Turkish Journal of Agriculture - Food Science and Technology, 11(s1): 2601-2608, 2023
DOI: https://doi.org/10.24925/turjaf.v11is1.2601-2608.6535

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Potential Biological Control Agents against Soft Rot Diseases Caused by Pectobacteria on Some Sugar Beet Cultivars

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ARTICLE INFO

Research Article

Received : 09.11.2023
Accepted : 22.12.2023

Keywords:
Sugar beet
Soft rot
Biological control
Climate change
Eco-friendly

ABSTRACT

Sugar beet is one of the most economically important agricultural crops cultivated in many provinces of Turkey. Especially in recent years, there has been an increase in bacterial tuber rot due to factors related to climate change. In preliminary trials, soft rot disease by *Pectobacterium caratovorum* subsp. *caratovorum* (Pcc) and *Pectobacterium betavasculorum* (Pb) were detected predominantly in sugar beets in Central Anatolia. Today, some cultural measures and copper compounds are used against soft rot agents in sugar beet, but successful results cannot be obtained in preventing the disease. In this study, a total of 270 soil samples were taken from the rhizosphere of 10 different fields in 3 different periods in 3 different ecologically diverse districts (Çumra, Altınekin and Seydişehir) of Konya, one of the provinces with the highest amount of sugar beet production in Turkey. As a result of the isolations, a total of 3064 bacterial isolates were purified and 262 of them showed antibacterial activity against Pcc and Pb *in vitro* conditions. In addition, 15 antagonist bacteria with the highest inhibitory effect on the development of both pathogens were tested in greenhouse conditions, and according to the results obtained from here, 3 antagonists with the highest effect were tested in field conditions in the cultivation areas of 3 different districts named above. Biochemical, morphological and molecular diagnoses of antagonist bacteria with high efficacy were made. According to the results obtained, it has been concluded that rhizospheric bacteria with antagonistic effect have a success rate of 33-90% against Pcc and Pb pathogens, and that the biological products to be prepared in future studies can be used in ecological, climate friendly and within sustainable agricultural practices in sugar beet production areas.

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Introduction

Sugar, a leading agricultural food resource, has been a fundamental food item with numerous applications of strategic importance for many countries for centuries. In Turkey, 95% of the sugar produced is derived from sugar beets. Sugar beet is a strategically important agricultural product in our country, both in terms of production and industry. Sugar beet (*Beta vulgaris* var. *saccharifera*) is an industrial plant that belongs to the Chenopodiaceae family, with origins in Central Europe. It is grown as an annual crop for sugar production and as a biennial crop for seed production. Additionally, sugar beet yields various by-products such as molasses, alcohol, yeast, antibiotics, bioethanol, apart from sugar (Sunulu and Sunulu, 2016). According to the Sugar Beet Product Report published by the Institute of Agricultural Economics and Policy Development in 2022, it is reported that approximately 18 million tons of sugar beet were produced in an area of about 300,000 hectares during the 2021-2022 production season, resulting in the production of 2.52 million tons of sugar (Anonymous, 2023).

According to the 2022 data in Turkey, sugar beet was cultivated in 56 provinces. The province of Konya, where the highest sugar beet production takes place, accounts for 25% of Turkey's cultivation area and covers 29% of the production. In a study that investigated the susceptibility of some commonly cultivated sugar beet varieties in Turkey to bacterial soft rot pathogens of the Pectobacterium genus, Bastas and Kaya (2019) obtained soft rot bacteria from diseased sugar beets during the 2016-2017 growing season in Konya and Karaman provinces. Biochemical, physiological, morphological, and PCR-based molecular analyses were performed for the identification and characterization of the isolates, and it was determined that the main pathogens causing soft rot disease in sugar beet plants are Pectobacterium caratovorum subsp. caratovorum and Pectobacterium betavasculorum.

Pectobacterium betavasculorum and *Pectobacterium carotovorum*, both of which are soft rot pathogens in sugar beets, are among the most destructive bacterial disease agents in sugar beet root diseases. These pathogens can also be transmitted through seeds and cause significant losses in plant growth, particularly in tuber yield and quality when suitable moisture and temperature conditions are present for pathogen development (Bastas and Kaya, 2019).

