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Isolation and identification of *Xanthomonas axonopodis* pv. *phaseoli* and *Pseudomonas savastonoi* pv. *phaseolicola* causing disease in common bean producing areas in Samsun province of Turkey

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ABSTRACT

In 2013-2014, surveys were conducted in order to investigate the presence of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) (common blight) and *Pseudomonas savastonoi* pv. *phaseolicola* (*Psp*) (halo blight) in common bean producing areas of Samsun province. Eight *Xap* and 5 *Psp* isolates were identified from single isolated pure colonies of the bacteria by means of biochemical, pathogenicity, molecular and 16S rDNA sequencing studies. Phylogenetic tree analysis demonstrated that the isolates of *Xap* and *Psp* obtained were clustered with their type strains. Göynük 98 was the most susceptible cultivar to *Xap* and *Psp* isolates among the five common bean cultivars tested in pathogenicity assays and selected for virulence assays. This work proved the presence of both disease agents in Samsun province, which supplies about 20 % of the total fresh bean production in Turkey, with molecular studies for the first time.

Keywords:
Detection
Common blight
Halo blight
PCR
16S rDNA

Fasulye üretim alanlarında hastalığa neden olan *Xanthomonas axonopodis* pv. *phaseoli* ve *Pseudomonas savastonoi* pv. *phaseolicola*'nın izolasyonu ve tanılanması

ÖZET

2013-2014 yıllarında, Samsun ili fasulye üretim alanlarında adi yanıklığa neden olan *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) ve hale yanıklığına neden olan *Pseudomonas savastonoi* pv. *phaseolicola* (*Psp*)'nin belirlenmesi için surveyler yapılmıştır. Elde edilen tek ve saf kolonilerle yapılan biyokimyasal, patojenisite, moleküler ve 16S rDNA sekanslama çalışmaları sonucunda 8 *Xap* ve 5 *Psp* izolatı tanılanmıştır. *Xap* ve *Psp* izolatları filogenetik ağaç analizinde tip kültürleri ile birlikte yer almıştır. Göynük 98 fasulye çeşidi in vitro patojenisite denemelerinde en hassas çeşit olarak belirlenmiştir ve virülens denemesi için kullanılmıştır. Yapılan bu çalışmada, Türkiye taze fasulye üretiminin % 20'sinin gerçekleştiği Samsun ilinde her iki hastalık etmenin varlığı moleküler çalışmalarla ilk kez ortaya konmuştur.

Anahtar Sözcükler:
Teşhis
Adi yanıklık
Hale yanıklığı
PCR
16S rDNA

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important legume species worldwide for direct human consumption and one of the most important crops grown all over the world. It is mainly consumed in developing countries in Latin America, Africa and Asia (Gepts, 2001). The crop is used primarily for human consumption of dry (mature) beans, shell beans (seeds at physiological maturity), and green pods (Mohammed, 2013). Its nutritional value is due to its high content of dietary fibres, minerals and certain vitamins (Gepts et al. 2008; Reynoso-Camacho et al. 2006). Fungal, viral and bacterial pathogens cause yield losses on common bean (Agrios, 1997; Fourie, 2002). Important bacterial pathogens cause severe economic losses in common bean growing regions, which are namely *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*), *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), *Pseudomonas syringae* pv. *syringae* (*Pss*), *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (*Cff*) (Schwartz and Galvez, 1980; Schuster and Coyne, 1981; Saettler, 1989; Park and Dhanvantari, 1987; Coyne et al. 1994; Ranalli and Parisi, 1998; Popovic, 2008). Contaminated seeds are

the main source of primary infection of these mentioned bacterial pathogens. Among those pathogens, the most predominant ones are *Xap* causes common bacterial blight and *Psp* causes halo blight on bean plants (Coyne et al. 1976; Schwartz and Galvez, 1980; Gilbertson et al. 1990; Dönmez et al. 2013). These pathogens can reduce yields under epidemic conditions, to different extents depending on geographic regions, with a reported average 43% yield loss under experimental conditions (Fourie, 2002). Seed contamination plays an important role in the development of an epidemic for both diseases (Schaad et al. 1995; Yu et al. 1998) and seed health testing is vital in preventing outbreaks of these diseases (Taylor, 1970). For instance, the common blight agent (*Xap*) was isolated from seeds stored for up to 15 years (Neergaard, 1979). Hence, the bacteria can survive during the germination of seed (Schuster et al. 1983).

