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RESEARCH ARTICLE

In vitro Effect of Bacterial Biocontrol Organisms against *Pectobacterium carotovorum* on Potato

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ABSTRACT

Soft rot is a disease that cause substantial economic losses in potato production. *Pectobacterium carotovorum* is known as one of the most common soft rot causative agents and the disease has not effective control methods being developed yet. In this study the effect of 3 different bacterial isolates of *Bacillus megaterium* (B60d, TV6D, TV91C), 2 of *Paenibacillus polymyxa* (Ç9, TV12E), 2 of *Bacillus subtilis* (TV6F, TV17C), 1 of *Pantoea agglomerans* (B79), 1 of *Agrobacterium radiobacter* (A16), 1 of *Bacillus megaterium* gc. subgroup A (FDG161), 1 of *Bacillus atrophaeus* (FD1), 1 of *Pseudomonas fluorescens* biotype F (FDG37) and 1 of *Bacillus pumilus* (TV3D) were tested against 5 pathogenic bacterial strains of *P. carotovorum* subsp. *carotovorum* (F37, F680, F741, F331, F742), using dual culture method in *in vitro* conditions. The most effective bacterial isolate against strains *Pectobacterium* were *P. carotovorum* F37 + *Bacillus megaterium* B60d (55,00 mm) isolate, followed by *P. carotovorum* F680 + *Bacillus megaterium* B60d (44,00 mm) and *P. carotovorum* F742 + *Bacillus megaterium* B60d (39,33 mm) isolates. Therefore, *Bacillus megaterium* has a promising biocontrol activity against tested plant pathogenic bacteria under *in vitro* conditions.

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Introduction

In terms of global production, potato (*Solanum tuberosum L.*) is the fourth most important food crop after corn, rice and wheat. This crop is grown throughout the world. According to FAO (Food and Agriculture Organization), in 2016 the world production was about 376 million tons potato. Asia and Europe are the world's major potato producing regions, accounting for more than 80% of world production (FAO, 2008).

Potato production globally is constrained by factors which cause substantial economic losses. These can be in the form of biotic and abiotic factors. The greatest losses are due to diseases and some of the important bacterial diseases are tuber soft rot, blackleg and aerial stem rot in the field (Ngadze, 2012).

For the soft rot, its characterized symptoms like sprouts of infected tubers that causes failure to emerge from the soil following planting and the emerged sprouts may show curled upper leaves, compact foliage, stunting, and fading from green to yellow-green. Infected plants later assume a distinct yellow color and gradually die as the lower stem rots away. When pulled, affected plants will have slimy, rotted, dark or inky black, mushy stems (Seebold, 2014). This disease mainly is caused by *Pectobacterium* and *Dickeya spp.*

Pectobacterium carotovorum (previously belonged to the genus *Erwinia*) is a Gram-negative plant-specific pathogen, causes soft rot diseases in monocot and dicot host plants in at least 35% of angiosperms (Marquez-Villavicencio et al., 2011). In potato it causes the disease by degradation of the plant cell wall (Aizawa, 2014).

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The effective control methods against the disease has not been developed, yet (Jeong-A et al., 2013). Various strategies such as chemical antibiotics and copper have been developed and used for many years (Cooksey, 1990). However, copper resistance has been reported in many bacterial pathogens and few effective bactericides have been developed (Cooksey, 1990; Bender et al., 1990). Chemicals are not effective in controlling soft rot pathogens; control strategies rely on the use of resistant cultivars, good agronomic practices such as planting certified disease-free seed, planting in well-drained soil and good sanitation (Ngazde, 2012; Rahman et al., 2017). Thus, novel strategies for the control of bacterial diseases are required and currently particular attention has been paid to biologically based strategies, such as bacteriocins or bacteriophages (Jeong-A et al., 2013).

In this context, the focus of biological control studies reflects the desire of several sectors to develop sustainable methods for plant disease control. However, efficient antagonists must be obtained for biological control to become a reality (Mota et al., 2016). Development of *in vivo* biocontrol agent selection is not a simple task due to the diversity of agents and interactions with the host plant, and therefore, efficient search methods are required. Therefore, it is necessary to develop efficient selection strategies to reduce costs and increase the possibility of selecting organisms that can be produced in a large scale at low cost and that maintain their viability and efficiency for long periods (Mota et al., 2016; Schisler and Slininger, 1997).

