Romanian Journal for Plant Protection, Vol. XVI, 2023
ISSN 2248 – 129X, ISSN-L 2248 – 129X

IN VITRO ANTAGONIST ACTIVITY OF *BACILLUS* SPP. STRAINS AGAINST *BOTRYTIS CINEREA* IN STRAWBERRY CROP

Maria - Cristina Lumînare[*] [1,2], *Oana - Alina Boiu - Sicuia* [1,2], *Daniel Nicolae Cojanu* [1,2], *Lavinia - Diana – Nicoleta Buturugă - Barbu* [1,2], *Stelica Cristea* [1]

[1]Research - Development Institute for Plant Protection, Bucharest, Romania,
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania,

*Correspondence address:
Research-Development Institute for Plant Protection 8 Ion Ionescu de la Brad 013813, Bucharest, Romania Phone: + 40 21269 32 31
Fax: + 40 21269 32 39
E-mail: cristina_tane@yahoo.com

http://www.doi.org/10.54574/RJPP.16.07

Abstract. The in vitro antagonistic activity of ten beneficial bacterial strains, including *Bacillus subtilis* (LC14), *Bacillus amyloliquefaciens* (LC13), *Bacillus cereus/thuringiensis* (LC1, LC2, LC4, LC5 and LC8), *Bacillus safensis* (LC7), *Paenibacillus castaneae* (LC6) and *Paenibacillus lautus* (LC3), against the pathogenic fungus *Botrytis cinerea*, which causes grey mold in strawberry crops, was investigated. The ability of *Bacillus* spp. strains to inhibit the growth of *Botrytis cinerea* was determined using a dual culture method and radial growth was measured at intervals of 5, 10, 15, and 18 days. The results showed that *Bacillus amyloliquefaciens* (LC13) and *Bacillus subtilis* (LC14) were effective in suppressing the vegetative growth of *Botrytis cinerea*, while the other *Bacillus* spp. strains, as well as *Paenibacillus castaneae* (LC6) and *Paenibacillus lautus* (LC3), did not affect the growth of the fungus. These findings demonstrate the potential of specific strains of *Bacillus amyloliquefaciens* (LC13) and *Bacillus subtilis* (LC14) to act as efficient biocontrol agents against *Botrytis cinerea* in strawberry crops. Further studies should focus on field trials to assess the practical application of these beneficial bacteria in controlling grey mold.

Key words: *Bacillus subtilis*, *Botrytis cinerea*, *antagonism, strawberry*

INTRODUCTION

Botrytis cinerea, often referred to as grey mold, is a destructive fungal pathogen that affects a range of crops, including strawberries. Without the application of fungicides, it has been observed that over 80% of the flower buds and fruits of strawberries may be lost due to infection with *B. cinerea* under humid conditions (Petrasch et al., 2019). Fungicides are the primary method of controlling gray mold; however, the disease has evolved resistance to several fungicides over time. According to Shao et al. (2021), substitution mutations in the target genes of fungicides are primarily responsible for the development of resistance to fungicides in *B. cinerea*. Furthermore, the accumulation of residues from fungicide use poses risks to both humans and the environment. As a result, alternative methods, such as microbial control agents, have emerged as a means of controlling the disease without the use of chemicals.

Plant growth-promoting rhizobacteria (PGPR), also known as rhizospheric microorganisms, are capable of producing substances that promote plant growth, fix nitrogen, regulate hormone levels, facilitate nutrient absorption, dissolve minerals, stimulate internal signals to control antibiotic production, and suppress pathogenic infections (Patwardhan et al., 2022). *Bacillus* strains, including *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus amyloliquefaciens*, and *Bacillus megaterium*, are commonly used to produce PGPR products (Borriss, 2011). Beneficial bacteria of the genus *Bacillus* have demonstrated potential as

biocontrol agents because of their ability to produce secondary metabolites with antifungal properties (Salazar et al., 2023).

A study conducted by Asraoui et al. (2019) demonstrated that the presence of *Bacillus amyloliquefaciens* strain in the roots of strawberry plants confers systemic protection against *Botrytis cinerea*. This protection is achieved by directly activating specific defence reactions in plants, such as systemic acquired resistance (SAR). Activation of SAR in turn leads to enhanced regulation of defense-related genes, including those responsible for the production of pathogenesis-related proteins such as PR1, chitinases, and glucanase. The observed "priming" effect suggests that plants induced with the *Bacillus amyloliquefaciens* strain exhibit more rapid and intense defense-related gene expression compared to non-induced plants.

To achieve the highest possible yield and quality of strawberries, it is essential to adopt methods that are not only effective in controlling diseases, but also adhere to the principles of sustainable and eco-friendly agriculture. In this regard, the present study sought to investigate the potential use of certain strains of beneficial bacteria, specifically those belonging to the *Bacillus* spp. genus, as microbial control agents to mitigate the impact of *Botrytis cinerea* on strawberry crops.