Both dry and fresh edible common bean are also one of the most important crops in Turkey with a production of 632,000 tons on an area of about 506,619 ha (FAO, 2014), being cultivated in different regions (Kahveci and Maden 1994, Ertugrul and Guven, 1998). Samsun province produces a large amount of fresh consumed bean, accounting for approximately 20% of the total production of Turkey (TUIK, 2013). *Xap* and *Psp* are also the most important bacterial pathogens causing losses in yield and quality of bean production in Turkey (Bastas and Sahin, 2017). Up to date, the bacterial diseases have been reported in different parts of Turkey, and both pathogens were occurred as the widespread in the growing areas (Benlioglu et al. 1994; Demir and Gundogdu, 1994; Kahveci and Maden, 1994; Bozkurt and Soylu, 2001; Donmez et al. 2013; Bastas and Sahin, 2017).

The present research aimed at surveying for the presence of *Xap* and *Psp* in the Samsun province, isolating them and characterise the obtained isolates at biochemical, pathogenic and molecular level. The results obtained from this study provide certain information that both pathogens occur on bean crops in the surveyed area and also reveal that precautions need to be taken by growers to prevent the spread of the detected pathogens in the region.

2. Materials and Methods

2.1. Sampling and bacterial isolation

Bacterial isolates of *Xap* and *Psp* were isolated from common bean leaves which were collected during surveys in Carsamba, Terme, Bafra, Tekkekoy and Ladik districts, covering 88% of total bean production areas in Samsun province. In these surveys, 87 common bean fields in 27 villages were surveyed for halo blight typical leaf symptoms contained small, round and reddish-brown necrotic lesions with yellow borders (Figure 1A) and common bacterial blight included irregular necrotic lesions with yellow borders (Figure 1B). The leaves showing signs of typical symptoms were collected for further analysis. From lesion (margins of healthy and diseased tissue), 3–5 small pieces of infected tissue were aseptically removed, placed into a tube with 0.3 ml of sterile saline solution, and left to soak for 30 minutes. The suspensions were plated on King's B (KB) and Nutrient agar (NA) plates, which were then incubated at 26°C for 24 hours. White-creamy and mucoid colonies raised with diffusible yellowish-green pigment that fluoresces green-blue under ultraviolet light (366 nm) were selected from each leaf sample for *Psp* and subcultured on KB. Yellow and mucoid colonies were selected from each leaf sample for *Xap* and subcultured on NA.

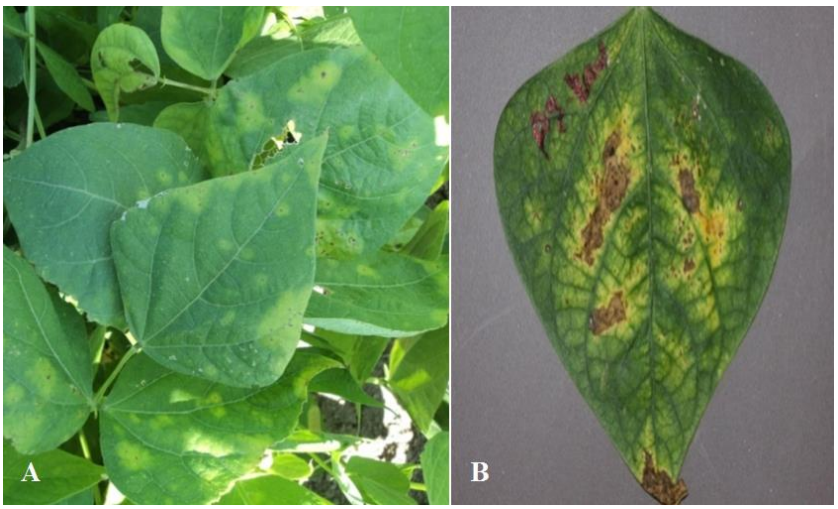

Figure 1. Diseased common bean plants; **A)** Typical halo blight caused by Psp, **B)** Common blight containing necrotic areas under the infection of Xap.

2.2. Identification of isolates

2.2.1. Biochemical and physiological tests

Biochemical and physiological tests; Levan formation, oxidase reaction, potato soft rot, arginine dihydrolase and induction of the hypersensitive reaction in tobacco leaves (LOPAT tests) were done as described by Lelliott and Stead (1987) and Schaad et al. (2001). Fluorescent pigment production was evaluated on KB plates on a UV transilluminator. Acid production from carbon sources were tested using methods described by Dye (1968). Reference isolate TE17 (*Xap*) and isolate SB5 (*Psp*) were used as positive control for each test.

