Scientific Papers. Series A. Agronomy, Vol. LXIV, No. 1, 2021
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

BACTERIAL STRAINS INVOLVED IN SOILBORNE PHYTOPATHOGENS INHIBITION

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Abstract

Soil borne phytopathogens are a continuous threat to plant health. Most soil borne pathogens have a broad spectrum of plant hosts being capable to infect cereals, oil crops and legumes of various botanical families. Biological measurements capable to reduce plant pathogens growth and development are a sustainable way to prevent crop infections with minimum risks for farmers, consumers and environment. In the present study, several bacterial strains isolated from different sources were evaluated for their potential to reduce the growth of Fusarium oxysporum, Fusarium graminearum, Rhizoctonia solani, Sclerotinia sclerotiorum and Sclerotium bataticola. Tested strains revealed clear aspects of fungal cell wall and cell membrane alteration.

Key words*: biocontrol bacteria, soil borne phytopathogens.*

INTRODUCTION

Plants are exposed to a large spectrum of phytopathogens, and many of them are found in the soil. Such soil-borne disease complexes are especially difficult to control. Once established, they significantly reduce microbial diversity and consequently affect rhizosphere and endosphere of plants, increasing the phytosanitary risks for the crops (Wolfgang et al., 2019).

Among soil-borne pathogens, this study is focused on two *Fusarium* species, *F. oxysporum* and *F. graminearum*, on *Rhizoctonia solani, Sclerotinia sclerotiorum* and *Sclerotium bataticola*.

Fusarium oxysporum causes vascular wilts in more than 100 host plants (Joshi, 2018), having many *formae speciales* which are host specific. *Fusarium graminearum* is an important pathogen of cereals, also involved in mycotoxin contamination. Although it produces *Fusarium* head blight of cereal crops, it can survive in the soil, on plant debris (Leplat et al., 2013).

Rhizoctonia solani is a ubiquitous soil-borne necrotroph, able to damage a wide range of economically important crops, from *Poaceae, Fabaceae, Solanaceae, Amaranthaceae, Brassicaceae, Rubiaceae, Malvaceae, Asteraceae, Araceae, Moraceae* and *Linaceae* families. Depending on the host plant and infection points, symptoms can include seed, root, hypocotyl, crown and stem rot, stem canker, black scurf, seedling blight, or damping off (Ajayi-Oyetundea & Bradley, 2018).

Sclerotinia sclerotiorum is a devastating soil-borne fungal pathogen causing stem rot of a wide range of plant species, such as oilseed rape, sunflower, soybean, and numerous vegetable crops (Willbur et al., 2019).

Sclerotium bataticola, also known as *Macrophomina phaseolina*, is a soil borne fungus causing charcoal rot to various plants, such as soybean, sunflower, corn, potato, or sweet potato (Lodha & Mawar, 2019).

The aim of this study is to characterize various plant-associated bacterial strains with potential biocontrol qualities, able to inhibit soil borne phytopathogenic fungi.

MATERIALS AND METHODS

Plant associated bacteria

A total of 30 bacterial strains isolated from different vegetal sources were used in this study (Table 1), one of which is a reference strain, *Bacillus subtilis* ATCC 6633, from the American Type Culture Collection. Some of these strains were previously identified at

specie level. BW, OS15, OS17, BIR and BPA are *Bacillus amyloliquefaciens* strains (Sicuia et al., 2017) and 1T2 strain is affiliated to *Bacillus endophyticus* (Boiu-Sicuia et al., 2017).

Table 1. Plant associated bacterial strains

Bacterial strains	Isolation source	Bacterial strains	Isolation source
ATCC6633	reference strain	LT MYM1	endophytes of various plant species
BN7	agricultural wastes	LFF MYM1	
B7.2		LFF MYM5	
E1Ps	pea endophyte	E1Pv	
BVFs3	fava endophyte	E2Pv	
B4	compost tea	1T2	
B5		c	
B6		BAHs1	seed endophytes
BIR	plant pathogen antagonists	BPVs2	
BPA		BTAs3	
BW	soil	E2Ms	root nodules endophytes
OS15	onion rhizosphere	E1Ml	
OS17		E2Ml	
FL400	root nodules	E2Vh	
T2	plant pest	MC2	momordica root

Routinely, these bacteria were grown on Luria Bertani agar medium at 28°C. However, they were also able to grow on Potato-Dextrose-Agar (PDA).

