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Biological control of potato soft rot caused by *Erwinia carotovora* subsp. *carotovora*

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Abstract

Four bioagents, *Bacillus subtilis*, *Pseudomonas fluorescence*, *P. aeruginosa*, and *Streptomyces* spp., were used in vitro and in vivo against two pathogenic isolates of *Erwinia carotovora* subsp. *carotovora* (Ecc1 and Ecc2), the causal agent of potato soft rot. In vitro *Streptomyces* spp. showed the strongest effect against Ecc1 and Ecc2 and gave the highest values of the inhibition zones, being 37 and 40 mm, respectively followed by *P. fluorescence*, *B. subtilis*, and *P. aeruginosa*, where the inhibition zones reached, respectively, 32, 28, and 24 mm against Ecc1 and 35, 29, and 26 mm against Ecc2. Also, these results confirmed those of the in vivo experiment (in pots) since *Streptomyces* spp. bioagent exhibited the lowest number of infected tubers followed by *P. fluorescence*, *B. subtilis*, and *P. aeruginosa*, respectively, against the two isolates Ecc1 and Ecc2. Also, disease severity of soft rot caused by each of the two isolates, Ecc1 and Ecc2, was decreased by using bioagents, and the lowest disease severity values were obtained by using *Streptomyces* spp., *P. fluorescence*, *B. subtilis*, and *P. aeruginosa,* respectively.

Keywords: Biological control, *Erwinia carotovora* subsp. *carotovora*, Potato soft rot

Background

Potato, *Solanum tuberosum* L., is one of the most important food and crops worldwide, and its production in developing countries increased at the rate of 2.8% annually (CIP 1995). Bacterial soft rot and black leg are probably the most serious diseases in terms of crop losses. Infection by late blight and dry rot pathogens is significant, not only because of the damage they cause to potato tubers, but also because they provide potential avenues of entrance of secondary invasion by *Erwinia carotovora* subsp. *carotovora* and *E. carotovora* subsp. *atroseptica* (Lui et al. 2005).

Microorganisms that can grow in potato rhizosphere are ideal to be used as biocontrol agents, since the rhizosphere provides the front line defense for roots and tubers against attack by pathogens (Kabeil et al. 2008).

Pseudomonas fluorescence, *Bacillus subtilis*, and *E. herbicola* showed activity against *E. carotovora* subsp. *carotovora* (Vanneste and Yu et al. 1996). Streptomyces is a well-known genus of the order Actinomycetales family. They usually inhabit soil and commonly enhance soil fertility. These prokaryotes have characteristics which make them useful as biocontrol agents against bacterial plant pathogens (Keiser et al. 2000). Biological control is considered as one of the most important methods to control bacterial soft rot disease in potato tubers (Algeblawi and Adam 2013).

In this study, we aimed to evaluate in vitro and in vivo effectiveness of four bioagents, *B. subtilis*, *P. fluorescence*, *P. aeruginosa*, and *Streptomyces* spp., against *E. carotovora* subsp. *carotovora* (Ecc1 and Ecc2), the causal agent of potato soft rot.

Materials and methods

Potato tubers

Potato seeds (*Solanum tuberosum* L., Diamante variety) were obtained from the Horticulture Department, Agricultural Research Center, Giza, Egypt.

Bacterial strains

Two isolates of *E. carotovora* subsp. *carotovora* (Ecc1 and Ecc2) WPP17 were obtained from the Bacterial Disease Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

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Antagonistic organisms

The antagonistic bacteria and actinomycetes (*B. subtilis*, *P. fluorescence*, *P. aeruginosa*, and *Streptomyces* spp.) were obtained from Bacteriological Lab, Faculty of Science, Zagazig University, Egypt.