2.2.2. Polymerase chain reaction

For PCR amplification, primers were designed using Geneious 7 software (Biomatters, Auckland, New Zealand) based on manual identification of sequence alignment. BLAST analysis was also performed on chosen genes sequences in NCBI database to confirm the specificities of the primers (Table 1). For *Xanthomonas axonopodis* pv. *phaseoli*, XapF/R primers were designed for targeting the methyltransferase homolog M.XphI (xphIM) and restriction endonuclease homolog R.XphI (xphIR) genes (Genbank Accession No: AF042157.1) and for *Pseudomonas savastonoi* pv. *phaseolicola* PsppF/R primers were designed for targeting the phtO, phtP, and amtA genes involved in the phaseolotoxin synthesis (Genbank Accession No: FR691729.1). PCR recations were set up in a total volume of 25 µL as follows: 12.5 µL of 2x mastermix, 1 µL of each primer (10 pmol mL^{-1})and 10.5 µL sterile water. A tiny amount of the intended colony was picked up with a sterile toothpick and directly placed into the PCR reaction tube. A thermal cycler (Bio-Rad T100 thermal cycler, USA) and the following protocol were used: initial denaturation step at 94°C for 4 min followed by 25 cycles of 94°C for 45 s, 56°C for 30 s, 72°C for 45 s with a final extension step at 72°C for 10 min. PCR products were run by electrophoresis in 1% agarose gels at 100 V for 45 min in 1x Tris-acetate-EDTA buffer and DNA fragments were visualized with a gel imaging system (Vilber Lourmat, France).

Table 1. Primers used for the detection of bacteria by polymerase chain reaction

Species	Primers	Sequence (5'-3')	Size (bp)
Xap	XapF	ACGGTCACGCGAACCTATAC	870
	XapR	TTCTGTCGCATACAGCTTGG	
Psp	PsppF	CGTCCATCAGAGCTTTGTCG	790
	PsppR	GCAATGGTCGAAGGTAGCTG	

2.2.3. 16S rDNA sequencing and phylogenetic analysis

For sequence analyses, the partial 16S rDNA of representative isolates for each pathogen (*Xap* and *Psp*) was amplified in 50 µl volume as described for the PCR tests above, using the universal primers 27F (AGAGTTTGATC(AC)TGGCTCAG; positions 8 to 27 and 1492R (ACGG(CT)TACCTTGTTACGACTT; positions 1508 to 1492 (Weisburget al. 1991). Sequencing of PCR products was performed in both directions with the same primers, and carried out by the Medsantek Company, Turkey. The chromas Pro software (Technelysium Pty Ltd, Qld, Australia) was used for editing and regenerating the obtained sequences. Resulted partial 16S rDNA gene sequences of Turkish *Xap* and *Psp* isolates were searched in the GenBank database using blastn tool for confirmation of their identity.

A multiple alignment with sequences of this study and closely related species was performed by using the program CLUSTAL W 1.6 in MEGA 6.0 to analyse 16S rDNA datasets cooperatively (Tamura et al. 2013). Dendrograms were produced using the Maximum Likelihood method based on the Jukes Cantor model (Jukes and Cantor, 1969). Topologies of the constructed trees were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings. Published sequences of *X. translucens* strain XT 2 (NR_036968) and *P. viridiflava* strain ATCC 13223 (NR_114482) were used as an outgroup for *Xap* and *Psp*, respectively.

2.3. Tobacco hypersensitivity reaction (HR)

Hypersensitivity reaction was tested on tobacco leaves (*Nicotiana tabacum* L. cv. *benthamiana*) by injecting the bacterial suspension with a hypodermic syringe for evaluating tissue collapse after 24 hours (Klement et al. 1990).

2.4. Pathogenicity assays

Five common bean cultivars used for dry bean seed production, viz. Akman 98, Nihatbey, Göynük 98, Zulbiye and Karacaşehir 90 were kindly provided by Dr. Ilyas Deligoz, Black Sea Agricultural Research Institute, Samsun, Turkey, and screened for their reactions to *Xap* and *Psp* isolates in both petri and pot assays. Bacterial isolates were cultured on NA plates incubated for 24-48 hours at 28°C. Final bacterial densities were estimated from the OD_{600} = 0.1 assuming that to be equal to 10^8 cfu mL^{-1}. For petri experiment, two weeks old bean leaves were harvested to use in a 9 mm diameter petri dishes containing sterile whatman paper to determine the most susceptible ones among

these cultivars to screen virulence of isolates in the pot experiment. Bacterial inoculums of *Xap* and *Psp* isolates were sprayed onto leaves in petri dishes and incubated at 27°C. Leaves in petri dishes sprayed with distilled water were used as a negative control. Reactions of the plants against to the bacterial isolates were evaluated and scaled after 7 days in petri experiment (Abeysinghe, 2003). For pot experiment, two weeks old plants of cultivar Göynük 98 were applied by spraying bacterial inoculum of *Xap* and *Psp* isolates and then covered by transparent plastic bags to maintain high humidity for one day in order to facilitate bacterial infection using the method described by Schuster (1955). Reactions of the infected plants to the isolates were evaluated and scaled after 20 days. Symptoms were assessed according to Dursun et al. (2002) and Osdaghi et al. (2010) with some modifications. Experiments were performed as a randomized complete block design with three replicates and repeated twice. Sterile water was sprayed for control plants. Disease severity (%) of the isolates for both experiments was calculated by Thousend Heuberger formula.

