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AKADÉMIAI KIADÓ

Biological control of soft rot induced by *Dickeya solani* with *Trichoderma asperellum* on potato tubers: Relationship with susceptibility of variety

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RESEARCH ARTICLE

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ABSTRACT

Pectobacteriaceae are plant pathogens responsible for serious diseases on many crops of interest including potato. Currently, regarding the several disadvantages of conventional control by chemicals, the application of bio-agents as alternatives is increasingly being explored. The present investigation was conducted in order to evaluate the antibacterial activity of the fungal strain *Trichoderma asperellum* T34 as an antagonist against phytopathogen *Dickeya solani* on tubers of three potato varieties (Agata, Monalisa, and Picobello). For this, half-tubers were inoculated by T34 suspension with concentrations of 10^3 and 10^5 cells mL^{-1} at 6, 9, and 12 h before bacterial infection (*D. solani* at concentrations of 10^7 and 10^8 cfu mL^{-1}). The results of infection assays without the antagonist indicated that Picobello variety was the least sensitive to soft rot, Monalisa and Agata varieties showed medium and high sensitivity, respectively. The antagonism assays revealed strong antibacterial activities, manifested by the regression of softened tissues gradually with the time of preincubation with T34, leading to a complete disappearance of disease symptoms using 12 h.

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Hence, the application of antagonistic *Trichoderma* strains as antimicrobial agents in the control of harmful plant pathogens is a subject of great interest and can be considered a promising strategy to handle soft rot diseases.

KEYWORDS

Biocontrol, crop protection, *Dickeya* spp, *Trichoderma* spp, potato

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important agricultural plants throughout the world ranking 4th worldwide among food crops, in terms of economic importance, just after cereals (maize, wheat, and rice), with annual production reaching 360 million tons on a harvested area more than 16 million ha (FAOSTAT, 2020).

Given its wide distribution and growing conditions, potato is affected by more than 40 soilborne diseases caused by different bacterial, fungal, and viral agents (Fiers et al., 2012). Among these pathogens, pectinolytic bacteria *Pectobacterium* and *Dickeya spp.* are responsible for potato soft rot, one of the most severe plant diseases in the world. These macergens cause substantial ravages and serious economic losses by inducing rotting symptoms on all parts of the plant, particularly on tubers, leading each year to severe damage on potatoes during storage and transit (Czajkowski et al., 2015; Toth et al., 2011). The main species associated with a soft rot disease are *Pectobacterium atrosepticum* (Gardan et al., 2003), *Pectobacterium carotovorum* subsp. *carotovorum* (Hauben et al., 1998) and *Dickeya* spp. (Samson et al., 2005).

According to scientific literature, *Dickeya solani* emerged as a major threat to potato production that is considered the most aggressive among all soft rot bacteria (Toth et al., 2011). Indeed, many studies highlighted the great virulence of *Dickeya* species, especially *D. solani* (Garlant et al., 2013; Golanowska et al., 2017; Laurila et al., 2010).

As pathogens can infest potato crops in all stages of growth and conservation, tuber's protection is of crucial importance. Various strategies and approaches are used for disease control. Application of bactericide chemical products, breeding tolerant varieties, as well as cultural practices can reduce the percentage of infection, avoiding thus economic and agricultural losses. However, these measures cannot ensure complete and effective protection. In addition, excessive use of pesticides and antibiotics can lead to the apparition of microbial resistance among pathogens generating thereby toxic and environmentally harmful residues which induce hazardous concerns (Vinale et al., 2008). Hence, interest has been developed in safer non-chemical methods that are effective with less health risk to humans, animals, and environment (Talapatra et al., 2017). The replacement of chemicals with biological agents is an interesting alternative way to control plant pathogens, protect food, and reduce pollution. Among attractive microbial antagonists, species belonging to fungi *Trichoderma* spp. are one of the most widely used as biocontrol agents due to their large suppressive effect on several plant diseases. Many *Trichoderma* strains were studied for their antimicrobial activities against numerous fungal and bacterial phytopathogens (Schuster and Schmoll, 2010; Xiao-Yan et al., 2006), but the section of best strains and their industrialization need more exploration.