MATERIAL AND METHODS

Botrytis cinerea was isolated from affected strawberry fruits. The pathogen was grown on PDA medium for 5 days at 25 °C. The culture was identified by examining the colony morphology and microscopic features, including the structure of hyphae, conidia, and conidiophores. Ten *Bacillus* spp. strains were isolated from natural sources and identified using the Biolog Gen III (Table 1). After isolation and identification, the bacterial strains were grown on LB agar medium for 24 h at 28°C.

Table 1. *Bacillus* spp. strains used in the study

Test strains	Identified Strains Biolog Gen III
LC1	*Bacillus cereus/thuringiensis*
LC2	*Bacillus cereus/thuringiensis*
LC3	*Paenibacillus lautus*
LC4	*Bacillus cereus/thuringiensis*
LC5	*Bacillus cereus/thuringiensis*
LC6	*Paenibacillus castaneae*
LC7	*Bacillus safensis*
LC8	*Bacillus cereus/thuringiensis*
LC13	*Bacillus amyloliquefaciens*
LC14	*Bacillus subtilis*

A dual culture method (Dennis & Webster, 1971) was used to examine the antagonisitic effect of ten *Bacillus* spp. strains on the mycelial growth of *Botritys cinerea*. Petri plates were prepared using PDA medium. Discs of mycelia from actively growing *Botrytis cinerea* cultures were placed at the center of the plates. *Bacillus* spp. strains were streaked at equidistant points around the edge of the Petri plates, maintaining a sufficient distance from the center. As a control, PDA was inoculated with pathogen alone. Plates were incubated at 26°C for 5 days. The mycelial growth of *Botrytis cinerea* around *Bacillus* spp. colonies was measured at intervals of 5, 10, 15, and 18 days.

The percentage growth inhibition (%) of fungal growth was calculated according to the method described by Korsten et al., (1995):

$$GI\ (\%) = \frac{r1}{kr} \text{ x } 100$$

where: GI is percent growth inhibition; r1 is the colony diameter in the control and k_R the fungal colony diameter after treatment with *Bacillus* spp. strains.

Percent GI was categorized on a scale from 0 to 4 after 18 days, where 0 = no GI, 1 = 1–25% GI, 2 = 26–50% GI, 3 = 51–75% GI, and 4 = 76–100%.

RESULTS AND DISCUSSIONS

The antagonistic potential of *Bacillus* spp. strains against *Botrytis cinerea* was evident in the case of *Bacillus amyloliquefaciens* (LC13) and *Bacillus subtilis* (LC14), which effectively suppressed the vegetative growth of *Botrytis cinerea* (Figure 1). Radial growth was unaffected by *Bacillus cereus/Thuringiensis* (LC1, LC2, LC4, LC5, and LC8), *Bacillus safensis* (LC7), *Paenibacillus castaneae* (LC6), and *Paenibacillus lautus* (LC3) when compared to the control (Table 2). The results of this study demonstrated the ability of specific strains of *Bacillus amyloliquefaciens* (LC13) – 65,55% and *Bacillus subtilis* (LC14) – 74,16% to act as highly efficient biocontrol agents against *Botrytis cinerea* in strawberry crops (Figure 2).

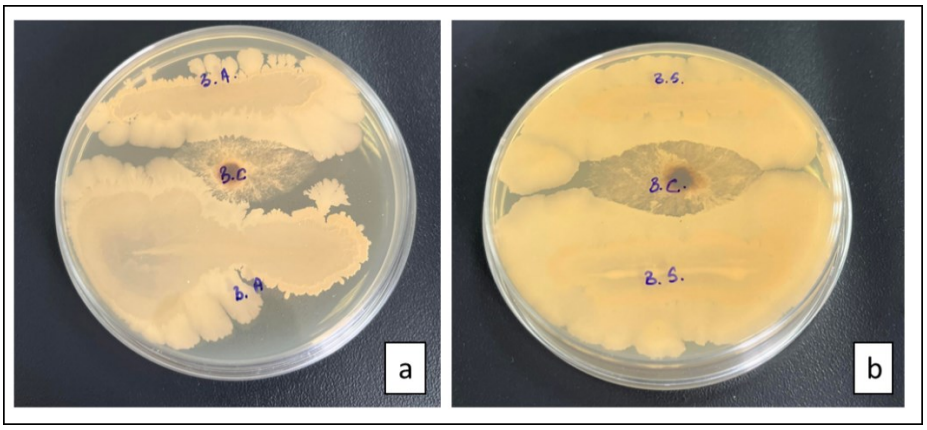


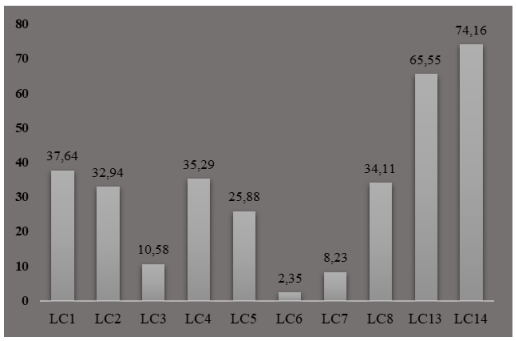
Figure 1. a - *Bacillus amyloliquefaciens* (LC13) vs. *Botritys cinerea; b - Bacillus subtilis* (LC14) vs. *Botritys cinerea*