2.5. Statistical analysis

Kolmogorov-Smirnov One Sample test results showed that all traits could be assumed normally distributed (P>0.001). Levene variance homogeneity test results indicated that all traits had homoscedasticity (P>0.001). Then, One-Way ANOVA test was applied to the all data. Duncan multiple comparison test was used to compare the means. Relations between traits were examined with Pearson correlation analyses. All analyses were evaluated using SPSS v.20.0 with the license of Ondokuz Mayıs University.

3. Results

3.1. Bacterial isolation and identification

Identification tests were performed on 58 isolates. The tests lead to identification of 8 *Xap* and 5 *Psp* isolates (Table 2). *Xap* isolates were maintained from infected leaves formed yellow, convex, mucoid colonies on the NA medium. They were Gram-negative (confirmed with KOH test), positive for catalase and levan production, aerobic, non-fluorescent after 48 hours of incubation on King's B medium, showed ability to cause hypersensitive reaction on tobacco leaves, could not macerate potato tuber slices, hydrolyzed esculin, starch and utilized oxidatively d-arabinose, d-mannose, d-trehalose, d-sorbitol, d-rhamnose, glucose, d-cellobiose, l-arabinose, d-mannitol and d-raffinose as a carbon source. All *Xap* isolates presented the same results like the reference isolate TE17. *Psp* isolates were retrieved from infected leaves formed white, creamy, flourescent shining under ultraviolet light on the King's B medium. They were Gram-negative (confirmed with KOH test), aerobic, catalase and oxidase positive, had an ability to produce levan and arginine, did not macerate potato tuber slices, could not hydrolyze starch and esculin, utilized oxidatively d-mannose, d-sorbitol, glucose, d-mannitol, d-raffinose, and did not use d-trehalose, d-rhamnose, d-cellobiose, l-arabinose, and d-arabinose as a carbon source. All *Psp* isolates gave same features as the reference isolate SB5 (Table 3).

3.2. PCR and 16S rDNA phylogenetic analysis

Eight isolates were positive for XapF/R primers with reference isolate TE17 producing the expected DNA fragments for *Xap* isolates – 870 bp. For 5 isolates the marker product of 790 bp was amplified with PsppF/R primers as occurred for reference isolate SB5. Other bacteria isolated from bean samples did not give amplification products with the same primer sets. Designed primers could be able to detect the bacterial colonies belonging to *Xap* and *Psp* isolated from infected plant material. The 16S rDNA fragments of four *Xap* and four *Psp* isolates were amplified and their sequences were analyzed with the NCBI Blast algorithm. All the sequenced *Xap* isolates had 99% identity with type strain *X*. a*xonopodis* pv. *phaseoli* ATCC 49119^T = G27. *Psp* isolates showed 99% sequence similarity to the type strain *P*. *syringae* pv. *phaseolicola* A1448^T at the nucleotide level. From 16S rDNA gene phylogeny, *Xap* isolates of this work clustered in the same clade with their type ATCC 49119^T (NR_104856) and were discriminated from other pathovars and its closely related var. *fuscans* in *Xanthomonas* species (Figure 2). All *Psp* isolates of this work grouped in the same clade with representative strain type *P*. *syringae* pv. *phaseolicola* A1448^T (NR_074598) and were distinguished from other *Pseudomonas syringae* pathovars (Figure 3). These results confirmed the identity and taxonomic assignment of the isolates belonging to the corresponding species at the pathovar level. Sequence data from 16S rDNA have been deposited in Genbank under the following accession numbers MF318487 to MF318490 for *Xap* isolates and MF318491 to MF318494 for *Psp* isolates.

3.3. Pathogenicity assays

The identified isolates belonging to *Xap* and *Psp* induced the HR that resulted in collapsed tissues of tobacco. Leaf reactions of the Akman 98, Nihatbey, Göynük 98, Zülbiye and Karacaşehir 90 cultivars to the different isolates of *Xap* and *Psp* were significantly different while ability of isolates to cause disease on these cultivars were nearly same (P>0.001). Average disease severity of inoculated *Xap* isolates was highest on Göynük 98 followed by Zülbiye cultivar, while lowest for Akman 98 followed by Karacasehir 90 (P<0.001).