Therefore, the aim of the present work was to investigate the bio-efficiency of the antagonistic fungus *Trichoderma asperellum* towards pectinolytic bacterium *D. solani* on potato tubers of three varieties (Agata, Monalisa, and Picobello) regarding their susceptibility to the disease, as part of integrated management strategies of post-harvest pathogens.

MATERIAL AND METHODS

Antagonist

T. asperellum strain T34 was used as the antagonistic agent in bio-assays (Spanish collection of type culture, C.E.C.T. 20417, European patent application EP 1 400 586bA1) (Trillas and Cotxarrera, 2003). T34 was kindly provided by Prof. M. Isabel Trillas Gay, head of the Laboratory of Plant Physiology, Faculty of Biology, University of Barcelona, Spain. The antibacterial effect was estimated in laboratory conditions, fungal cultures were routinely grown and maintained at 25 °C. T34 was stored under refrigeration temperature (4 °C).

Potato Dextrose Agar medium (PDA: 200 g potato Infusion, 4 g, 20 g dextrose, and 15 g agar) was used for T34 cultivation. The initial solution of T34 (10^9 cfu mL^{-1}) was diluted and adjusted with distilled water to concentrations of 10^3 and 10^5 cfu mL^{-1}.

Bacterial strain

D. solani CFBP 8199 (IPO 2222 isolated in 2007 from potato in the Netherlands) (van der Wolf et al., 2014) was used as the bacterial target strain. This strain was selected following a screening among a collection of different soft rot strains and isolates (including *Dickeya dadantii*, *Pectobacterium atrosepicum* and *P. carotovorum* subsp. *carotovorum*) by performing infection assays on potatoes (tubers, half-tubers and slices). *D. solani* exhibits the highest levels of virulence as well as the uppermost enzymatic activities (not published data).

D. solani was grown and cultivated on LB (LB-Luria medium/L: 10 g bactotryptone, 5 g yeast extract, 0.5 g NaCl, and 15 g molten Agarose) and King B (King B medium/L: 20 g bactotryptone, 10 mL glycerol, 1.5 g dipotassium hydrogen phosphate, 1.5 g magnesium sulfate, and 15 g molten Agarose) solid media at 28 °C for 24 h. After an overnight preculture in LB broth, bacterial concentration was adjusted spectrophotometrically at 600 nm to two optical densities (OD_{600} 0.1: 10^8 cfu mL^{-1} and 0.01: 10^7 cfu mL^{-1}).

Plant material

Three commercial varieties of fresh and healthy market potato tubers (*S. tuberosum* L.) were selected for inoculation experiments (Agata, Monalisa, and Picobello). All tubers used have similar average sizes: Agata (caliber 35–45 mm; very susceptible), Monalisa (caliber: 25–35 mm; moderately susceptible), and Picobello (caliber: 22 mm; slightly susceptible). The choice of these varieties over others was made according to their different degrees of sensitivity to maceration (not published data). The procured potato tubers were washed thoroughly under running tap water, then surface-disinfected by submersion in an alcoholic solution (10% ethanol) for 10 min. The samples were then air-dried under a laminar flow stream before use.

Inoculation and antagonistic assays

Inoculation bio-assay on potato half-tubers was adapted from combined protocols previously described (Lapwood et al., 1984). Briefly, the tubers were cut longitudinally, under aseptic conditions, using a sterile scalpel blade into two equal parts, then a uniform hole was made on the center of each half-tuber by a cork borer (diameter/depth: 5/10 mm). Samples were placed in plastic boxes covered by moistened absorbent paper to create conditions of saturated humidity. The half-tubers were inoculated with 50 µl of bacterial solution of *D. solani* calibrated at 10^7 or 10^8 cfu mL^{-1} (positive control). For antagonistic assay, 50 µl of two T34 concentrations (10^3 and 10^5 cells mL^{-1}) were inoculated on half-tubers as pretreatment, at an elapsed time of 6, 9, or 12 h before bacterial infection (50 µl of *D. solani* at concentrations of 10^7 or 10^8 cfu mL^{-1}). For infection assay or antagonistic trials, negative controls were prepared by replacing the bacterial solution with 50 µl of sterile distilled water.