Bacteria that secrete a high amount of pectolytic and hydrolytic cell wall-degrading enzymes such as polygalacturonases, pectinases, and cellulases, develop intracellularly, leading to the softening and complete rotting of root tissues (Fassihiani and Nedaeini, 2008). Symptoms of the disease can be noticed after the roots have rotted and turned wet, dark brown, and the leaves have wilted. The disease can occur at any time during the growing season when environmental conditions are favorable. In the later stages of vegetation, soft and wet darkened tissue develops within the root structure. In the final stage of infection, sugar beet roots are completely deteriorated, resulting in a structure that has no value in terms of yield and quality. Sugar beets infected with soft rot agents continue to cause infections in silos after harvest.

These diseases occurring in sugar beet roots, which are of great agricultural and economic importance, not only result in yield losses but also cause a significant reduction in sugar content. Despite the use of certain cultural measures and copper compounds against these pathogens, successful results in disease prevention have not been achieved. Therefore, biological control studies aimed at effective, environmentally friendly, and sustainable agricultural production against *Pectobacterium caratovorum* subsp. *caratovorum* and *Pectobacterium betavasculorum*, which cause soft rot in sugar beet, are of great importance.

In this study, antagonist bacterial agents isolated from soil samples collected from sugar beet cultivation areas in Konya, a central hub for sugar beet cultivation in our country, were investigated in vitro and in vivo to explore the possibilities of biological control against soft rot pathogens *Pectobacterium caratovorum* subsp. *caratovorum* and *Pectobacterium betavasculorum*.

Materials and Methods

Isolation of Bacterial Soft Rot Pathogens and Antagonistic Bacteria

In this study, a total of 270 soil samples were collected from the rhizosphere of both diseased and healthy sugar beet plants at three different critical growth stages of sugar beets, marked with GPS coordinates, from 10 different sugar beet fields in the Altınekin, Çumra, and Seydişehir districts. The samples were collected from each field at three different time periods corresponding to three critical growth stages, including post-thinning, the beginning of tuber formation, and pre-harvest periods. The first period, post-thinning samples were collected from May 26th to June 1st, 2021, the second period, tuber formation samples were collected from July 30th to August 5th, 2021, and the third period, pre-harvest samples were collected from October 8th to October 14th, 2021. The collected samples were transferred to sterile paper bags for the isolation of antagonist isolates and then transported to the laboratory under cold chain conditions.

In addition, reference pathogenic isolates, *Pectobacterium caratovorum* subsp. *caratovorum* S1 isolate, were obtained from Selçuk University Faculty of Agriculture Molecular Plant Bacteriology Laboratory (Prof. Dr. Kubilay Kurtulus Bastas), and *Pectobacterium betavasculorum* isolate were sourced from Bozok University Faculty of Agriculture, Department of Plant Protection (Assoc. Prof. Dr. Murat Öztürk).

Isolation of Bacterial Agents

During the isolation of bacterial isolates, 10 grams of soil sample was transferred to 90 ml of sterile water, and the mixture was horizontally shaken at 150 rpm for 30 minutes. After the shaking process, dilutions of 10^{-3} CFU ml^{-1} and 10^{-5} CFU ml^{-1} were prepared from the suspensions of each sample. Subsequently, 100 µl from each suspension was triple streaked onto nutrient agar (NA) culture plates. Petri dishes were incubated at 25°C for 24-48 hours, and the developed bacterial colonies were grouped based on color, shape, and distinct colony characteristics. The purified bacteria were stored at -80°C in nutrient broth (NB) containing 25% glycerol until further use.

Pathogenicity Test of Pathogens

Isolates of *Pectobacterium betavasculorum* (Pb) and *Pectobacterium caratovorum* subsp. *caratovorum* (Pcc), cultured in NA medium for 48 hours, were adjusted to an absorbance of 0.2 at 600 nm using a spectrophotometer. Prior to application, a 2 mm diameter wound was created in the root area using a metal punch, and 25 ml of bacterial suspension was inoculated into the wound tissue. The inoculated plants were left for two weeks at 25°C and 75% humidity along with negative control groups. The reference culture Pcc isolate was used as a positive control, and sterile distilled water was applied as a negative control (Khan and Siddiqui 2020).