Table 2. The isolates obtained from infected bean plants in this study

No	Species	Isolate	Plant part	Year and Place of isolation
1		Ca. Sa. 7	Bean, leaves	2013, Turkey, Çarşamba
2		Ca. Ko. 1	Bean, leaves	2013, Turkey, Çarşamba
3		Ca. Ko. 2	Bean, leaves	2013, Turkey, Çarşamba
4	*Xap*	Ca. Ko. 3	Bean, leaves	2013, Turkey, Çarşamba
5		Ca. As. 3	Bean, leaves	2013, Turkey, Çarşamba
6		Ca. Ba. 5	Bean, leaves	2013, Turkey, Çarşamba
7		Ca. Ba. 6	Bean, leaves	2013, Turkey, Çarşamba
8		Ca. Ba. 8	Bean, leaves	2013, Turkey, Çarşamba
1		Ba. Ka. 1	Bean, leaves	2014, Turkey, Bafra
2		Ba. Ka. 2	Bean, leaves	2014, Turkey, Bafra
3	*Psp*	Ba. Ku. 3	Bean, leaves	2014, Turkey, Bafra
4		La. Ak.8	Bean, leaves	2014, Turkey, Ladik
5		La. Ak. 9	Bean, leaves	2014, Turkey, Ladik
*1**	*Xap*	TE17	Bean, leaves	2015, Turkey, Erbaa, Tokat
*2**	*Psp*	SB5	Bean, leaves	2015, Turkey, Bafra

*Reference strains used in the study (provided by Dr. Demet Çelik Ertekin, Black Sea Agricultural Research Institute, Samsun, Turkey) were subjected to PCR test with listed primers for *Xap* and *Psp*. Pathogenicity of the selected isolates was evaluated on pure bacterial culture

Table 3. Biochemical properties of *Xap* and *Psp* isolates

Characters		Species														
		Xap									*Psp*					
		TE17*	Ca.Sa.7	Ca.Ko.1	Ca.Ko.2	Ca.Ko.3	Ca.As.3	Ca.Ba.5	Ca.Ba.6	Ca.Ba.8	SB5*	Ba.Ka.1	Ba.Ka.2	Ba.Ku.3	La.Ak.8	La.Ak.9
Gram reaction		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KOH[a]		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
OF[b]		A[c]	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Oxidase		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Levan production		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arginine hydrolysis		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flourescent pigment		-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
HR on tobacco		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Potato soft rot		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Esculin hydrolysis		+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Starch hydrolysis		+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Carbon sources[d]	D-Arabinose[a]	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
	D-Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	D-Trehalose	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
	D-Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	D-Rhamnose	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
	Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	D- Cellobiose	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
	L- Arabinose	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
	D- Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	D-Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a]KOH, potassium hydroxide test; [b]OF, oxidative-fermentative test; [c]A: aerobic; [d]Utilization of compounds as the sole carbon source; + , positive reaction; -, negative reaction; *reference isolates TE17, positive control for *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*); SB5, positive control for *Pseudomonas savastonoi* pv. *phaseolicola* (*Psp*)

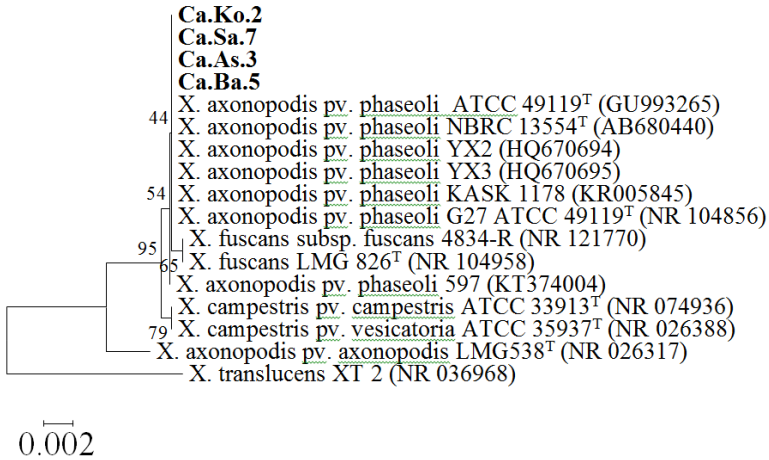

Figure 2. Phylogenetic tree based on partial 16S rDNA gene sequences showing the phylogenetic relationships of different *Xanthomonas* species. Accession numbers of reference strains in GenBank are given in parenthesis.

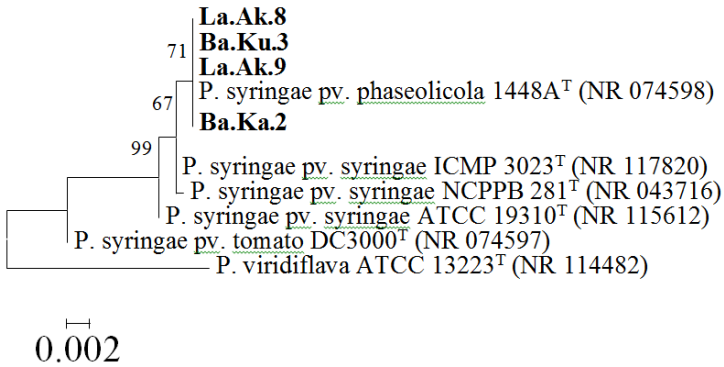

Figure 3. Phylogenetic tree based on partial 16S rDNA gene sequences showing the phylogenetic relationships of different *Pseudomonas syringae* pathovars. Accession numbers of reference strains in GenBank are given in parenthesis.

