's instructions. The purified DNA was then used as template for subsequent amplifications and stored in a 1.5 ml centrifuge tube at -20 °C until use. The 16 S rDNA genes of bacterial strains were amplified using the 27 F and 1492R universal bacterial primer set (Frank et al. 2008). PCR amplifications were carried out in 25 µl of reaction containing 12.5 µl of 2x master mix (K0171, Fermentas, Thermo Fisher Scientific, Vilnius, Lithuania), 2 µl of (10 µM) each primers, 1 µl of genomic DNA (20 ng/µl) and 7.5 µl of nuclease free water. PCR amplifications were performed with a thermocycler (BIO-RAD, T100). Prior to purifications and sequencing, PCR amplicons were separated by electrophoresis in a 1X TAE Buffer using agarose gel (1% w/v) stained with ethidium bromide at 0.1 µg/ml. The size of the PCR products was estimated by using a 100 bp size marker (SM0241, Thermo Fisher Scientific, Vilnius, Lithuania). PCR amplicons of each strain were sequenced in both forward and reverse directions via DNA sequencer (ABI 3100, Applied Biosystems) (Weisburg et al. 1991). The DNA sequences were aligned and the consensus sequences were generated with BioEdit version 7.0.5. Consensus sequences of the strains were subjected to the BLAST tool for analysis and compared with those available in NCBI (National Center for Biotechnology Information) GenBank using BLASTn program and deposited in GenBank. The lengths of the AY 2 – 1, ST 1–1 and KU 3 – 1 queries were 1081 bp, 1246 and 1266 bp, respectively. For phylogenetic analysis, 12 sequences of 16 S rDNA gene representing different known species or subspecies of the *Stenotrophomonas*, *Xanthomonas* and *Paenibacillus* genus were imported from GenBank database. The accession numbers of these type strains are shown in Table 1. Multiple sequence alignments were developed with ClustalW, one of the two algorithms was implemented in MEGAX (Kumar et al. 2018). The phylogenetic tree was constructed using the neighbor-joining (NJ) method with 1000 bootstrap repeats.