After that, all boxes of antagonistic assays and positive and negative controls were sealed and kept in dark for incubation at 28 °C for 72 h. After incubation, the rotten tissues of each half tuber were carefully collected, weighed, and the result was expressed in grams (g).

The experiments were carried out in six replications and reproduced two times per potato variety (Agata, Monalisa, Picobello), per concentration of pathogen (*D. solani* at 10^7 or 10^8 cfu mL^{-1}), and per time of pretreatment with antagonist T34 (6, 9, or 12 h) and its concentration (T34 at 10^3 and 10^5 cells mL^{-1}).

Statistical analysis

The statistical software package SPSS was used for data investigation (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY). Statistical treatment was carried out by multifactorial analysis of variance (MANOVA, LSD test), and significant differences between results were considered at $P < 0.05$. Sigmaplot 14 software was used for graphical presentations.

RESULTS AND DISCUSSION

In this study, the industrialized strain of *T. asperellum* T34 was used as the antagonist towards the highly pectinolytic bacterial strain *D. solani* responsible for potato tuber soft rot. For this, many bioassays were planned to check the effect of the bacterial load, the T34 concentration, and the time of pretreatment with T34 on three potato varieties with a variable sensibility to soft rot disease.

In the preliminary experiments, the impact of soft rot on potato tuber was evaluated using four parameters: weight of rotten tissues (g) representing the amount of macerated tissues, the volume (mL) corresponding to the volume of water needed to fill the wells formed after discarding all soften tissues, as well as calculation of the diameter and depth of macerated area (mm). After data analysis, it has been revealed that the weight and the volume of macerated tissues were found more representative and indicative of disease severity, giving more accurate results. These two parameters were closely related and exhibited a strong coefficient of correlation ($r = 0.988$). Indeed, several studies demonstrated that the assessment of potato soft rot susceptibility using the volume and/or weight of infected tissues are better and preferred

parameters than the calculation of linear dimensions of infection zone (Bourne et al., 1981; Wright et al., 1991). Taking into account the technical and practical considerations, the weight of macerated tissues was the only parameter used to express the results in the present study.

Infection assays

Infection assays using only *D. solani* CFBP 8199 without the antagonist revealed a considerable variation depending to bacterial concentration and potato variety. It was observed that soft rot symptoms were appeared after 24 h of infections and propagate on potato rapidly during the 72 h of incubation. Lesions caused by *D. solani* appeared with creamy to brown color on the surface and turn black around the rotting area upon exposure to air. Tissues are mushy, slimy and water soaked. Besides typical symptoms, some samples show an indigo blue color overhanging the macerated area (Fig. 1). This colored aspect is due to the secretion of indigoidine blue pigment. The production of this pigment is generally exclusive to Dickeya spp. Following their mutation and not found in cultures of other soft rot bacteria (Chu et al., 2010). Moreover, negative controls tubers, regarding the samples inoculated by sterile distilled water, no maceration symptoms were observed.

The results of infection showed that the virulent effect of *D. solani* depend significantly ($P \leq 0.05$) on potato cultivar (Fig. 2). Agata cultivar was the most susceptible variety, showing a great

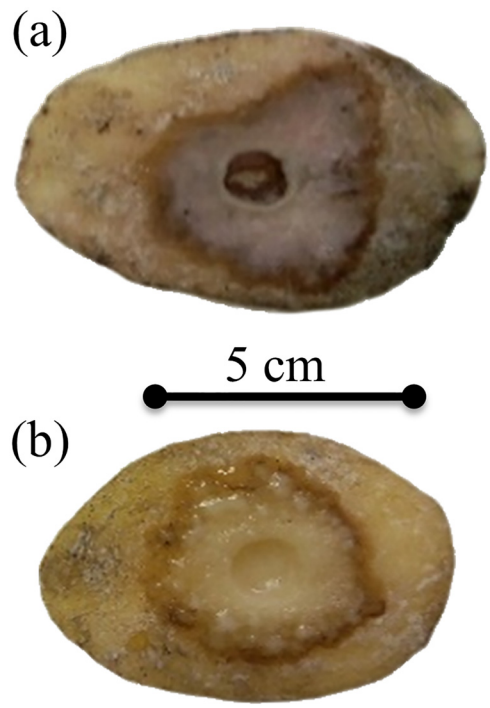

Fig. 1. Aspects of soft rot induced by *D. solani* on half-tubers of Agata potato with production of indigoidine (a) and without production of indigoidine (b)