Determination of Hiper-Sensitivity Reaction in Tobacco by Antagonists

Before pot experiments with antagonist isolates found to be effective in in vitro efficacy tests, over-sensitivity tests were conducted in tobacco to determine the possibility of being a phytopathogen. Suspensions of 10^{8} CFU ml^{-1}density in saline buffer were prepared from the isolates with antibacterial activity developed in NA medium for 48 hours. Each prepared suspension was injected into the interveinal area between two veins of the tobacco plant (*Nicotiana tabacum* cv. Samsun). Necrosis formation in the inoculated area was considered a positive reaction 24-48 hours after inoculation (Lelliot and Stead, 1987). Sterile water was applied as a negative control, and Pcc isolate was used as a positive control.

Identification of Soft Rot and Antagonistic Bacterial Agents

For all bacterial agents obtained in the study, biochemical, morphological, and physiological tests were conducted according to Sachaad et al. (2001), and preliminary identifications of pathogenic and antagonistic bacteria were performed. In molecular diagnosis, bacterial

DNA isolation was carried out following the method of De Boer and Ward (1995), and the PCR primers used for Pb and Pcc are listed in Table 1.

After conducting biochemical, morphological, and biochemical tests for antagonist bacterial agents, molecular level MALDI-TOF-MS diagnoses were performed at the Mustafa Kemal University Plant Health Application and Research Center, and 16SrRNA diagnoses were obtained through service procurement from Subgenomic Analysis company.

Determination of in vitro Antagonistic Activity of Antagonist Bacterial Isolates

The biocontrol activities of candidate antagonist bacterial isolates, isolated from the sugar beet rhizosphere microbiota, were determined under in vitro conditions using a binary comparison test in NA medium. In the experiments, the effectiveness values for each isolate were calculated by dividing the zone diameter formed around the bacterial colony by the diameter of the bacterial colony (Bora and Özaktan, 1998; Aysan et al., 2003; Bozkurt and Soylu, 2019; Bitgen and Mirik, 2021). The trial was conducted with three replications. After incubation, suspensions of Pb and Pcc pathogens at a density of 10^8 CFU ml^{-1} were sprayed onto petri dishes at equal distances around the bacterial colonies using a hand sprayer for each application. Blank disks containing 25 µg streptomycin sulfate were used as a positive control (Umarusman et al., 2019). After 48 hours of incubation at 25°C, the inhibition zones formed around the isolates and the colony diameter of the isolates were measured in millimeters and recorded. All isolated isolates were tested separately for both pathogens.

In vivo Experiments of Antagonists

Greenhouse Experiments

In controlled greenhouse conditions, pot experiments were set up in a randomized complete block design. In 3-liter pots containing a 1:1:1 mixture of sterile peat, perlite, and soil, 3 sugar beet seeds belonging to the Mohican (sensitive) and Rodeo (resistant) varieties were sown. Two weeks after the cotyledon stage of sugar beet seedlings, thinning was performed to leave one plant per pot. Each treatment was planned with 5 replications, with one plant in each replication. Separate experiments were conducted for Pb and Pcc applications, resulting in a total of 340 pots. Fifteen antagonist isolates with the highest combined effectiveness ratio against Pb and Pcc, as determined in *in vitro* efficacy tests, were selected and tested in pot experiments.

Fifteen common antagonist isolates were prepared at a concentration of 10^9 CFU ml^{-1} in the NA medium. Prior to antagonist applications, a 1 cm wound tissue with a thickness of 2 mm was created in the root-shoot of each plant using a metal punch, 3 cm below the soil surface. Then, 25 ml of antagonist applications were made to the root area. Negative control applications included 25 mg/liter of 25 ml streptomycin sulfate, and positive control applications consisted of 25 ml of water (Ganiyu et al., 2023).

One week after the antagonist application, Pectobacteria cultured for 48 hours in NA medium were inoculated into slices at a density of 10^9 CFU ml^{-1} using a syringe. After inoculation, 25 ml of pathogen bacterial suspensions were applied to the root area of each plant. Negative control applications included 25 mg/liter of 25 ml streptomycin sulfate, and positive control applications involved the inoculation of pathogen bacteria into sugar beet seedlings that had been watered one week earlier (Ganiyu et al., 2023).

Field Experiments

Field experiments were set up in the Altınekin, Çumra, and Seydişehir districts where antagonist isolates were obtained. These field trials were established to compare the disease suppression potential of the same antagonists in areas with ecological differences.

Seeds of sugar beet varieties Mohican and Rodeo were sown with row spacing of 1×0.75 meters, with 20 cm between rows (6 plants) and 25 cm between rows (4 plants), resulting in a total of 24 plants per plot. As the basal fertilizer, DAP (Diammonium Phosphate) was applied at a rate of 20 kg/da. Herbicide treatment with Stomp Extra herbicide was conducted prior to crop emergence for weed control. The field trials were planned with a randomized complete block design, with three replications, and a total of 24 plants in each replication.