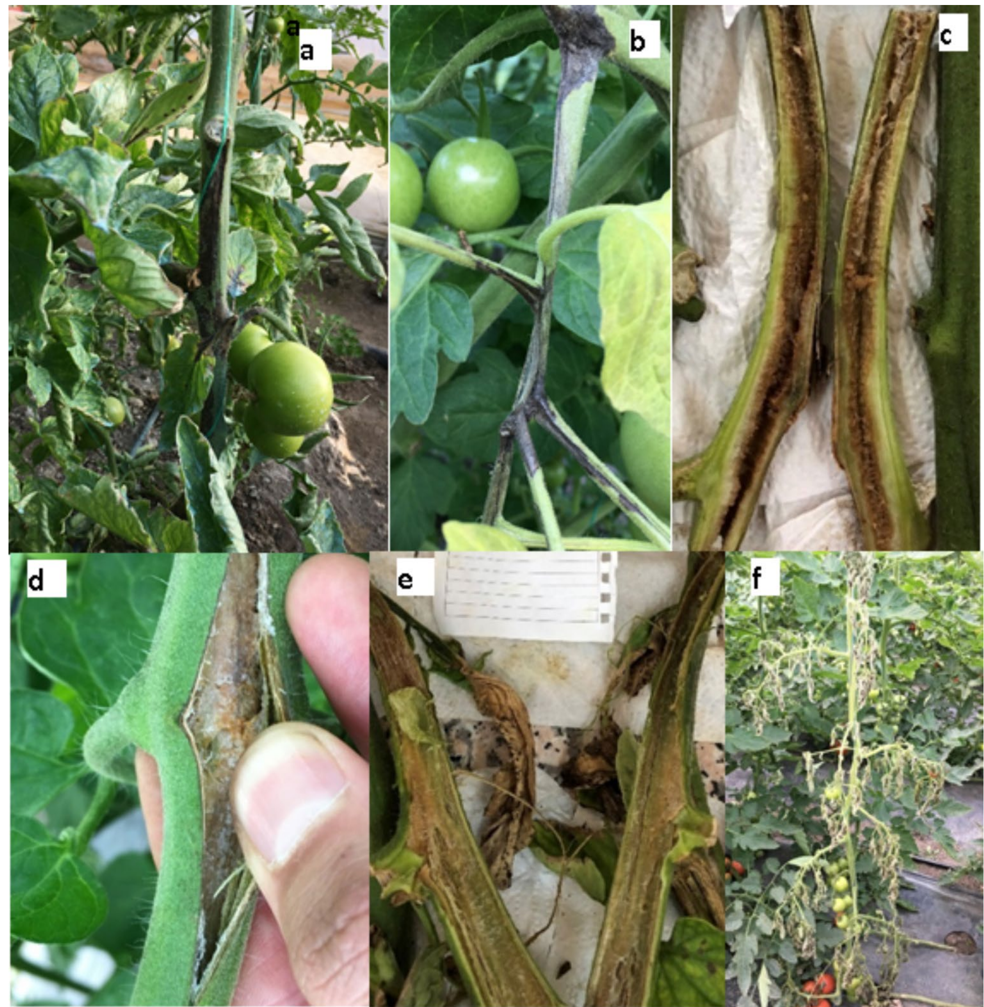

Fig. 1 Natural disease symptoms observed during surveys, necrosis on outer tomato stem (**a-b**), pith necrosis (**c**), tomato stem and core rot (**d-e**) and wilting (**f**)

Fig. 2 Growth of bacterial colonies in KB medium after 72 h, SG 2 – 1 (**a**), AY 2 – 1 (**b**) KU 3 – 1 (**c**)

Results

Sampling and bacterial isolations

In this study, 130 individual greenhouses in which tomatoes grown were surveyed in neighborhoods of Silifke, district of Mersin, Turkey, and symptomatic twelve plants were sampled for isolations. When the stem was cut longitudinally, outer and inner symptoms of pith necrosis were observed in tomato plants (Fig. 1a-c). In some greenhouses, symptoms of stem and core rot (Fig. 1d-e) and wilting (Fig. 1f) were screened in plants. Twenty representative bacterial strains were isolated from the symptomatic tomato plant tissues. Bacterial colonies were round, slow growing, white, cream, or creamy-yellow in color and 1 mm in diameter after 72 h in KB medium (Fig. 2a-c). Fourteen out of twenty strains had developed colonies that were yellow, mucoid, fluid, round, with entire margins on YDCA medium after 72 h.

Identification of bacterial strains isolated from symptomatic tomato plants

Pathogenicity study

When the tomato stem was cut longitudinally after 2–3 weeks following stem inoculations with 14 representative bacterial strains, pith necrosis (Fig. 3a-c), stem rot and pith necrosis (Fig. 3d-e) were observed on tomato stem. However, inoculations of six strains on tomato stem resulted in water-soaked and grey-green lesions on stem (Fig. 3f), wilt, inner and outer stem rot (Fig. 3g) 3–7 days post-inoculations.

In the pathogenicity tests, sterile deionized saline buffer inoculated control seedlings were asymptomatic. Bacteria were reisolated from infected seedlings.

Phenotypic characteristics

Colonies of all 14 bacterial strains isolated from pith necrosis were round with an entire margins, creamy, slightly raised, mucoid on YDCA and were 1–2 mm diameter on KB culture medium after 72 h. The bacterial strains were gram negative, oxidative, positive for oxidase and negative for fluorescent pigmentation on KB, levan type colony, pectolytic activity on potato slices. The activity of arginine dihydrolase, hydrolysis of aesculin, and nitrate reduction were varied, and most of the strains resulted in hypersensitive reaction on tobacco leaves 24 h post-inoculations. All the strains were pathogenic on tomato plants. All tested strains could grow on 1–10% NaCI; however, carbon source utilization from mellibiose, raffinose, D-sorbitol, D-manitol, L-arabinose and sucrose varied among strains (Table 2).

The six strains isolated from basal stem rot and inner pith necrosis appearing tomato plants were Gram negative, positive for pathogenicity on tomato seedlings, potato soft rot, HR on tobacco, starch and aesculin hydrolysis; however negative for fluorescent pigment production on KB, oxidase, and indole production, respectively. The acid production from different carbon sources, arginine dihydolysis, nitrate reduction test and O/F reaction differed among six strains. Since all the strains tolerated at 1–3% NaCl, neither of them had any growth at 5–10% NaCl (Table 4).