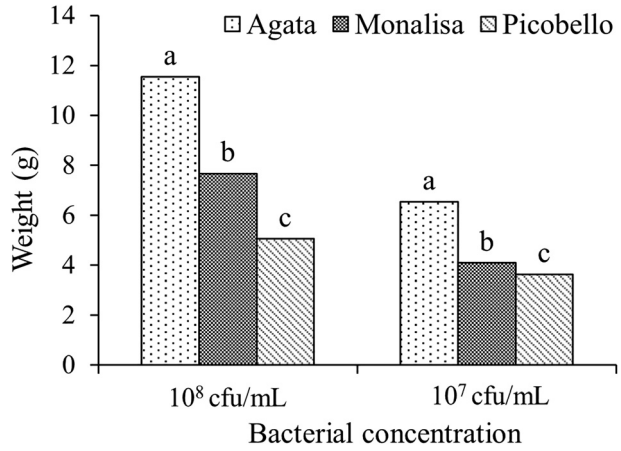

Fig. 2. Effect of pretreatment with *T. asperellum* on soft rot weight induced by *D. solani* on potato tubers of Agata (a), Monalisa (b), and Picobello (c) varieties. *The results of each bacterial concentration with different letters are statistically different (Test LSD, P < 0.05, with a>b > c)*

level of sensitivity with the highest amounts of maceration (11.54 g for 10^8 cfu mL^{-1} and 6.54 g with 10^7 cfu mL^{-1}), followed by Monalisa cultivar. Whereas, Picobello cultivar was the less affected indicating on its lowest sensitivity.

A high maceration tissues were observed also by Shoeib et al. (2022) on potato tuber infected by *D. solani* (250 µL of culture at 10^6 cfu mL^{-1}) reaching a percentage of disease severity up than 37%. The obtained macerated tissue weights were higher than data reported by Tsror et al. (2012), who established a high variation between the 40 Dickeya strains compared. *D. solani* present a high ability of infection, quick tissues maceration, and propagation through potato tuber even under lower temperatures (Toth et al., 2011).

From the results, it can observe that there was a relationship between the quantity of soft rot tissue weight and the used bacterial concertation for infection assay. Agata and Monalisa inoculation with 10^8 cfu mL^{-1} produced two times more rotten tissues than that treated with 10^7 cfu mL^{-1}. According to Poiatti et al. (2009), Agata cv is very sensitive to most potato diseases, including soft rot and bacterial wilt caused by *Ralstonia solanacearum*. Andrivon et al. (2003) and Friedman (2006) reported that potato resistance to soft rot is linked to the high content of bioactive secondary metabolites including glycoalkaloids and phenolic compounds. Moderate tolerance of Picobello cv to soft rot can be explained by its relative richness of some constitutive components like glycoalkaloids (α-solanine and α-chaconine). Ginzberg et al. (2009) suggested that potato steroidal glycoalkaloids are highly involved in plant resistance against pests and pathogens.

Antagonism assays

Tubers preventive treatment by T34 application showed a high inhibitory effect against *D. solani* by regression of soft rot symptoms after 6 and 9 h post-inoculation, and the complete absence of disease after 12 h (100% of inhibition for all assays) as illustrated in the photographs of Fig. 3.