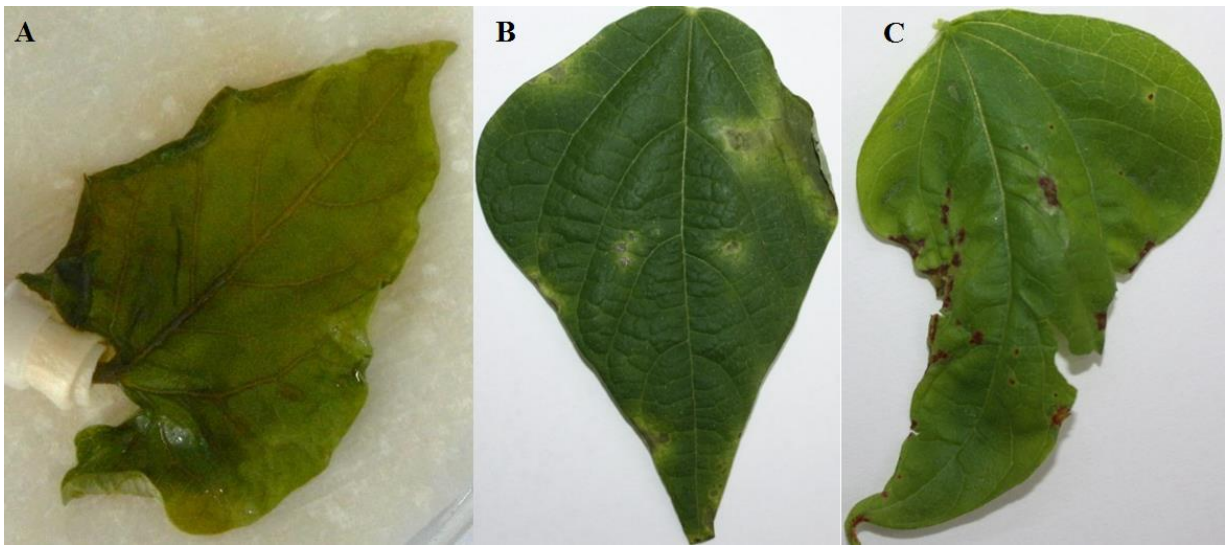

Figure 4.**A)** Diseased leaf of Göynük 98 cultivar caused by isolate Ca. Ko. 2 (*Xap*) in petri assay, **B)** Necrotic, light Brown and irregular shaped common blight caused by *Xap* isolates, **C)** Halo blight spots around necrotic areas inoculated by *Psp* isolates, on Göynük 98 cultivar in pot assay.

Leaves of Göynük 98 inoculated with *Xap* and *Psp* isolates have a wet appearance in petri assay (Figure 4A). There was significant difference for disease severity caused by Ca.Sa.7 and Ca.As.3 isolates on Nihatbey, Ca.As.3 isolate in respect to other three one on cultivar Karacasehir 90, and also between the Ca.Ko.3 and Ca.Sa.7 isolates on Zulbiye cultivar (P<0.001). Differences in leaf reactions of the cultivars to the isolates of *Psp* were significant (P<0.001). They caused higher disease on Göynuk and Nihatbey cultivars, respectively (P<0.001) and lowest capability of pathogenicity on Akman 98 that was the only one showed highest resistant reaction to all *Xap* and *Psp* isolates. No differences in the degree of virulence was observed between two *Psp* isolates on Göynük 98 as noticed the same between the four tested *Xap* isolates for their leaf reaction (P>0.001) (Table 4).

Table 4. Disease severity of *Xap* and *Psp* isolates on cultivars in petri assay