Rapid identification of bacteria with MALDI-TOF MS

In order to have a proper generic and probable taxonomic status of the strains, MALDI-TOF MS score values should exceed the confidence threshold. The protein similarity

Fig. 3 Typical disease symptoms after pathogenicity tests, pith necrosis on stem and vascular tissue (**a-c**), stem rot and pith necrosis (**d-e**), water-soaked and grey-green lesions on stem (**f**), inner and outer stem rot (**g**)

Table 2 Phenotypic characteristics and copper tolerance of the 14 strains isolated from tomato pith necrosis in Turkey

Characteristics	AY1-1	AY2-1	AY3-1	AY4-1	AY5-1	SG1-1	SG2-1	SG3-1	SG4-1	MS 1–1	HU1-1	HU2-1	ST1-1	ST2-1
Gram reaction														
Fluorescent pigment production on King's B medium														
Levan										+				
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Potato soft rot														
Arginine dihydrolase										+	+	+	+	+
HR on tobacco						+	+	+	+	+	+	+	+	+
Starch hydrolysis							+	+	+	+				
Growth on 1% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth on 3% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth on 5% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth on 10% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidative/Fermentative growth	o	o	o	o	o	o	o	o	o	o	o	o	o	o
Aesculin hydrolysis	+	+	+	+	+	+	+	+	+	+				
Nitrate reduction test	+	+	+	+	+	+	+	+	+					
Acid production from:														
D-mannitol						+	+			+				
Mellibiose														
D-sorbitol														
Sucrose						+	+				+	+	+	+
L-arabinose											+	+		
Raffinose														
Pathogenicity on tomato	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mucoid growth on YDCA	+	+	+	+	+	+	+	+	+	+	+	+	+	+
In vitro copper tolerance (mM)														
0.16	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.32	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.4	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.8	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.4	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.6	+	+	+	+	+	+	+	+	+	+	+	+		
1.8	+	+	+	+	+	+	+	+	+	+	+	+		

Note: +, positive reaction

of representative bacterial strains (n = 14) varied about 1.50–2.30 score values representing no species consistency, thus, determined as *Stenotrophomonas* spp. However, six bacterial strains (KU1-1, KU2-1, KU3-1, MS1-1, MS2-1 and MS3-1) were confirmed as *Paenibacillus polymxa* and *Paenibacillus amylolyticus* with high score values (≥ 2.00) exceeding the threshold value of 2.0 for secure species identity (Table 3).

Sequence analysis

For sequencing, we selected the highly virulent representative bacterial strains (n = 3) isolated from three individual greenhouses in Mersin. The cloned fragments of bacterial strains (AY 2 – 1, ST 1–1 and KU 3 – 1) were sequenced, and sequences were deposited in GenBank under the accession numbers MZ710642- MZ710644 (Table 1). The constructed tree is shown in Fig. 4.

Based on the BLAST search results, the strain AY 2 – 1 was identical (100%) with *Stenotrophomonas rhizophila* strain e-p10 (Accession number NR_121739) in GenBank. Since the strain ST 1–1 shared 99.71% sequence similarity with *Stenotrophomonas chelatiphaga* strain LPM-5 (Accession number NR_116366), and strain KU 3 – 1 was identical to *Paenibacillus amylolyticus* strain JCM9906 (Accession number NR_112163) and *Paenibacillus amylolyticus* strain NBRC 15,957 (Accession number NR_112728) at 99.06% similarity.