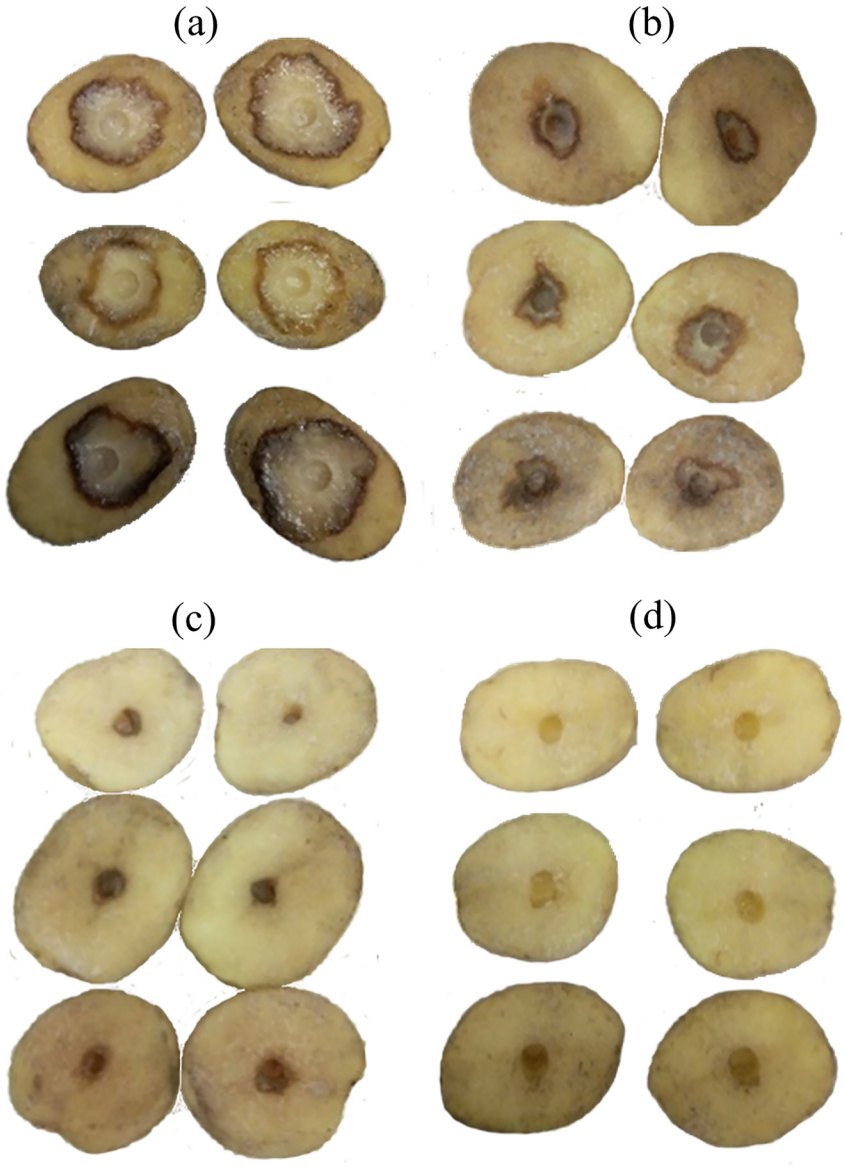

Fig. 3. Aspects of half-tubers pretreated by *T. asperellum* before infection with *D. solani* at elapsed time of 6 (a), 9 (b), and 12 h (c) as well as the control using distilled water (d)

Results of soft rot weight for antagonism tests assessed on the three potato varieties infected by *D. solani* were presented in Fig. 4.

For half-tubers of Agata cv treated 6 h before infection with T34 at concentration 10^5 cells mL^{-1} induced disease in soft rot weight of more than 86% for both samples inoculated by 10^7 and