In the greenhouse experiments, three antagonists were selected that were effective against both pathogens and exerted the highest disease suppression. The same method and bacterial inoculum density used in the greenhouse experiments were applied in field experiments. Similarly, bacterial pathogens were applied using the same method and bacterial density, but two applications were made to each plant, with a one-week interval between them, using 50 ml of pathogen bacterial suspensions each time.

Effectiveness Evaluations

All plants in the experiment were harvested, and the lesion length in the longitudinally cut tubers was measured with the help of a digital caliper and expressed as a ratio to tuber length. Disease severity (%) was calculated accordingly. The effectiveness of antagonist isolates was determined by comparing them to the positive control using the Abbott formula (% efficacy: (control-treatment/control) ×100) (Karman, 1971).

Statistical Analysis

The % efficacy of different treatments was analyzed using the Anova statistical program with the LSD multiple comparison test at a significance level of $P \leq 0.05$ (Umarusman et al., 2019).

Table 1. PCR Primers Used for Pb and Pcc

Primer	Pathogen	PCR Products (bp)	Reference
L1(5'CAAGGCATCCACCGT3') G1(5'GAAGTCGTAACAAGG3')	Pb	540 620	(Toth et al., 2011)
Y1('TTACCGGACGCCGAGCTGTGGCGT') Y2('CAGGAAGATGTCGTTATCGCGAGT')	Pcc	434 434	(Darrasse et al., 1994)

Table 2. PCR Protocols for Pb and Pcc

L1 G1 Primers			Y1 Y2 Primers		
Initial denaturation	94°C - 5 dk		Initial denaturation	94°C - 5 dk	
Denaturation	94°C –1 dk		Denaturation	94°C –30 sn	
Annealing	55° C –2 dk	28 Cycles	Annealing	55° C –45 sn	34 Cycles
Extension	72° C –2 dk		Extension	72° C –45 sn	
Final extension	72°C - 7 dk		Final extension	72°C - 7 dk	

Table 3. Reactions of isolated Pb and Pcc isolates in diagnostic tests

Analysis	Pb (Ref.)	Pcc S1 (Ref.)	Pb A2	Pb A5	Pcc Ç4	Pcc Ç11	Pb Sy9	Pcc Sy4	Pcc Sy7	Pcc Sy9
Gram Reaction	-	-	-	-	-	-	-	-	-	-
CVP Deepening	+	+	+	+	+	+	+	+	+	+
Pectolytic Activity	+	+	+	+	+	+	+	+	+	+
Bacterial Growth at 37°C	+	+	+	+	+	+	+	+	+	+
Bacterial Growth in 5% NaCl	+	+	+	+	+	+	+	+	+	+
Oxidase Reaction	-	-	-	-	-	-	-	-	-	-
Levan Production	+	+	+	+	+	+	+	+	+	+
Presence of Catalase	+	+	+	+	+	+	+	+	+	+
Hypersensitivity Reaction (HR)	+	+	+	+	+	+	+	+	+	+
Erythromycin Sensitivity	-	-	-	-	-	-	-	-	-	-
Utilization of a-Methyl-Glucoside	+	+	+	+	+	+	+	+	+	+
KB Fluorescent Pigmentation	-	-	-	-	-	-	-	-	-	-
Reduction of Substances from Sucrose	+	+	+	+	+	+	+	+	+	+
Indole Production	-	-	-	-	-	-	-	-	-	-
Phosphatase Production	-	-	-	-	-	-	-	-	-	-
Acid Production from Lactose	+	+	+	+	+	+	+	+	+	+
Acid Production from Maltose	+	+	+	+	+	+	+	+	+	+
Acid Production from Trehalose	+	+	+	+	+	+	+	+	+	
Acid Production from Sorbitol	-	-	-	-	-	-	-	-	-	-
Acid Production from Malonate	-	-	-	-	-	-	-	-	-	-

Results

Identification of Soft Rot Pathogens

The soft rot isolates obtained were identified as Pb and Pcc based on biochemical, morphological, physiological, HR, and pathogenicity tests (Sachaad et al., 2001). Diagnostic tests of pathogens are listed in Table 3.