Table 3 Maldi-TOF MS characteristics of the strains isolated from pith necrosis, core necrosis and stem rot of tomato in Silifke, Mersin, Turkey

Number	Strain	Colony morphology	Disease symptoms	Identity with MALDI-TOF MS
1	AY 1–1	Creamy Yellow	Pith necrosis	*Stenotrophomonas* sp.
2	AY 2 – 1	Creamy Yellow	Pith necrosis	*Stenotrophomonas* sp.
3	AY 3 – 1	Creamy Yellow	Pith necrosis	*Stenotrophomonas* sp.
4	AY 4 – 1	Creamy Yellow	Pith necrosis	*Stenotrophomonas* sp.
5	AY 5 – 1	Creamy Yellow	Pith necrosis	*Stenotrophomonas* sp.
6	SG 3 – 1	Creamy Yellow	Pith necrosis	*Stenotrophomonas* sp.
7	SG 4 – 1	Creamy Yellow	Pith necrosis	*Stenotrophomonas* sp.
8	MS 1–1	Creamy Yellow	Pith necrosis	*Stenotrophomonas* sp.
9	SG 1–1	Creamy Yellow	Pith necrosis	*Stenotrophomonas* sp.
10	SG 2 – 1	Creamy Yellow	Pith necrosis	*Stenotrophomonas* sp.
11	HU 1–1	Cream	Pith necrosis	*Stenotrophomonas* sp.
12	HU 2 – 1	Cream	Pith necrosis	*Stenotrophomonas* sp.
13	ST 1–1	Cream	Pith necrosis	*Stenotrophomonas* sp.
14	ST 2 – 1	Cream	Pith necrosis	*Stenotrophomonas* sp.
15	KU 1–1	White	Pith necrosis	*Paenibacillus amylolyticus*
16	KU 2 – 1	White	Pith necrosis	*Paenibacillus amylolyticus*
17	KU 3 – 1	White	Pith necrosis	*Paenibacillus amylolyticus*
18	MS 2 – 1	Cream	Stem rot	*Paenibacillus polymyxa*
19	MS 3 – 1	Cream	Stem rot	*Paenibacillus polymyxa*
20	MS 4 – 1	Cream	Stem rot	*Paenibacillus polymyxa*

In vitro copper tolerance of bacterial strains

The bacterial strains (n = 20) were resistant to 1 mM copper amended KB media. Pith necrosis causing bacterial strains (n = 14) were tolerant to 1.8 mM (except ST1-1 and ST2-1) (Table 2). Inner pith necrosis and stem and core rot causing bacterial strains (= 6) were susceptible to 1.2 mM copper (Table 4). In order to interpret, the effectiveness of copper should be tested on tomato plants in the field. As it is well-known that copper compounds have a contact effect, low efficacy is expected to be against vascular bacterial strains. Copper compounds can only avoid from dissemination and transmissions in the field.

Discussion

Our study confirms the presence of multiple bacteria associated with tomato pith necrosis and basal stem rot diseases in Mersin province of Turkey. The bacterial strains of *Stenotrophomonas* spp., and *Paenibacillus amylolyticus* isolated from discoloured stem tissue, pith necrosis, and basal stem rot symptoms (n = 20) on tomato plants. To date, the presence of tomato pith necrosis and stem rot diseases caused by *Pseudomonas* spp. have been reported in Turkey (Sahin et al. 2005). Pith necrosis and basal stem rot of tomato as well as other important crop species caused by several *Pseudomonas* species were known as serious diseases which can lead to the destruction of tomato plants in any stage of development and in different cultivation systems (Kudela et al. 2010) However, the occurrence and pathogenicity of *Stenotrophomonas* species, and *Paenibacillus amylolyticus* on tomato plants were revealed for the first time in this research. Little information is available about bacterial diseases caused by these pathogens on tomato plants.