Fungal plant pathogens

Five fungal species of plant pathogens were used in this study: *Fusarium graminearum* DSM4527, *Fusarium oxysporum* ZUM2407, *Rhizoctonia solani* DSM63002, *Sclerotinia sclerotiorum* and *Sclerotium bataticola*. The first three are reference strains from international microbial collections. The fourth strain is a Romanian isolate from USAMV collection, and the fifth belongs to RDIPP microbial collection. All of these fungi were routinely grown on PDA medium.

Antifungal activity evaluation

All 30 bacterial strains were analysed for their antifungal activity against previously mentioned soil-borne fungi. The test was performed *in vitro*, on PDA medium, similar to the dual culture technique (Soria et al., 2012).

The antagonism test was performed in 9 cm Petri dishes, using fresh fungal and bacterial biomass. Mycelia plugs of 5 mm in diameter were inoculated on PDA, in the centre of the plates. Against each fungus, at 2.5 cm distance from the centre of test plates, five bacterial strains were co-inoculated per dish, in equidistant distributed spots. Control plates were also prepared for each fungal strain.

Cultures were incubated at 28°C for 10 days, and antifungal activity was periodically evaluated according to Dinu et al. (2012). Bacterial efficacy to inhibit fungal growth (E%) was calculated using the following equation:

$$E\ (\%) = (Rc\text{-}Ri)/Rc\ *100$$

where Rc is the radius of fungal growth in control plates, and Ri is the radius of fungal growth influenced by the bacterial strain.

Clear inhibition zones between fungal and bacterial colonies were also measured, and the mycelia edge was microscopically analysed.

RESULTS AND DISCUSSIONS

The antifungal potential of several plant associated bacteria was evaluated *in vitro*, using direct confrontation method. A total of 30 strains of plant associated bacteria were evaluated against five important phytopathogens.

Among all bacterial strains tested, 60% were endophytic isolates.

Taking into account the antagonistic activity towards the tested fungi, only half of the strains were able to inhibit all five pathogens, with at least 50% efficacy (Figure 1).

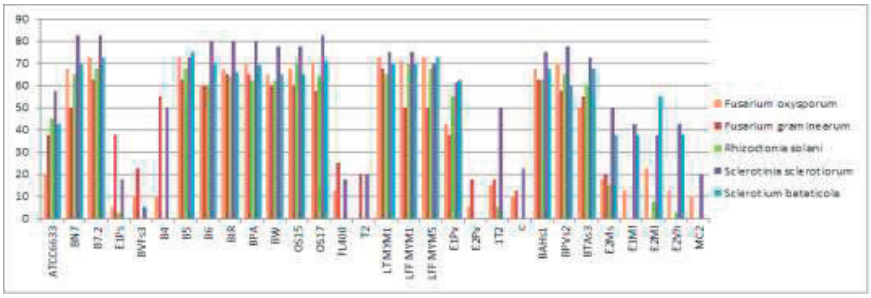

Figure 1. Bacterial efficacy (%) in fungal growth inhibition (after one week of co-cultivation)

Between the tested fungi, *S. sclerotiorum* was most vulnerable to the studied bacteria, where 66.7% of the strains reduced fungal growth with at least 50% and up to 82.5% efficacy (Table 2). *S. bataticola* was inhibited with at least 50% efficacy by 56.7% of the bacterial strains. *R. solani* and *F. graminearum* were inhibited by almost the same strains, representing 53.3% of the total bacteria tested, and *F. oxysporum* by 50% of the tested bacteria.

Table 2. Bacterial potential to inhibit fungal growth (after one week of co-cultivation)

Plant pathogenic fungi	Efficacy interval	Percentage of antagonistic bacteria	Antagonistic bacterial strains
F. oxy	50-72.5%	50 %	BN7, B7.2, B5, B6, BIR, BPA, BW, OS15, OS17, BAHs1, LT.MYM1, BPVs2, LFF.MYM1, BTAs3, LFF.MYM5
F. gram	50-67.5%	53.3%	BN7, B7.2, B4, B5, B6, BIR, BPA, BW, OS15, OS17, BAHs1, LT.MYM1, BPVs2, LFF.MYM1, BTAs3, LFF.MYM5
R. s.	50-70%	53.3%	BN7, B7.2, B5, B6, BIR, BPA, BW, OS15, OS17, BAHs1, LT.MYM1, BPVs2, LFF.MYM1, BTAs3, LFF.MYM5, E1Pv
S. s.	50-82.5%	66.7%	ATCC6633, BN7, B7.2, B4, B5, B6, BIR, BPA, BW, OS15, OS17, BAHs1, LT.MYM1, BPVs2, LFF.MYM1, BTAs3, LFF.MYM5, E1Pv, E2Ms
S. b.	50-75%	56.7%	BN7, B7.2, B5, B6, BIR, BPA, BW, OS15, OS17, BAHs1, LT.MYM1, BPVs2, LFF.MYM1, BTAs3, LFF.MYM5, E1Pv, E2Ml