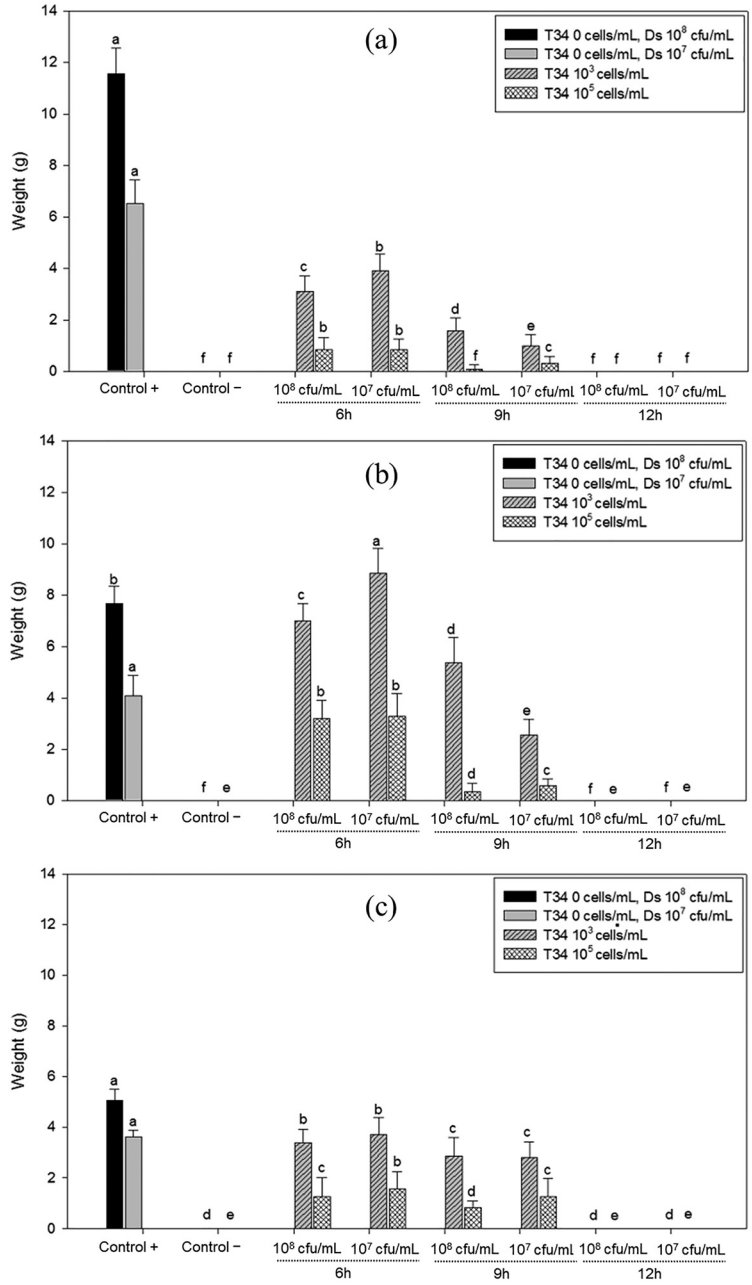

Fig. 4. Effect of pretreatment with *T. asperellum* on soft rot induced by *D. solani* on potato tubers of Agata (a), Monalisa (b), and Picobello (c) varieties. *The results of each bacterial concentration with different letters are statistically different (Test LSD, P < 0.05, with a>b > c > d > e > f)*

10^8 cfu mL^{-1} of *D. solani*. Whereas, for the samples pretreated with T34 at 10^3 cells mL^{-1} we scored inhibition rates of 72.94% (10^8 cfu mL^{-1}) and 66.23% (10^7 cfu mL^{-1}). T34 at 10^5 cells mL^{-1} gave almost the same maceration reduction on the Monalisa variety, for both bacterial inocula with 21.64% for 10^8 cfu mL^{-1} and 22.67% for 10^7 cfu mL^{-1}, while 10^3 cells mL^{-1}, gave regressions of 8.88 and 10.89%, respectively. Concerning Picobello cv, inhibitions obtained with T34 at 10^5 cells mL^{-1} were two-fold higher than 10^3 cells mL^{-1} for both bacterial concentrations.

Samples infected 9 h after T34 application showed the same trend for disease suppression as for treatment at 6 h with the best results. The pretreatment of Agata with T34 provides an average inhibition of 93% for both concentrations of antagonist and bacterial concentrations.