	Isolates	Cultivars	Disease severity (%) (Mean ± SD)
Xap	Ca.Ko.2	Akman 98	0± 0 ^{f}
		Nihatbey	10.67 ± 1.76 ^{def}
		Göynük 98	58.33 ± 4.41 ^{a}
		Zülbiye	33.33 ± 6.01 ^{b}
		Karacaşehir 90	6 ± 1.15 ^{def}
	Ca.Ko.3	Akman 98	4.33 ± 1.2 ^{ef}
		Nihatbey	16.67 ± 1.67 ^{de}
		Göynük 98	55 ± 2.89 ^{a}
		Zulbiye	18.33 ± 4.41 ^{cd}
		Karacasehir 90	6.67 ± 1.76 ^{def}
	Ca.As.3	Akman 98	5.33 ± 1.33 ^{ef}
		Nihatbey	0 ± 0 ^{f}
		Göynük 98	51.67 ± 6.01 ^{a}
		Zulbiye	30 ± 2.89 ^{bc}
		Karacasehir 90	18.33 ± 4.41 ^{cd}
	Ca.Sa.7	Akman 98	0 ± 0 ^{f}
		Nihatbey	36.67 ± 3.33 ^{b}
		Göynük 98	51.67 ± 6.01 ^{a}
		Zulbiye	8.33 ± 1.67 ^{def}
		Karacasehir 90	5 ± 2.89 ^{ef}
Psp	La.Ak.9	Akman 98	5.0 ± 0.87 ^{ef}
		Nihatbey	48.33 ± 4.41 ^{a}
		Göynük -98	55 ± 8.66 ^{a}
		Zulbiye	15 ± 2.89 ^{de}
		Karacasehir 90	28.33 ± 4.41 ^{bc}
	Ba.Ka.2	Akman 98	5 ± 0 ef
		Nihatbey	56.67 ± 3.33 ^{a}
		Göynük 98	60 ± 5.77 ^{a}
		Zulbiye	15 ± 5 ^{de}
		Karacasehir - 90	30 ± 2.89 ^{bc}
	Control (Water)	Akman 98	0 ±0 ^{f}
		Nihatbey	0 ±0 ^{f}
		Göynük 98	0 ±0 ^{f}
		Zulbiye	0 ±0 ^{f}
		Karacasehir 90	0 ±0 ^{f}
	Significance level		P<0.001

Eight Xap and 5 Psp isolates identified in this study caused disease on cultivar Göynük 98 in pot trials. Leaf chlorosis and necrosis as young lesion symptoms were observed on bean plants, caused by *Xap* isolates after 5-7 days (Figure 4B). Lesions became brown and elongated and extended at the margin of leaves. A big part of top foliage necrosis occurred on 14–16th day after inoculation in comparison to control plants. There was no significant difference between virulences of *Xap* isolates except for the isolate Ca.Sa.7. Severity for Ca.Ko.2, Ca.Ko.3 and Ca.As.3 varied from 56.13% to 62.50% (P> 0,001) when compared with Ca.Sa.7 that

had the least ability of virulence (P<0,001). For Psp isolates, chlorotic halos were observed at 4th day (Figure 4C), spreading outwards from the spots, following days haloes from adjacent spots joined up at 10 day and turned to brown lesions covered on 15-17th day. Disease severity of Psp isolates La.Ak.9 and Ba.Ka.2 were rated from 68.33% to 75.00% with no significant difference (P<0,001). No disease symptoms were observed on the control plants (Table 5).

Table 5. Virulence of the isolates on Göynük 98 cultivar in pot assay

Pathogen	Isolates	Disease severity (%) (Mean ± SD)
Xap	Ca.Ko.2	62.50 ± 0.65^{ab}
	Ca.Ko.3	56.13 ± 2.07^{b}
	Ca.As.3	62.50 ± 1.85^{ab}
	Ca.Sa.7	43.80 ± 1.41^{c}
	Control (Water)	0 ±0 d
Psp	La.Ak.9	75.00 ± 3.42^{a}
	Ba.Ka.2	68.73 ± 0.96^{a}
	Control(Water)	0 ± 0^{b}
Significance level	P<0.001	

4. Discussion

The overall objective of our study was to determine the occurrence of Xap and Psp on common bean in the Samsun province situated in Black Sea Region. Purified colonies of a total 58 isolates from common bean leaves collected during surveys in different locations of Samsun province were examined and characterised. According to the tests, 8 *Xap* and 5 *Psp* isolates, each representing different field, were identified from diseased bean leaves. Bacterial isolates belonging to *Xap* and *Psp* were confirmed to be *Xap* and *Psp* through biochemical, pathogenicity, PCR amplifications and BLAST searches. Biochemical properties of *Xap* and *Psp* isolates were congruent with other studies (Wortmann and Allen, 1994, Güven et al. 2004; Popovic et al. 2010). Phylogenetic tree analysis ascertained other identification methods by placing sequenced isolates of this study, grouping in the same clade with *Xap* and *Psp* strains derived from GenBank database. In this study, obtained 16S rDNA gene sequences of Turkish *Xap* and *Psp* isolates are first available data from Turkey.