Sensitivity of E. carotovora subsp. carotovora isolates to antagonistic microorganisms (in vitro)

Antagonistic effect of *B. subtilis*, *P. fluorescence*, *P. aeruginosa*, and *Streptomyces* spp. on two isolates of *E. carotovora* subsp. *carotovora* (Ecc1 and Ecc2) was studied. The suspension representing each of the two isolates of *E. carotovora* subsp. *carotovora* (1×10^5 CFU/cm^3) was spread on the surface of the Czabe-dox agar media in petri dishes by a sterilized L-shaped glass rod spreader followed by placing a 7-mm diameter agar disk cut from the margin of a culture grown in a plate on which the biocontrol strain had been grown for 48 h at 28 °C for bacteria and for 7 days for actinomycetes in the center of each plate. Inhibition zone diameter of two isolates of *E. carotovora* subsp. *carotovora* (Ecc1 and Ecc2) was measured after incubation at 28 °C for 48 h, and mean value of three replicates was calculated.

Effect of bacterial bioagents on infected potato tubers with E. carotovora subsp. carotovora (in vivo)

Pots (30 cm diameter) were sterilized by soaking in formalin (5%) for 5 min and left for 1 week to get rid of the poisonous effect of formalin. Pots were filled with autoclaved soil (autoclaved for 3 h for three successive days). Inoculation of both bioagents or pathogenic two isolates of *E. carotovora* subsp. *carotovora* (Ecc1 and Ecc2) was prepared by growing each bacterium in a conical flask containing 200 ml autoclaved sucrose-peptone liquid medium incubated at 30 °C for 48 h. Actinomycetes inoculum was prepared by growing *Streptomyces* sp. in flasks containing autoclaved starch nitrate liquid medium and incubated at 30 °C for 7 days.

Ten potato tubers (Diamante) were washed with tap water and surface sterilized by ethyl alcohol 70%. The antagonistic bacteria or actinomycetes (2×10^8 CFU/ml) were mixed with 1% (*W*/*V*) carboxymethyl cellulose as sticker agent for 1 h (Anuratha and NSS 1990). Tubers of potato after surface sterilization were soaked for 1 h in the above mixture. These tubers were left overnight in jars for drying, then cultivated in potted soil inoculated by the two isolates of *E. carotovora* subsp. *carotovora* (Ecc1 and Ecc2) each alone; the treatments were as the follows:

1. Control (untreated tubers planted in sterilized soil),
2. Tubers coated by each of the antagonistic microorganisms alone (bacteria or actinomycetes) and planted in soil,
3. Tubers planted in soil inoculated by *E. carotovora* subsp. *carotovora* (Ecc1),
4. Tubers planted in soil inoculated by *E. carotovora* subsp. *carotovora* (Ecc2),
5. Tubers coated by each of the antagonistic bacteria or actinomycetes and planted in soil inoculated by *E. carotovora* subsp. *carotovora* (Ecc1)
6. Tubers coated by each of the antagonistic bacteria or actinomycetes and planted in soil inoculated by *E. carotovora* subsp. *carotovora* (Ecc2).

Three tubers were planted in each pot, and five pots were used as replicates for each treatment; the pots left in a glass house at 28 ± 2 °C, 80% humidity, and irrigation were carefully carried out. The percentages of infection as well as healthy survivals and disease severity of the disease were recorded at the end of the experiment according to Chastanger and Ogawa (1979) based on visual inspection of each tuber infection. Infected fruits were placed in one of five categories:

0 = superficial flack (no rot)
1 = 1–24% of the surface decayed
2 = 25–49% of the surface decayed
3 = 50–74% of the surface decayed
4 = 75% or more of the surface decayed

The decay index (DI) for each treatment was obtained as follows:

$$\mathrm{DI} = \frac{\text{Sum (number of tuber per category} \times \text{category number}}{\text{Total number of infected tuber}}$$

$$\%\text{severity of infection} = (\mathrm{DI}/4 \times 100)$$

Experimental design and statistical analysis

All treatments in this study were arranged in a complete randomized design. The obtained data were subjected to analysis of variance using the general linear module procedure of Anonymous (1985), where appropriate treatment means were separated using Duncan's multiple range test (Duncan 1955) and all percentages were transferred to the analysis before statistical analysis.