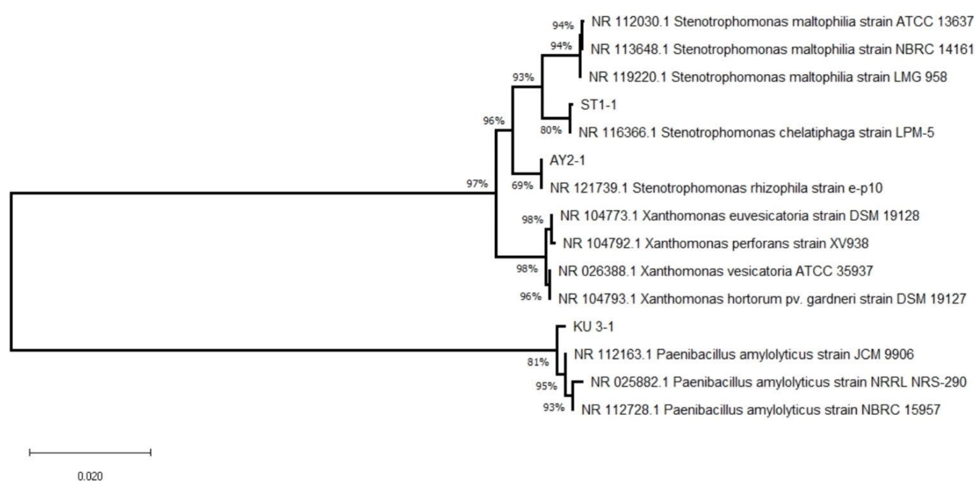

Fig. 4 A phylogenetic tree was constructed by the Neighbor Joining Method and Tamura-3 parameter model based on 16 S rDNA sequences of tomato strains (AY 2 – 1, ST 1–1, KU 3 – 1) and other 12 references obtained from GenBank. The bootstrap values obtained for 1,000 replicates

Table 4 Phenotypic characteristics and copper tolerance of the strains isolated from tomato core necrosis and stem rot in Turkey

Characteristics	KU1-1	KU2-1	KU3-1	MS2-1	MS3-1	MS4-1
Gram reaction						
Fluorescent pigment production on King's B medium						
Levan				+	+	+
Oxidase						
Potato soft rot	+	+	+	+	+	+
Arginine dihydrolase	+	+	+			
HR on tobacco	+	+	+	+	+	+
Indole production						
Starch hydrolysis	+	+	+	+	+	+
Aesculin hydrolysis	+	+	+	+	+	+
Nitrate reduction test	+	+	+			
Growth on 1% NaCl	+	+	+	+	+	+
Growth on 3% NaCl	+	+	+	+	+	+
Growth on 5% NaCl						
Growth on 10% NaCl						
Oxidative/Fermentative growth	F	F	F	O	O	O
Acid production from:						
D-mannitol				+	+	+
Melibiose				+	+	+
D-sorbitol						
Sucrose				+	+	+
L-arabinose						
Raffinose				+	+	+
Pathogenicity on tomato	+	+	+	+	+	+
In vitro copper tolerance (mM)						
0.16	+	+	+	+	+	+
0.32	+	+	+	+	+	+
0.4	+	+	+	+	+	+
0.6	+	+	+	+	+	+
0.8	+	+	+	+	+	+
1.0	+	+	+	+	+	+
1.2						

Note: –, positive reaction; +, negative reaction

The genus *Stenotrophomonas* (in the class of Gammaproteobacteria) is widespread in the environment, soil, and plant tissues. The species belonging to that genus were formerly named in the genus *Pseudomonas* and *Xanthomonas* (Swings et al. 1983). In 1993, the bacteria were transferred to the genus *Stenotrophomonas* (Palleroni and Bradbury 1993). The genus now consists of a number of species including *S. maltophilia, S. nitritireducens*, *S. rhizophila*, *S. acidaminiphila*, *S. koreensis*, *S.chelatiphaga*, *S. terrae* and *S. humi* according to the phenotypic and genotypic studies (Ryan et al. 2009). Stenotrophomonas spp. have many features that could be used in different processes. Some *Stenotrophomonas* spp. can produce antimicrobial substances that protect plants from infections, or propagate plant promoting factors. Moreover, many *Stenotrophomonas* spp. have a high degree of resistance to heavy metals and antibiotics. The bacterium, *Stenotrophomonas maltophilia* is also known to cause human disease and has been proved to be virulent in a nematode model (Ryan et al. 2009). *Stenotrophomonas* species, especially *S. maltophilia*, was isolated from tomato fruits (Stoyanova and Bogatzevska 2012) and tomato seeds, and found to be the sole causal agent of the disease in seeds (Stoyanova et al. 2018) In Zimbabwe, *S. maltophilia* has also been isolated from tomato seeds and proved that it was pathogenic on tomato plants (Sibiya et al. 2003). Since *Stenotrophomonas* species are phylogenetically closely related to the phytopathogenic genera *Xanthomonas* and *Xylella*, *S. maltophilia* has also been reported to be pathogenic on tomato (Stoyanova et al. 2018). To our knowledge, this paper presented the first report of phytopathogenic bacteria *Stenotrophomonas rhizophila* and *Stenotrophomonas chelatiphaga* on tomato plants. The prevalence of tomato pith necrosis caused by *Stenotrophomonas* spp. was 4.6% in the surveyed area.