Materials and Methods

Material

Pathogen bacteria and biological control agent bacteria

Pathogen isolates used in this study were identified by using microbial identification system (MIS) and BIOLOG system. 5 pathogenic bacterial isolates were obtained from the culture collection in the Department of Plant Protection, Faculty of Agriculture at Ataturk University, Erzurum, Turkey. Pathogenic bacteria isolates information are given in Table 1.

Table 1. Pathogenic bacteria isolates (Dadaşoğlu, 2013)

Bacteria No	Identification Results
F- 37	*P. carotovorum* subsp. *carotovorum*
F- 331	*P. carotovorum* subsp. *carotovorum*
F- 680	*P. carotovorum* subsp. *carotovorum*
F-741	*P. carotovorum* subsp. *carotovorum*
F-742	*P. carotovorum* subsp. *carotovorum*

Bacterial biocontrol isolates were obtained from the Cultural Collection of the Department of Plant Protection, Faculty of Agriculture, Ataturk University. Bioagent bacteria isolates were grown on nutrient agar (NA) for routine use, and maintained in Luria Bertani (LB) Broth with 30% glycerol at –80°C for long-term storage. These isolates have been determined to be used as a plant growth agent and biosystems against pests and bacterial and fungal plant pathogens. In this study, 13 bacterial isolates were used among the numerous bacterial isolates considering the results of the previously conducted studies (Table 2).

Table 2. Bioagent bacteria isolates

Bacteria No	Identification Results
B60d	*Bacillus megaterium*
B79	*Pantoea agglomerans*
B16	*Agrobacterium radiobacter*
Ç9	*Paenibacillus polymyxa*
FDG161	*Bacillus megaterium* **gc subproup** *A*
FD1	*Bacillus atrophaeus*
FDG37	*Pseudomonas fluorescens* biotype F
TV6F	*Bacillus subtilis*
TV12E	*Paenibacillus polymyxa*
TV17C	*Bacillus subtilis*
TV6D	*Bacillus megaterium*
TV3D	*Bacillus pumilus*
TV91C	*Bacillus megaterium*

Method

Bioagent bacterial isolates test in petri against pathogens

Frozen pathogens and potential bioagent bacterial cultures were placed in petri plates containing NA and left to incubation at 25-27°C, 24 hours later fresh culture were obtained for *in vitro* dual culture method. Fresh pathogens developed cultures were taken and spread on the surface of the nutrient agar and the bacterial potential bioagent was applied in the middle of the petri plates (diameter 6 mm). Petri dishes were wrapped with parafilm and allowed to incubate for 48 hours at 27 ° C. Finally, for the evaluation of the inhibition zones or the hyperparasite effects, the propagation of the colonies of bacterial bioagent on the surface of the Petri dishes was measured.

Pectolytic activity tests

Fresh and healthy potato tubers were sterilized in 5% sodium hypochlorite for 10 minutes. Sterilized tubers were sliced 5 mm diameter and placed onto petri dishes containing sterilized wet papers. Bacterial broth cultures of 24 hours incubation were injected into potato slices in 1 ml volume and these slices were incubated at 28°C. After 24-72 h observed softness was assessed as positive result. As a control solution, sdH_2O was used (Figures 1 and 2).

The data analyses

The data obtained were subjected to transformation with arcsin, then one-way variance analysis was applied and the differences between the means were compared to LSMeans Student test at P <0.01 significance level. Data analysis in the present study were processed using statistical software JUMP IN (SAS Institute, cary, NC,% .0 PC version).

Figure 1. Pectolytic activity

Figure 2. Control

Results and Discussion

Pectobacterium sp. has a large list of hosts, including many species of agricultural and scientifically important plants. Pathogen produces pectolytic enzymes which hydrolyze pectin between individual plant cells. This causes the detachment of cells and soft rot. In this study, the objective was control diseases without using chemicals. 23 bacterial isolates were used as bioagents. In this study, 13 different bacterial species were applied in vitro against the pathogen. Table 2 shows the 13 bacterial biocontrol isolates tested for activity against *P. carotovorum* subsp. *carotovorum* F37, F680, F741, F331, F742.

According to MIS, bacterial isolates were identified as 3 *Bacillus megaterium* (B60d, TV6D, TV91C), 2 *Paenibacillus polymyxa* (Ç9, TV12E), 2 *Bacillus subtilis* (TV6F, TV17C), 1 *Pantoea agglomerans* (B79), 1 *Agrobacterium radiobacter* (A16), 1 *Bacillus megaterium* gc. subgroup A (FDG161), 1 *Bacillus atrophaeus* (FD1), 1 *Pseudomonas fluorescens biotype* F (FDG37) and 1 *Bacillus pumilus* (TV3D).