where: F. oxy = *Fusarium oxysporum*, F. gram = *Fusarium graminearum*, R. s. = *Rhizoctonia solani*, S. s = *Sclerotinia sclerotiorum*, S. b. = *Sclerotium*

Clear zones (CZs), between plant associated bacteria and each plant pathogenic fungi, were considered an antagonistic effect of the beneficial strains, and were also measured (Table 3).

Table 3. Clear zones of microbial inhibition (after one week of co-cultivation)

Bacterial strain	*F. oxy*	*F. gram*	*R. s.*	*S. s.*	*S. b.*
	Clear zone (mm)				
ATCC 6633	0	0	0	1	0
BN7	2	0	1	8	2
B7.2	3	0	2	9	3
B5	4	2	1	7	3
B6	1	1	1	9	2
BIR	3	1	1	11	2
BPA	5	2	0	9	3
LT MYM 1	5	2	1	7	3
LFF MYM 1	5	0	1	8	3
LFF MYM5	4	0	2	6	3
E1Pv	0	0	0	3	1
BAHs1	4	1	2	9	2
BPVs2	4	1	2	9	2
BTAs3	0	0	2	7	2
BW	3	0	2	9	3
OS15	2	0	3	7	1
OS17	4	0	2	10	3

where: F. oxy = *Fusarium oxysporum*, F. gram = *Fusarium graminearum*, R. s. = *Rhizoctonia solani*, S. s = *Sclerotinia sclerotiorum*, S. b. = *Sclerotium bataticola*

Among the evaluated bacteria, 17 strains maintained a clear inhibition zone, unable to be colonized by the fungi. The wider CZs were noticed against *S. sclerotiorum*, the same pathogen with most severely inhibited mycelia growth. According to the biometric evaluation of the CZs, the bacterial strains inducing wider inhibition zones were the same expressing higher antifungal activity: BN7, B7.2, B5, B6, BIR, BPA, BW, OS15, OS17, LT.MYM1, LFF.MYM1, LFF.MYM5, BAHs1, BPVs2, and BTAs3 (Figure 2).

Figure 2. Bacterial antagonistic activity against five fungal phytopathogens

In order to evaluate more accurately the antifungal activity and understand fungal growth alterations, mycelia was analysed under the microscope.

The inhibited mycelia growth of *F. oxysporum* revealed fungal cells ulceration and lysis, with cytoplasmic content leaks (Figure 3). Such aspects were previously described (Boiu-Sicuia et al., 2017, 2018a, 2018b) and similar results were also mentioned in other several studies (Giorgio et al., 2015).

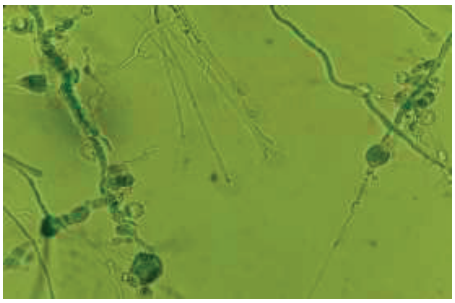

Figure 3. *F. oxysporum* cells swelling and lysis caused by antagonistic plant associated bacteria.

F. graminearum mycelia modifications also revealed cells ulceration and lysis (Figure 4), leakage and/or inactivation of *F. graminearum* cellular contents.