Even though *Xap, Psp, Pss* and *Cff* isolates are reported as seed borne and economically important pathogens of common bean, the most prevalent agents are Xap and *Psp* (Bastas and Sahin, 2017). The results indicated that Xap and *Psp* are present in the region. Even though many biochemical and molecular techniques can be adequate to determine the species level of bacteria, its paramount to perform pathogenicity experiments for determination the pathovar level of *Xanthomonas* and *Pseudomonas* species, because it is crucial to perform pathogenicity assays of isolated colonies in detection works for emerging risks in the surveyed region (Sheppard et al. 1989; Popovic et al. 2008; 2010). As observations were recorded from our pathogenicity assay, *Xap* isolates caused blight necrosis at the edge of leaves surrounded with irregular shaped yellowish areas and typical haloes on leaves were seen by *Psp* isolates. Haloes around necrotic areas caused by *Psp* are very characteristic disease symptom of halo bacterial blight. Primers PsppF/R designed for genes responsible for phaseolotoxin production was able to amplify target DNA of Psp which means that our *Psp* isolates have an ability to produce phaseolotoxin (Schaad et al. 2007). Primers used in this work have an ability to produce PCR amplicon from isolated colonies of *Xap* and *Psp* with their reference isolates which their pathovar level was approved by pathogenicity assay on common bean. Even though primers designed in this study have not been tested on other pathovars of *Xanthomonas* and *Pseudomonas*, they were useful for identification of *Xap* and *Psp* isolates supported with further pathogenicity assays to confirm the obtained PCR results.

In the surveyed area, number of obtained isolates might be assumed small, but the area can be considered to be at risk of these pathogens because only one or two infected seeds are enough to cause a severe outbreak (Webster et al. 1983). One infected plant in 10.000 could be sufficient to cause epidemic for the common blight disease caused by *Xap* in bean fields (Saettler, 1991). All *Xap* isolates of this work were detected in Carsamba district where in 10-20 meters altitude there are favourable conditions for pathogens, especially for *Xap* which causes extreme yield losses at high humidity and temperature. Most of the farmers use certificated commercial seeds as a primary control of pathogens in Carsamba, Terme and Bafra districts, where sprinkler irrigation systems are used in the region that can helpfor splashing of bacterium by water in the presence of an inoculum source and leads to contamination of healthy plants (Akhavan et al. 2013). *Psp* isolates from Ladik and Bafra districts were detected on plant leaves showing halo blight symptoms. The *Psp* infection most probably originated from the use of contaminated seeds from the previous season, because the visited grower showed the sown seeds that were in wrinkled shape. Use of disease free seeds does not always guarantee disease control due to the existence of other inoculum sources

(Allen et al. 1998). Availability of cultivars resistant to the pathogens is the most effective method to decrease losses caused by *Xap* and *Psp* (Saettler, 1989; Fourie, 2002). In our pathogenicity assays, five local Turkish cultivars of common bean were evaluated for their reaction to *Xap* and *Psp* isolates in petri and pot conditions. The cultivar Akman 98 that was determined to be most resistant where as Göynük 98 was the most susceptible to *Xap* and *Psp* isolates among the cultivars tested in this work. Bozkurt (2009) found Göynük 98 cultivar as susceptible to three and resistant to two *Xap* isolates in petri experiment. In the other study, Bozkurt and Soylu (2001) showed that none of the tested local Turkish cultivars were resistant to all nine tested races of *Psp* and determined Göynük 98 to be susceptible for 1, 6, 8 and 9 races, but resistant or moderately resistant to other races of *Psp*. Hence, it is crucial to correctly identify the isolates of pathogens and their pathogenicity profiles, and consider a diverse array of races to evaluate responses of common bean during resistance and sensitivity screening (Keen, 1990; Arnold et al. 2011). Because of the differences between the races of *Xap* and *Psp* regarding their interaction with bean genotypes, the emergence of new races and increased variability of pathogens, developing long-term resistance is extremely difficult. Isolates express different levels of pathogenicity and virulence within and between geographical locations (Smale and Worley, 1956; Schwartz and Corrales, 1989). Therefore, the control of *Xap* and *Psp* in Turkey is especially essential for a large number of small scale farmers who require an effective and cost-efficient way to protect local bean varieties from pathogens adapted to specific environmental conditions (Bozkurt and Soylu, 2001). In Turkey, resistance levels of some lines/genotypes and cultivars were tested for their resistance level to *Xap* and *Psp* pathogens. Dursun et al. (2002) determined only one source among 22 genotypes that was resistant to *Xap*, and furthermore Donmez et al. (2013) detected the 36K source to be resistant for both pathogens among thirty six genotypes. For this purpose, the use of local resistant genotypes in breeding trials has a great significant for long term protection.

The results of this study based on morphological, biochemical, pathogenicity and molecular properties of *Xap* and *Psp* isolates could be used for further detection and characterisation of these isolates and disease resistance studies in the future.

5. Conclusion

Seed borne major pathogens of common bean *Xap* and *Psp* can reduce yield and seed quality under favourable conditions which may turn into an epidemic. Combined control measures such as the use of clean or certified seeds, growing resistant or moderately resistant cultivars to prevent or reducing the development of bacterial population, spraying of copper-based products, crop rotation with resistant crops, deep ploughing of all bean refuse left after harvest or avoiding wrong application of cultural practices should be applied in the surveyed regions of Samsun province where humidity and temperature are convenient for both bacterial pathogens.

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