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Biocontrol Activity of Actinomycetes Strains against Fungal and Bacterial Pathogens of *Solanum lycopersicum* L. and *Daucus carota* L.: In Vitro and In Planta Antagonistic Activity †

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Abstract: Plants are affected by various biotic and abiotic stresses due to climate change. Tomato and carrots are important crops that are attacked by various pathogens. Fourteen plant growth promoting bacteria (PGPB) belonging to the genera *Streptomyces* sp. and *Nocardiopsis* sp. were selected for the biocontrol of several common fungal and bacterial pathogen. Antifungal activity was assessed against *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) and *Rhizoctonia solani* (RHS). Antibacterial activity was evaluated against *Pseudomonas syringae*, *Pseudomonas corrugata*, *Pseudomonas syringae* pv. *actinidiae*, *Pectobacterium carotovorum* subsp. *Carotovorum*. *In vitro* antifungal and antibacterial antagonistic activities were evaluated by dual culture method. Fungal-bacterial interaction areas were analysed by scanning electron microscopy (SEM). Cell-free culture filtrates (CF) from strains showing good biocontrol potential, were produced and investigated for their *in vitro* antifungal and antibacterial activity. Two most effective strains were also combined in consortium and utilized for *in planta* pre-emergence biocontrol assays on both *S. lycopersicum* and *D. carota*. For each pathogenic strain, four experimental conditions were compared: CNT (no bacterial inoculation/no infection), PGPB (with bacteria/no infection), PGPB+INF (with bacteria/and infection), INF (with infection/no bacteria). The PGPB strains *Streptomyces albidoflavus* H12 and *Nocardiopsis aegyptica* H14 showed good *in vitro* antifungal (inhibition > 50%) and antibacterial (inhibition halo > 10 mm) activity. The SEM micrographs showed deterioration of fungal filaments and modification of hyphal structures. The CFs of both strains were also able to inhibit FORL and RHS in *in vitro* growth (minimum inhibitory concentration of 0.2–0.8%). *In planta* biocontrol assessments showed that, the consortium was effective in reducing the infection effects of both fungal and bacterial pathogens. Dual onsortium allowed normal plant development compared to the control. These results confirm the usefulness of actinomycetes strains as a bio-control agent and can therefore be an alternative to chemicals used in agriculture.

Keywords: PGPB; actinomycetes; bio-control activity; fungal pathogens; pathogenic bacteria; SEM; culture filtrates; tomato; carrot

1. Introduction

Plants are affected by various biotic and abiotic stresses due to climate change. Areas affected by temperature increase are more susceptible to pathogens attack [1]. The use of microorganisms in agriculture is a sustainable strategy to control phytopathogens. These bacteria can improve plant health and growth, providing a long-term protection [2,3]. Several rhizospheric microorganisms act as biostimulants, and show antagonistic properties against several pathogens [4]. Among them, actinomycetes have the ability to produce a wide range of secondary metabolites (e.g. antibiotics and extracellular enzymes) [5], that inhibit the growth of several fungal and bacterial pathogens [6]. Moreover, biocontrol activity is obtained through the induction of systemic resistance [5]. The present study is aimed at evaluating the biocontrol capability of actinomycetes isolates against several fungal and bacterial pathogens of *Solanum lycopersicum* and *Daucus carota*. *In vitro* antifungal and antibacterial antagonistic activities were evaluated by dual culture method. Fungal-PGPB interaction areas were also analysed by scanning electron microscopy (SEM). From strains with good biocontrol potential, cell-free supernatant (CFS) were produced and investigated for their *in vitro* antifungal and antibacterial activity. The most effective strains were also combined in consortium and utilized for the seed treatment for *in planta* pre-emergence biocontrol assays on both *S. lycopersicum* and *D. carota*. For each pathogenic strain, four experimental conditions were compared: CNT (without PGPB/infection), PGPB (with PGPB/no infection), PGPB+INF (with PGPB/infection), INF (with infection/no PGPB). The induced protection was assessed by estimation of plant survival, morpho-biochemical parameters, damages and chlorophyll contents.

2. Experiments

In vitro antagonistic activity by diffusible and volatile compounds was carried out by dual culture method on PDA culture medium using fourteen actinomycetes strains of the genus *Streptomyces* sp, and *Nocardiopsis* sp. The fungal pathogens tested were *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), and *Rhizoctonia solani* (RHS). The morphological deterioration of PGPB-fungus interaction areas were analyzed by SEM microscopy. The pathogenic bacteria tested were: *Pseudomonas syringae*, *Pseudomonas corrugata*, *Pseudomonas syringae* pv. *actinidiae*, *Pectobacterium carotovorum* subsp. *carotovorum*. The inhibition percentages of fungi were calculated after incubation until the complete growth of the control plate [7]. The bacterial inhibition halos were assessed after 48 hours. Cell-free supernatant (CFS) of *Streptomyces albidoflavus* H12 and *Nocardiopsis aegyptica* H14, which showed good *in vitro* biocontrol, were investigated for Minimal Inhibitory Concentration (MIC), and for Minimum Bactericidal Concentration (MBC) as described by CLSI guidelines [8]. Using polystyrene microplate, 100 µL of media were introduced in each well, then 100µL of CFS of single strains, and consortium were inserted in the first wells and the dilutions were maintained. Finally, the fungi and test-bacteria were inoculated.