The bacterium *S. chelatiphaga* strain ST 1–1 and *Stenotrophomonas rhizophila* strain AY 2 – 1 were highly virulent on tomato plants ten days post inoculations under 24 °C and 80% RH in a controlled climatic cabinet.

In this study, tomato pith necrosis causing *Paenibacillus amylolyticus* (n = 3) and stem rot causing *Paenibacillus polymxa* (n = 3) species were isolated from two different tomato greenhouses and diagnosed via pathogenicity on tomato, phenotypic, and genotypic characterizations. We have described white on KB, pectolytic activity positive and gram negative *P. amylolyticus*, designated strain KU 3 – 1 (GenBank accession number MZ710643), belonging to the genus *Paenibacillus* and isolated from the inner of tomato stem symptoms in Mersin Province, Turkey. Tomato inner pith necrosis and stem rot causing bacteria were prevalent at 0.8% in the surveyed area. The phenotypic characteristics of the strains were variable (Table 4). In 1993, the members of "group 3" within the genus *Bacillus* were transferred to the genus *Paenibacillus* (belonging to the family *Paenibacillaceae*), and it was proposed *Paenibacillus polymyxa* as the type species for that genus (Ash et al. 1993). This genus has been identified with more than 200 species to date (www.bacterio.net/paenibacillus.html). Members of this genus are Gram-positive, Gram negative or Gram-variable, spore-forming, and facultatively anaerobic or strictly aerobic (Yang et al. 2018). To date, the pathogen *P. amylolyticus* was isolated from soil (Teeraphatpornchai et al. 2003), stonewool substrate (Validov et al. 2006), and coffee cherries (Sakiyama et al. 2001) as a beneficial microroganism; in contrast, little is known about its pathogenicity on crop plants.

In organic and conventional agriculture, copper based compounds are the unique and most widely used fungicides for its wide spectrum of activity to combat plant bacterial diseases (Gessler et al. 2011). However, copper resistance has developed gradually in phytopathogenic bacteria due to wide and irregular use of copper based bactericides for plant disease control. Copper resistant phytopathogenic bacterial strains have been reported in Turkey (Mirik et al. 2007; Husseini and Akköprü 2020; Egerci et al. 2021; Ozturk and Soylu 2022). In this study, *Stenotrophomonas* spp. grew up to 1.8 mM copper amended culture media (except ST 1–1 and ST 1–2), *Paenibacillus amylolyticus* strains were resistant for 1.0 mM copper, and neither of the stem rot causing bacteria grew at 1.2 mM copper amended petri dishes. According to the results obtained from this research, all the bacterial strains (n = 20) were copper-resistant since they grew over 1.0 mM copper in vitro. In order to find out the sources and levels of copper resistance among strains, future studies like plant inoculations and copper response genes transfer in plasmids need to be carried out.

In conclusion, our study is of pivotal importance since it stresses that *Stenotrophomonas rhizophila*, *Stenotrophomonas chelatiphaga* and *Paenibacillus amylolyticus* are emerging tomato-infecting bacteria in Turkey. The pathogenicity of these strains on other important plant species, prevalence, and severity in the other tomato production areas, interaction with other pathogenic and beneficial microorganisms on tomato should be monitored as a future plan. In addition, this is the first report of phylogenetic characterisation of newly introduced bacterial strains based on 16 S rDNA isolated in Turkey, since the identification of plant pathogen is an essential requirement for an effective disease control.

Although the incidence and prevalence of both diseases are very low and restricted to few greenhouses in the region inspected, major tomato production areas should be monitored for this emerging threat. The identification of newly emerging bacterial strains could be also valuable for breeding programs and cultivation of tomato globally.

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Author contributions The author, Sumer Horuz contributed to the supervision process of the research, designing the methodology, planning the study, surveying the greenhouses, making the isolations and identifying the strains, interpreting the data, and writing the initial manuscript. The author Mehmet Serin contributed to surveying the greenhouses, running the tests for identification, and performing the experiments.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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