Table 2. Percent growth inhibition scale of *Bacillus* spp. strains against *Botritys cinerea*

Bacillus spp. strain	Percent growth inhibition %	Growth inhibition category[a]
Control	0	No GI
Bacillus cereus/thuringiensis LC1	37,64	2
Bacillus cereus/thuringiensis LC2	32,94	2
Paenibacillus lautus LC3	10,58	1
Bacillus cereus/thuringiensis LC4	35,29	2
Bacillus cereus/thuringiensis LC5	25,88	1
Paenibacillus castaneae LC6	2,35	1
Bacillus safensis LC7	8,23	1
Bacillus cereus/thuringiensis LC8	34,11	2

Bacillus amyloliquefaciens LC13	65,55	3
Bacillus subtilis LC14	74,16	3

[a] The percentage growth inhibition was calculated using the method described by Korsten et al., (1995) after 18 days. The GI percentage was classified using a scale ranging from 0 to 4, where 0 = no GI, 1 = 1–25% GI, 2 = 26–50% GI, 3 = 51–75% GI, and 4 = 76–100% GI.



Variant	Colony diameter, cm			
	5 days	10 days	15 days	18 days
Control	7	9	9	9
Bacillus cereus/thuringiensis LC1	4,9	5,3	5,5	5,6
Bacillus cereus/thuringiensis LC2	5,1	5,6	6	6
Paenibacillus lautus LC3	7,1	7,4	7,8	8
Bacillus cereus/thuringiensis LC4	4,6	5,6	5,9	5,9
Bacillus cereus/thuringiensis LC5	6	6	6,5	6,6
Paenibacillus castaneae LC6	6,7	8,4	9	9
Bacillus safensis LC7	6,1	6,9	9	9
Bacillus cereus/thuringiensis LC8	4,9	5,4	6	6
Bacillus amyloliquefaciens LC13	3,1	3,1	3,1	3,1
Bacillus subtilis LC14	2,1	2,3	2,4	2,5

Figure 2. Mycelial growth inhibition of *Bacillus* spp. against *Botritys cinerea*

Several studies have shown that various strains of *Bacillus* spp. have a general inhibitory effect on *B. cinerea* growth in strawberry crops. Maung et al. (2021) found that *Bacillus amyloliquefaciens* Y1 effectively inhibited the growth of *B. cinerea* (61.22 %), promoted strawberry growth, and showed promising ability to suppress grey mold disease under in vivo conditions, reducing disease severity in flowers and fruits. Based on the work of Es-Soufi et al. (2020), *Bacillus amyloliquefaciens* Bc2 efficiently controlled *B. cinerea* in strawberries cultivated under field conditions, resulting in increased plant growth and fruit yield.

Hang et al. (2005) discovered that *Bacillus subtilis* S1-0210 could be used as a biological agent to combat *B. cinerea* in strawberries. The wettable powder formulation effectively decreased infection rates and managed grey mold occurrence in both pots and fields. Pre-treatment yielded superior results compared to post-treatment, indicating that *Bacillus subtilis* S1-0210 has the potential to be used as a biocontrol agent. Grahovac et. al. (2019) evaluated the use of *Bacillus subtilis* strain BS10 as a biocontrol agent against *B. cinerea* in open-field strawberry crops in Serbia. The results showed that BS10, applied at a concentration of 2%, was highly effective in controlling *B. cinerea*, with an efficacy ranging from 79% to 85%. Vicente-Hernández et al. (2019) investigated the potential of *Bacillus methylotrophicus* M4-96, a beneficial rhizobacterium found in maize, as a plant growth promoter for strawberry plants. Research has revealed that it promotes the formation of callose in the leaves, leading to a decrease in the severity of *Botrytis cinerea* infection. Acetoin, the primary volatile compound generated by bacteria, has been found to enhance the growth and development of strawberry explants.

CONCLUSIONS

This study demonstrated the in vitro antagonistic activity of *Bacillus amyloliquefaciens* LC13 and *Bacillus subtilis* LC14 strains against *Botrytis cinerea*, offering a promising eco-friendly approach for managing gray mold in strawberry crops. Further studies should focus on

field trials to assess the practical application of these beneficial bacteria in controlling gray mold.

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