In molecular diagnosis, specific bands of approximately 540 and 620 bp were obtained for the Pb isolates using L1/G1 primers, and for Pcc isolates, approximately 434 bp bands were obtained using Y1/Y2 primers. According to the results obtained, it was determined that 2 isolates from the Altınekin region were Pb, 2 isolates from the Çumra region were Pcc, 3 isolates from the Seydişehir region were Pcc, and 1 isolate was Pb.

Isolation of Antagonist Bacterial Agents

In this study, a total of 3064 bacterial isolates were obtained from 270 soil samples collected from Altınekin, Çumra, and Seydişehir districts. According to their regional distribution, Altınekin region obtained 378 isolates from the post-thinning period, 356 isolates from the beginning of tuber formation period, and 315 isolates from the pre-harvest period samples. Çumra region obtained 318 isolates from the post-thinning period, 360 isolates from the beginning of tuber formation period, and 345 isolates from the pre-harvest period samples. Seydişehir region obtained 316 isolates from the post-thinning period, 328 isolates from the beginning of tuber formation period, and 348 isolates from the pre-harvest period samples.

Determination of Antagonist Bacterial Isolates' Antagonistic in vitro Activity

In the *in vitro* conditions with Petri dishes containing NA agar, a total of 3064 bacterial isolates from sugar beet rhizosphere were tested against Pb and Pcc separately. As a result of these tests, 64 isolates from the I. period, 136 isolates from the II. period, and 60 isolates from the III. period showed antagonistic effects. Of all antagonist isolates, 45% were effective against Pcc, 50% against Pb, and 28% showed antagonistic effects against both pathogens (Figure 1).

The results of the tests showed that the IS.Ç.5.1.C isolate had a higher efficacy value than the streptomycin antibiotic used as the positive control. The most successful 15 antagonists that showed antagonistic effects against both pathogens are listed in Table 4.

Effect of Antagonists on Soft Rot Disease in Greenhouse Trials

Sugar beet plants of the Mohican and Rodeo varieties that underwent antagonist and pathogenic bacterial inoculations were observed for typical soft rot disease symptoms around the inoculation points three weeks after inoculations, and the study was terminated.

According to the results of the sugar beet pot trials established under greenhouse conditions with the 15 antagonist isolates that had the highest efficacy values in in vitro effectiveness tests and showed common effects on Pb

and Pcc (Table 4), Pb caused a disease rate of 97.27% in the susceptible Mohican variety. In the antagonist applications, the IS.A.4.3.O isolate was found to suppress the disease by 89.99%. Pb caused a disease rate of 56.67% in the resistant Rodeo variety. Among the applications, YB.S.7.2.F isolate showed the most successful effect with a suppression rate of 88.00% (Figure 1). According to the results of the Pcc greenhouse trials, Pcc caused a disease rate of 87.39% in the susceptible Mohican variety. In the antagonist applications, the IS.Ç.5.1.C isolate was found to suppress the disease by 90.00%. Pcc caused a disease rate of 95.30% in the resistant Rodeo variety. Similar to the susceptible variety, the IS.Ç.5.1.C isolate showed the most successful effect with a suppression rate of 84.09%.

Table 4. *In vitro* activity value of antagonistic bacterial agents against soft rot agents in sugar beet and their effectiveness under greenhouse conditions (%)