Results and discussion

In vitro antagonistic effect of bacterial bioagents on two isolates of *E. carotovora* subsp. *carotovora*

Data in Table 1 show that in vitro antagonistic activity of *Streptomyces* spp. exhibited the highest inhibition zones (mm) against the two isolates of *E. carotovora* subsp. *carotovora* (Ecc1 and Ecc2), being 37 and 40 mm, respectively, followed by *P. fluorescence* (32 mm and 35 mm),

Table 1 In vitro antagonistic activity of *Bacillus subtilis*, *Pseudomonas fluorescence*, *Pseudomonas aeruginosa*, and Streptomyces spp. with two isolates of *Erwinia carotovora* subsp. *carotovora* (Ecc1 and Ecc2)

Treatment	Inhibition zone (mm)	
	Erwinia carotovora (Ecc1)	Second strain (Ecc2)
Bacillus subtilis	28 ± 0.47^{e}	29 ± 0.57^{e}
Pseudomonas fluorescence	32 ± 0.35^{d}	35 ± 0.45^{c}
P. aeruginosa	24 ± 0.47^{g}	26 ± 0.52^{f}
Streptomyces spp.	37 ± 0.35^{b}	40 ± 0.42^{a}

Various superscript letters indicate significant differences (Duncan, $p < 0.05$)

respectively, but *B. subtilis* and *P. aeruginosa* exhibited the lowest values of inhibition zones against the two isolates of *E. carotovora* subsp. *carotovora* (Ecc1 and Ecc2), being 28, 29, 24, and 26 mm, respectively.

In vivo antagonistic effect of bacterial bioagents against the two isolates of *E. carotovora* subsp. *carotovora*

Data in Table 2 show that *Streptomyces* spp. was the most potent bioagent against the two isolates of *E. carotovora* subsp. *carotovora* (Ecc1 and Ecc2) since they gave the lowest numbers of infected tubers. Meanwhile, the percentage of infected tubers recorded 10% and 5% for Ecc1 and Ecc2, respectively, followed by *P. fluorescence* which percentage recorded for infected tubers was 15% for two isolates (Ecc1 and Ecc2), respectively. *B. subtilis* reduced percentage of infected tubers to 30% and 25% (Ecc1 and Ecc2), respectively. But *P. aeruginosa* reduced percentage of infected tubers to 35% and 40% for Ecc1 and Ecc2, respectively.

Data in Fig. 1 show the severity of soft rot caused by the first isolate (Ecc1) of *E. carotovora* subsp. *carotovora* was decreased from 5 to 0.5% by using *Streptomyces* spp. as bioagent. Meanwhile, disease severity was also decreased due to using other bioagents from 5 to 0.75, 1.5, and 1.75 due to using *P. fluorescence*, *B. subtilis*, and *P. aeruginosa*, respectively. Also by using the second isolate of *E. carotovora* subsp. *carotovora* (Ecc2), the disease severity was decreased from 5 to 0.5, 0.75, 1.25, and 2 due to using *Streptomyces* spp., *P. fluorescence*, *B. subtilis*, and *P. aeruginosa*, respectively.

The obtained results show that soft rot disease caused by the two isolates of *E. carotovora* subsp. *carotovora* (Ecc1 and Ecc2) can be biologically controlled by *Streptomyces* spp., *B. subtilis*, *P. fluorescence*, and *P. aeruginosa*, respectively. These results are in agreement with those recorded by Ryan et al. (2001) who reported that *B. subtilis* GBO3 and *B. amyloliquefaciens* IN937a were able to promote plant growth indirectly through induced systematic resistance (ISR); this happens through secretion of volatiles which in turn activate an ISR pathway in Arabidopsis seedlings challenged with the soft rot pathogen *E. carotovora* subsp. *carotovora*. Lemessa and Zeller (2007) showed that using antagonistic isolates like *B. subtilis* and *P. macerans* has potential in potato bio protection or as a part of an integrated disease management package for bacterial diseases. Seaf Elyazel (2008) revealed that *Streptomyces* spp. (gram-positive filamentous bacteria) can produce and secrete a biologically active compound including antibiotics, ionophores, hydrolytic enzymes (protease, nuclease, lipase, and a variety of enzymes hydrolyzing polysaccharides), and enzyme inhibitors. These characteristics make *Streptomyces* spp. attractive candidates for biological control agents against soil-borne plant pathogens. Salem and Askora (2012) confirmed that the brown rot disease in Egyptian potato tubers caused by *Ralstonia solanacearum* can biologically be controlled by using the bioagents *P. fluorescence*, *B. subtilis*, *P. aeruginosa*, and *Streptomyces* spp., and the latter gave the effective results in controlling the brown rot in

Table 2 In vivo biological control of potato soft rot caused by two isolates of *Erwinia carotovora* subsp. *carotovora* by using *Bacillus subtilis*, *Pseudomonas fluorescence*, *Pseudomonas aeruginosa*, and Streptomyces spp.