The best results against *P. carotovorum* subsp. *carotovorum* (F37, F680, F741, F331, F742) are shown in Figures 3, 4, 5 and Table 3. Inoculated zone values with bacterial bioagent were found to vary between 55.00-11.67 mm. The highest activity was observed between F37 + B60d (55.00 mm) isolate, followed by F680 + B60d (44.00 mm) and F742 + B60d (39.33) isolates. The lowest activity were obtained between F37 + Ç9 (11.67 mm), F741 + Ç9 (13.33 mm) and F331 + Ç9 (16.67 mm) isolates.

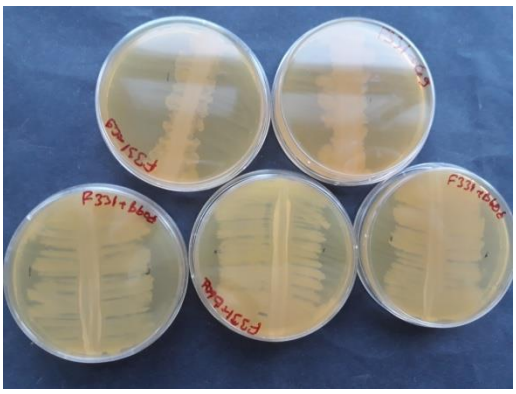

Figure 3. Hyperparasitic activity results (1)

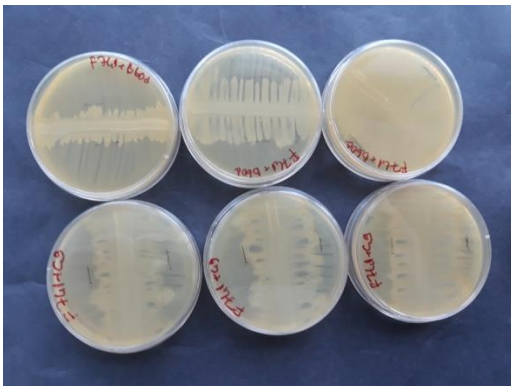

Figure 4. Hyperparasitic activity results (2)

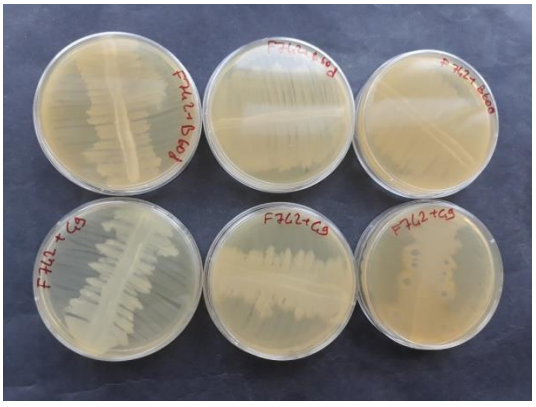

Figure 5. Hyperparasitic activity results (3)

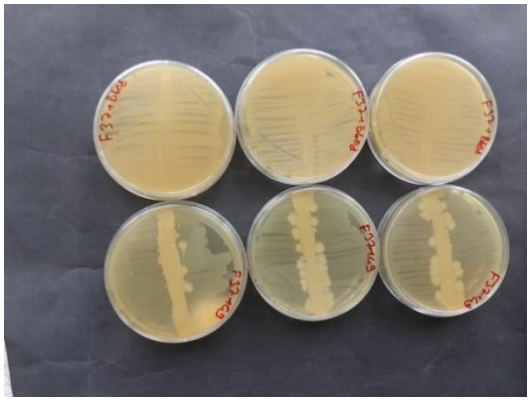

Figure 6. Hyperparasitic activity results (4)

Table 3. Inhibition rates of *P. carotovorum* subsp. *carotovorum* isolates in the dual culture tests of bacterial biocontrol isolates

No. Pathogen and bioagent bacterial isolate	ZONE (mm)	
F37 and B60d	55.00	A
F680 and B60d	44.00	B
F742 and B60d	40.00	B
F331 and B60d	39.33	BC
F741 and B60d	29.33	CD
F742 and Ç9	26.33	DE
F680 and Ç9	20.00	DEF
F331 and Ç9	16.67	EF
F741 and Ç9	13.33	F
F37 and Ç9	11.67	F
Control B60d	0.00	G
Control and Ç9	0.00	G
LSD	10.01	
CV	24.01	

Bacillus megaterium was the bacterial biocontrol isolates that had the greatest growth zone and prevented the pathogen development.

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