İzolat Kodu	Pcc *In vitro* Activity	Pcc Mohican		Pcc Rodeo	
		Disease Incidence (%)	Disease Control (%)	Disease Incidence (%)	Disease Control (%)
N.C.		0		0	
P.C.	60	87.39a		95.3a	
İS.Ç. 5.1.C	63	8.74h	90.00	15.17f	84.09
HÖ.A.9.2.G	40	43bc	50.80	17.23ef	81.92
İS.A.4.3.O	40	26.16defg	70.07	27.92bc	70.71
YB.A.9.3.D	30	25.16defg	71.22	26.59bcd	72.09
YB.A.8.1.N	23	27.41def	68.64	15.98ef	83.23
YB.A.9.2.O	23	22.23efg	74.56	19.64def	79.39
YB.A.10.2.L	23	14.65gh	83.24	20.16cdef	78.84
YB.S.7.2.F	23	19.68fgh	77.48	28.72b	69.86
YB.S.7.2.N	23	28.7def	67.17	16.99ef	82.17
YB.A.4.1.H	22	25.3defg	71.05	13.62f	85.71
İS.Ç.8.3.A	22	20.56fgh	76.48	25.6bcd	73.13
YB.A.7.3.G	20	20.37fgh	76.69	29.81b	68.72
YB.A.3.1.N	20	36.96bcd	57.71	20.81cdef	78.16
YB.Ç.2.1.A	20	34.21cde	60.85	23.75bcde	75.08
YB.S.7.2.K	20	47.82b	45.29	25.29bcd	73.46
İzolat Kodu	Pb *In vitro* Activity	Pb Mohican		Pb Rodeo	
		Disease Incidence (%)	Disease Control (%)	Disease Incidence (%)	Disease Control (%)
N.C.		0		0	
P.C.	60	97.27a		56.67a	
İS.Ç. 5.1.C	68	16.09f	83.46	23.73b	58.13
HÖ.A.9.2.G	30	18.57f	80.92	12.21bc	78.46
İS.A.4.3.O	35	9.74f	89.99	10.15bc	82.09
YB.A.9.3.D	25	18.85f	80.63	13.96bc	75.36
YB.A.8.1.N	23	49.31b	49.33	7.97bc	85.94
YB.A.9.2.O	23	35.22cd	63.80	11.4bc	79.89
YB.A.10.2.L	23	37.42bcd	61.54	19.62bc	65.38
YB.S.7.2.F	22	33.11de	65.97	6.8c	88.00
YB.S.7.2.N	20	41.06bc	57.80	19.21bc	66.10
YB.A.4.1.H	20	19.22f	80.24	12.24bc	78.41
İS.Ç.8.3.A	20	13.03f	86.61	9.72bc	82.85
YB.A.7.3.G	18	16.11f	83.45	13.2bc	76.70
YB.A.3.1.N	20	36.64cd	62.34	10.54bc	81.40
YB.Ç.2.1.A	17	22.1ef	77.29	9.94bc	82.45
YB.S.7.2.K	18	46.29bc	52.42	9.89bc	82.55

Means followed by the same letter within a column are not significantly different from each other at $P \leq 0.05$ according to Duncan's Multiple Range Test

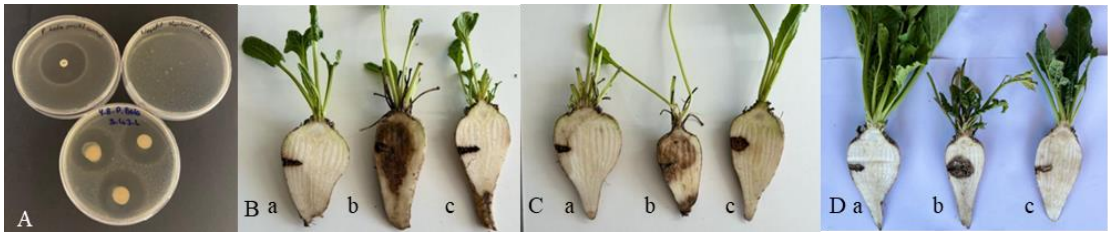

Figure 1. Biological activity assays on sugar beet

A. Antibacterial activity of antagonists against pathogens in *in vitro* effectiveness tests, B. Effect of YB.S.7.2.F isolate on disease severity of *Pectobacterium betavasculorum* in sugar beet variety Rodeo under greenhouse conditions, C. Effect of IS.Ç.5.1.C isolate on disease severity of *Pectobacterium caratovorum* in sugar beet variety Rodeo under greenhouse conditions, D. Effect of IS.Ç.5.1.C isolate on disease severity of *Pectobacterium betavasculorum* in sugar beet variety Rodeo under field conditions (a: Negative Control, b: Positive Control, c: Antagonist Application).

Table 5. Effectiveness of antagonistic bacterial agents against soft rot agents in field conditions in Altınekin, Çumra and Seydişehir sugar beet cultivation areas in Konya province (%)