Treatment	No. of healthy tubers	No. of infected tubers	Infection % to control
Tubers only (control)	20	0	0
Tubers + Ecc1	0	20	100
Tubers + Ecc1 + *B. subtilis*	14	6	30
Tubers + Ecc1 + *P. fluorescence.*	17	3	15
Tubers + Ecc1 + *P. aeruginosa*	13	7	35
Tubers + Ecc1 + *Streptomyces spp.*	18	2	10
Tubers + Ecc2	0	20	100
Tubers +Ecc2 + *B. subtilis*	15	5	25
Tubers + Ecc1 + *P. fluorescence*	17	3	15
Plant + Ecc1 + *P. aeruginosa*	12	8	40
Plant + Ecc1 + *Streptomyces spp.*	19	1	5

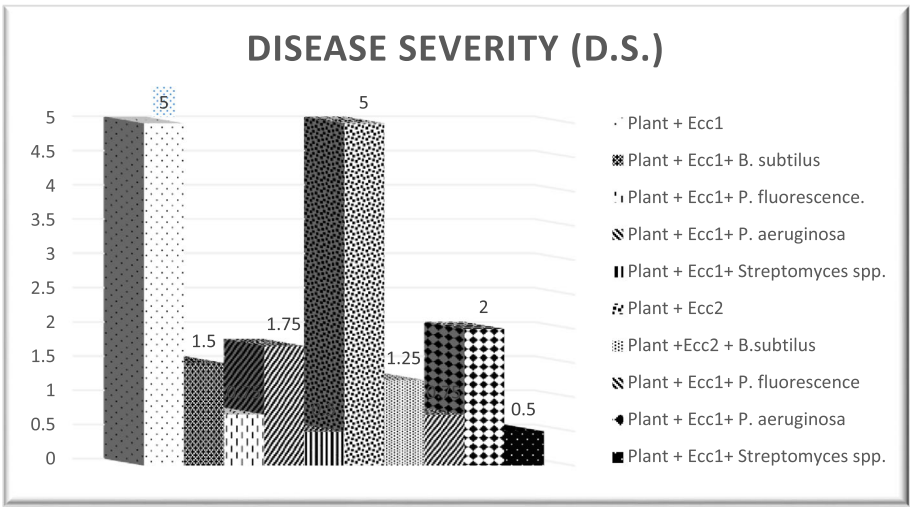

Fig. 1 Disease severity of soft rot due to using *Streptomyces spp.*, *Bacillus subtilis*, *Pseudomonas fluorescence*, and *Pseudomonas aeruginosa*

potato tubers. Algeblawi and Adam (2013) reported that the bioagents, i.e., *P. fluorescence*, *B. subtilis*, and *B. thuringiensis*, reduced soft rot disease in potato tubers caused by *E. carotovora* subsp. *carotovora* in pot experiment. The best results were obtained when isolates of *P. fluorescence and B. subtilis* were applied against *E. carotovora* subsp. *carotovora* compared to control treatment.

Conclusion

The results of the current study indicated that the usage of four bacteria as bioagents was effective in decreasing the severity of *E. carotovora* subsp. *carotovora* (Ecc1 and Ecc2) infection in potato tubers. It is worthy to note that *Streptomyces spp*. showed more pronounced effects against *E. carotovora* subsp. *carotovora* (Ecc1 and Ecc2) in either in vivo or in vitro studies than the other three bacterial bioagents.

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Availability of data and materials

The dataset supporting the conclusions of this article are included within the article.

Authors' contributions

First author EA is responsible for the implementation and conception or design of the experimental work and for designing and supervising the study. Second author YM is responsible for revising the paper scientifically, checking analysis, and interpreting data. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

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Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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