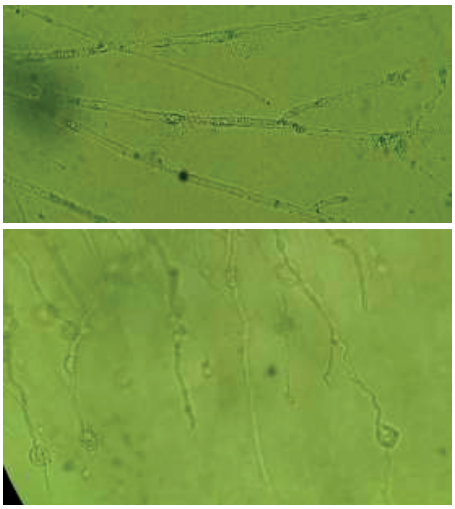

Figure 4. Cells ulceration and lysis of *F. graminearum* due to antagonistic bacteria

Ntushelo et al. (2019) suggest that iturin and other lipopeptides cause leakage of cellular contents and/or inactivation of *F. graminearum* conidia. Morphological distortions in conidia and hyphae, and severe damage of the cell coat, were already associated with iturin producers (Gong et al, 2015).

Mycelia growth and cell morphology modification were also seen on *R. solani* in presence of antagonistic bacteria. Similar aspects were previously described in similar studies mentioning *Rhizoctonia* growth inhibition (Boiu-Sicuia et al., 2017, 2018b).

Antifungal activity against *S. sclerotiorum* induced cell wall and plasma membrane damage, which lead to cell contents' leakage (Figure 5).

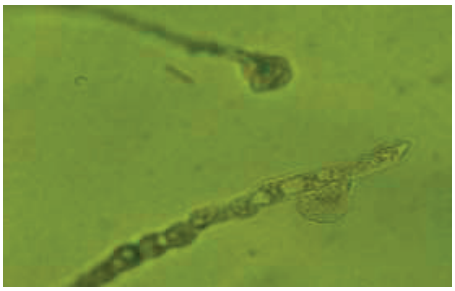

Figure 5. *Sclerotinia sclerotiorum* cells damage caused by antagonistic plant associated bacteria

Reviewed studies on *S. sclerotiorum* biocontrol (Kamal et al., 2016; Smolińska & Kowalska, 2018) describe bacterial antagonists involved in hyphal disintegration, cytoplasm leakage, delayed formation of infection cushion, weakening or killing of sclerotia as well as germination restriction (Saharan & Mehta, 2008; Gao et al., 2013; Chen et al., 2014). Among bacterial antagonists several endophytic strains were listed, best described being *B. subtilis* EDR4 strain (Chen et al., 2014).

The microscopic analysis of *S.bataticola* mycelia inhibited growth revealed cells ulcerations, fungal perforation and leaks of cytoplasmic content (Figure 6). Similar aspects were previously described against this pathogen (Singh et al., 2008; Boiu-Sicuia et al., 2018a, 2018b)

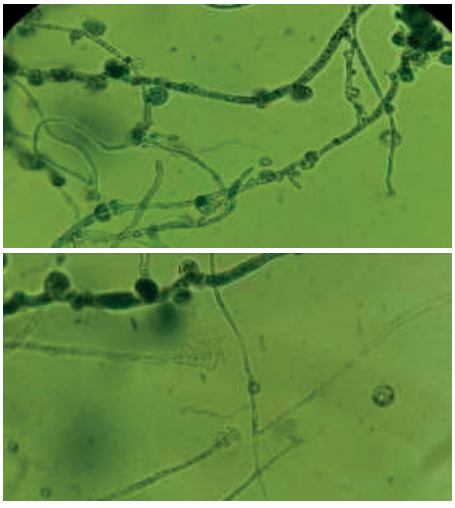

Figure 6. Cells ulceration, hyphal disintegration and cytoplasm leakage of *Sclerotium bataticola*

The mycelia growth modifications and cell alteration are probably due to the antifungal metabolites delivered by the biocontrol bacteria (Calderón et al., 2014). Similar perturbations of the fungal growth were also described in the presence of various volatile organic compounds released by *Pseudomonas* spp. and *Bacillus* spp. strains (Giorgio et al., 2015). Cell wall and plasma membrane damage could also be caused by the lytic enzymes released by the biocontrol bacteria (Boiu-Sicuia et al., 2018c).

CONCLUSIONS

Plant associated bacteria have an important role in plant protection against phytopathogens. Among the bacterial strains used in this study, the *Bacillus* spp. express a moderate to high biocontrol activity against important soil-borne phytopatogens, such as *Fusarium oxysporum, Fusarium graminearum, Rhizoctonia solani, Sclerotinia sclerotiorum and Sclerotium bataticola*. Antifungal properties were seen in both rhizospheric and endophytic bacteria. However, rhizobia-like strains did not reveal antifungal potential.

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