Treatments	Altınekin Field Trials							
	Pb Mohican		Pb Rodeo		Pcc Mohican		Pcc Rodeo	
	DI	DC	DI	DC	DI	DC	DI	DC
N.C.	0.0		0.0		0.0		0.0	
P.C.	31.84a		40.41a		72.36a		73.38a	
İS.Ç.5.1.C	10.07c	68.38	9.53c	76.42	16.5b	77.18	12.07b	83.55
YB.A.4.1.H	16.13b	49.35	15.26b	62.24	18.52b	74.39	13.42b	81.71
İS.Ç.8.3.A	16.88b	46.98	11.4bc	71.79	10.46b	85.54	16.46b	77.57
Treatments	Çumra Field Trials							
	Pb Mohican		Pb Rodeo		Pcc Mohican		Pcc Rodeo	
	DI	DC	DI	DC	DI	DC	DI	DC
N.C.	0.0		0.0		0.0		0.0	
P.C.	20.84a		34.07a		35.73a		42.88a	
İS.Ç.5.1.C	6.23c	70.12	5.66bc	83.38	7.3c	79.56	8.68b	79.75
YB.A.4.1.H	7.1c	65.91	10.45b	69.33	12.17b	65.94	7.29b	83
İS.Ç.8.3.A	13.92b	33.18	7.75bc	77.24	8.72b	75.6	7.99b	81.37
Treatments	Seydişehir Field Trials							
	Pb Mohican		Pb Rodeo		Pcc Mohican		Pcc Rodeo	
	DI	DC	DI	DC	DI	DC	DI	DC
N.C.	0.0		0.0		0.0		0.0	
P.C.	15.5a		14.59a		62.78a		44.02a	
İS.Ç.5.1.C	6.19c	60.04	6.9c	52.7	16.9ab	73.07	16.61b	62.28
YB.A.4.1.H	8.71b	43.8	10.64ab	27.08	8.33b	86.73	8.92bc	79.74
İS.Ç.8.3.A	7.67bc	50.53	8.79b	39.74	8.77b	86.02	6.14c	86.05

DI: Disease Incidence (%); DC: Disease Control (%); Means followed by the same letter within a column are not significantly different from each other at $P \leq 0.05$ according to Duncan's Multiple Range Test

Effect of Antagonister on Soft Rot Disease in Field Trials

In the study, field trials with Mohican and Rodeo varieties were conducted in Altınekin, Çumra and Seydişehir districts, where the antagonist isolates were collected, and the study was terminated when typical soft rot symptoms were observed in the positive control plants four weeks after the inoculations.

According to the results of Altınekin field trials; Pb caused disease at a rate of 31.88% in the Mohican sensitive variety. Pb caused disease at a rate of 40.55% in the Rodeo resistant variety. Among the treatments, the IS.5.1.C isolate showed the most successful effect with a suppression level of 76.38%, similar to the sensitive variety. IS.C.8.3.A isolate was found to suppress Pcc disease by 84.03%. Pcc caused disease at a rate of 72.28% in the Rodeo resistant variety.

According to the results of Cumra field trials; Pb caused disease at a rate of 20.50% in the Mohican sensitive variety. It was observed that the IS.C.5.1.C isolate suppressed the disease by 70.14%. Pb caused disease at a rate of 34.08% in the Rodeo resistant variety. Similarly, among the treatments, the most successful effect on the sensitive variety was the IS.Ç.5.1.C isolate, with a suppression level of 72.74%. Pcc caused disease at a rate of 35.85% in the Mohican sensitive variety. It was observed that the application of IS.C.5.1.C isolate suppressed the disease by 79.26%. Pcc caused disease at a rate of 44.10% in the Rodeo resistant variety. Among the applications, unlike the sensitive variety, the YB.A.4.1.H application showed the most successful effect, with a suppression level of 83.39%.

According to the results of Seydişehir field trials; Pb caused disease at a rate of 15.72% in the Mohican sensitive variety. It was observed that the application of IS.C.5.1.C isolate suppressed the disease by 60.31%. Pb caused disease at a rate of 14.57% in the Rodeo resistant variety. Among the treatments, the IS.C.5.1.C isolate showed the most successful effect with a suppression level of 55.98%, similar to the sensitive variety. Pcc caused disease at a rate of 64.24% in the Mohican susceptible variety. Pcc caused disease at a rate of 44.24% in the Rodeo resistant variety. Among the applications, unlike the sensitive variety, the most successful effect was the IS.Ç.8.3.A isolate application, with a suppression rate of 90.38% (Table 5).

Re-isolation Studies

In accordance with Koch's postulates, reisolation of soft rot bacteria from sugar beet plants was carried out following in vivo greenhouse and field trials. Samples were collected from plant tissues displaying typical soft rot symptoms, and reisolation was performed. The obtained agents were identified as the disease agents in plants as Pb and Pcc through biochemical, morphological, physiological, and molecular tests.