For the Monalisa variety, a significant difference was found between treatments at 10^5 cells mL^{-1} (91.09%) and 10^3 cells mL^{-1} (30.08%) for the bacterial concentration of 10^8 cfu mL^{-1}. While 10^7 cfu mL^{-1} allowed a reduction of 85.61 and 66.82% using 10^5 and 10^3 cells mL^{-1}, respectively. The lowest effect was scored on Picobello cv with values of 77.35 (10^8 cfu mL^{-1}) and 43.33% (10^7 cfu mL^{-1}) using 10^5 cells mL^{-1} of T34.

The antagonistic effect of T34 with 12 h pretreatment before bacterial infection revealed a complete inhibition of soft rot development for both *T. asperellum* and *D. solani* concentrations. All treated half-tubers does not present any symptom for the three varieties. The time elapsed between pretreatment with T34 and infection by *D. solani* was a key parameter in the interaction antagonist-plant-pathogen and have more incidence of disease suppression than the concentration of T34 or the bacterial inoculum.

The pretreatment of potato tubers with T34 was increased as the time to infection with *D. solani* longer. Indeed, the rapid growth of *Trichoderma* spp. was an important advantage through space occupation and nutrient uses on plant hosts before the development of pathogens and deploying their arsenal of toxins and enzymes (Barbosa et al., 2001). *Trichoderma* species can demonstrate a great adaptation to environmental conditions, growing generally faster than plant pathogens and inhibiting their growth (Howell, 2003). The fast growth of *T. asperellum* explains their ability to colonize tissues and take nutriments at the early time just after their introduction, thus depriving the pathogen of many sources necessary for its development.

The effect of pretreatment with T34 was not similar for the three potato varieties. The response of potato varieties towards treatment was linked to their degree of sensitivity to soft rot. In fact, the antagonistic activity was higher on sensitive Agata tubers followed by Monalisa and Picobello varieties characterized respectively by a medium and low sensitive to soft rot disease. These findings can be explained by the better opposition of potato cultivars not only to soft rot pathogens but also to the used antagonist. The tubers use also their resistance compounds as glycoalkaloids and other secondary metabolites like phenolic compounds to oppose the development of T34, inducing a decrease in its antagonistic abilities. However, the varieties with high sensitivity allowed the development of antagonists that provided thus more efficient protection against *D. solani*.

Antagonistic activity of T34 against *D. solani* can be explained by several mechanisms with direct effects, including the use of volatile and non-volatile compounds as well as antibiotic production, and the indirect actions by triggering the plant defense responses by induction of systemic resistance (ISR) (Howell, 2003; Vinale et al., 2008, 2012) and promotion of growth hormones production (Shoresh et al., 2010). Furthermore, *Trichoderma* can degrade pectinases and other enzymes that are essential for phytopathogens responsible of soft rot (Yedidia et al., 2001).

Several kinds of secondary metabolites isolated from *Trichoderma* spp. are involved in its biocontrol action, including toxins, phenols, and particulars amino acids (Mukherjee et al., 2012).

Among these inhibitory compounds, peptaibols that playing a great role in antimicrobial activity (Daniel and Filho, 2007). Peptaibols are able to increase membrane permeability by forming voltage-dependent ion channels in plasma membranes, inducing consequently cell death by cytoplasmic leakage (Rahimi Tamandegani et al., 2020).

CONCLUSION

The antibacterial activity of *T. asperellum* T34 assessed on potato tubers (Agata, Monalisa, and Picobello varieties) applied at different intervals before bacterial infection indicated excellent levels of antagonism towards *D. solani*. The most efficient treatment was observed after 12 h of T34 application, leading to total inhibition of pathogen development. Both tested concentrations of T34 (10^3 and 10^5 cells mL^{-1}) showed a high antagonistic effect, with better inhibition levels for 10^5 cells mL^{-1} after 6 and 9 h pretreatment. Therefore, the preventive application of *T. asperellum* enhance potato protection by reducing disease incidence until complete suppression. The phytopathogen bacteria *D. solani* was highly implicated in the soft rot of potatoes, causing significant losses economic, thus, the use of *T. asperellum* T34 can be considered as an efficient biocontrol tool against soft rot bacteria and might considered to constitute a tool of disease management strategy for potato cultivation and storage.

Competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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