Discussion and Conclusion

Sugar beet soft rot disease agents *Pectobacterium betavasculorum* and *Pectobacterium carotovorum* subsp. *caratovorum*, which have great economic importance in our country as well as in the world, cause significant yield and quality losses. While the disease generally causes around 15-30% damage (Agrios, 2006), Pb, specific to sugar beet, has been reported to cause over 40% crop loss

in some growing areas (Thomson et al., 1981). As a matter of fact, it is known that bacterial soft rot disease is increasing in the Konya region, which has the largest cultivation and production area in our country (Bastas and Kaya, 2019).

In the managements against the plant pathogens, apart from cultural measures, the recommended copper preparations and limited chemical control practices are insufficient in the management against these diseases. For this reason, the development of alternative methods such as biological control is of great importance in terms of producing effective biopesticides and protecting environmental health (Li et al., 2018; Safara et al., 2022; Kim et al., 2023). In our country, there are successful studies on the use of antagonist bacteria in the fight against many pathogenic bacterial disease factors (Bora and Özaktan, 1998; Aysan et al., 2003; Bozkurt and Soylu, 2019; Bitgen and Mirik, 2021; Aktepe and Aysan, 2023).

In biological control studies of sugar beet root rot disease agents in different regions of the world, bacteria (Xiao et al., 2011), fungus (Bagy et al., 2019), virus (Kim et al., 2023) and yeast (Hassan et al., 2019). It has been reported that different prokaryotic and eukaryotic microorganisms such as) give successful results. However, to date, no information has been found on biological control studies against soft rot disease in the sugar beet production areas of our country. With increasing temperatures due to global climate change, the emergence or infections of some plant pathogenic bacterial agents have begun to increase. Especially heat-loving bacterial factors, one of which is Pectobacterium spp. It causes significant yield and quality problems (Schaad et al., 2001).

In our study, for the first time in our country, the effectiveness of antagonistic bacterial factors isolated from Konya sugar beet cultivation areas against soft rot disease was investigated. For this purpose, 3064 bacterial isolates were obtained from 270 soil samples collected from 3 different districts with the highest production amounts and different ecological conditions (Altınekin, Çumra and Seydişehir), 15 of which showed antagonistic effects on both Pcc and Pb.

In *in vitro* tests against the sugar beet soft rot disease agents Pb and Pcc of antagonist isolates specific to our country and Konya province, the effectiveness value of the streptomycin antibiotic was 60%, while İ.S. Ç.5.1.C isolate showed 68% effectiveness for Pb and 63% effectiveness for Pcc. The disease severity suppression rates of 15 antagonist isolates applied in greenhouse trials showed effectiveness varying between 49-86% depending on resistant and sensitive varieties.

In *in vivo* experiments conducted in Altınekin, Çumra and Seydişehir districts where the antagonist isolates were collected, the isolate's disease suppression rates were 33-90%, depending on the resistant and susceptible varieties and the districts. It has been demonstrated by many studies that biochemical substances, secondary metabolites, enzymes or volatile organic compounds produced by friendly microorganisms used in biological control studies of plant pathogenic bacteria significantly reduce disease severity by targeting the growth, proliferation, biofilm formation or quorum-sensing mechanisms of pathogenic bacteria (Garge et al. Nerurkar, 2016; Kumar et al., 2016; Zhang et al., 2019; Vesuna and Nerurkar, 2020; Safara et al., 2022).

Studies are continuing on the mechanism of action of bacterial antagonists, which were determined to be highly effective against soft rot disease in our trials, in preventing the disease. The findings to be obtained can be used effectively in the production of biological preparations. The methods of antagonist microorganisms of application, their high potential to colonize the applied area, and their survival time are important factors affecting the success of biological control. In addition, determining the virulence mechanisms of the target pathogen and using antagonist microorganisms that have a specific effect on these mechanisms increases the success of biological control.

While the results we obtained in this study form the basis for the development of effective bioformulations in the control against soft rot factors, which have caused significant losses in sugar beet cultivation in our country in recent years, it is thought that these data will enable the development of biopesticides within the scope of sustainable, organic and environmentally friendly control practices.

Acknowledgements

This research was presented at the 3rd International Congress of the Turkish Journal of Agriculture - Food Science and Technology, Malatya, Turkiye, held on 13 and 16 September 2023 (as an oral presentation).

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