In planta antagonistic activity of H12 and H14 consortium–the most actives strains against the different tested pathogens - was assessed on S. *lycopersicum*, and D. *carota* against the abovementioned pathogens in pre-emergence [7]. The experiment was organized as follows: (i) CNT (without PGPB/infection), PGPB (with PGPB/no infection), PGPB+INF (with PGPB/infection), INF (with infection/no PGPB). Each experimental unit was realized in 25 pots with two seeds per pot under natural light conditions until the disease's symptoms showed up. The induced protection was assessed by estimation of plant survival, morpho-biochemical parameters, damages and chlorophyll contents. The plants were analyzed for the morpho-physiological characters; damages, and total chlorophyll contents [7].

3. Results

Almost all 14 strains (64%) showed good *in vitro* antagonistic activity by producing diffusible and volatiles compounds against fungal pathogens (inhibition percentage up to 85%). Most of the

tested strains (70%) exhbit at least one activity against pathogenic bacteria (inhibition halo up to 25mm) (Table 1).

Table 1. *In vitro* antagonism assay of actinomycetes strains against fungul and pathogenic bacteria (n = 3).

Pathogenic strains	D14	G10	G22	G33	H12	H14	J4	J13	J21	J27	K12	K23	S2	T45
FORL	-	++	-	-	++	++	-	+	-	-	-	-	-	++
RHS	++	-	++	++	++	++	-	+	-	-	-	-	++	++
P. corrugate	-	++	-	++	++	++	-	++		-	-	-	++	-
P. carotovorum	-	++	-	++	+	-	-	++		+	-	-	-	-

+, moderate inhibition; **++**, high inhibition; **-**, no inhibition.

In Figure 1 are shown the SEM micrographs that highlight the comparison of the structures of fungal hyphae in the presence and in the absence of PGPB. Micrographs of FORL (1A) and RHS (1B) control show normal and continuous fungal structures. While, the micrographs of FORL-PGPB (1C) and RHS-PGPB (1D) interactions areas show clear morphological deterioration of hypae, that appear sparse and discontinuous. *S. albidoflavus* H12, and *N. aegyptica* H14 strains also showed good antibacterial activity against *Pseudomonas corrugate*, and *Pectobacterium carotovorum* (inhibition halos > 10mm). The CFSs of combined strains were effective to inhibit growth of both FORL and RHS fungi (MBC up to 0.8 %) and most of the bacterial pathogen growth.

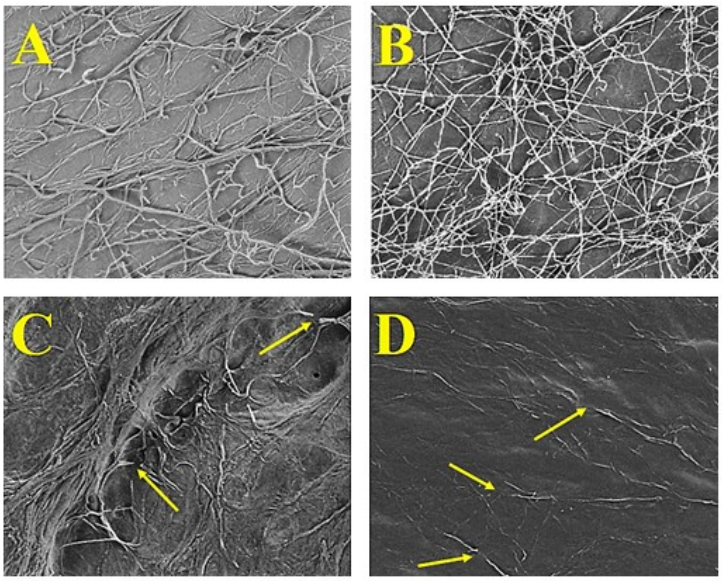

Figure 1. SEM micrographs of FORL and RHS hyphae. The panels show the fungal hyphal branching following a normal growth in FORL and RHS control plates (**A**, **B**, respectively). In the presence of H12 and H14 consortium, the hyphal structures change in the interaction zones of both FORL and RHS with the PGPB (**C**, **D**, respectively).

Concerning the *in planta* experiment, the inoculation with the consortium (PGPB) improved development and growth of both tomato and carrot plants compared to the control (better germination rates, morpho-physiological characters, and chlorophyll content) (Figure 2,3).

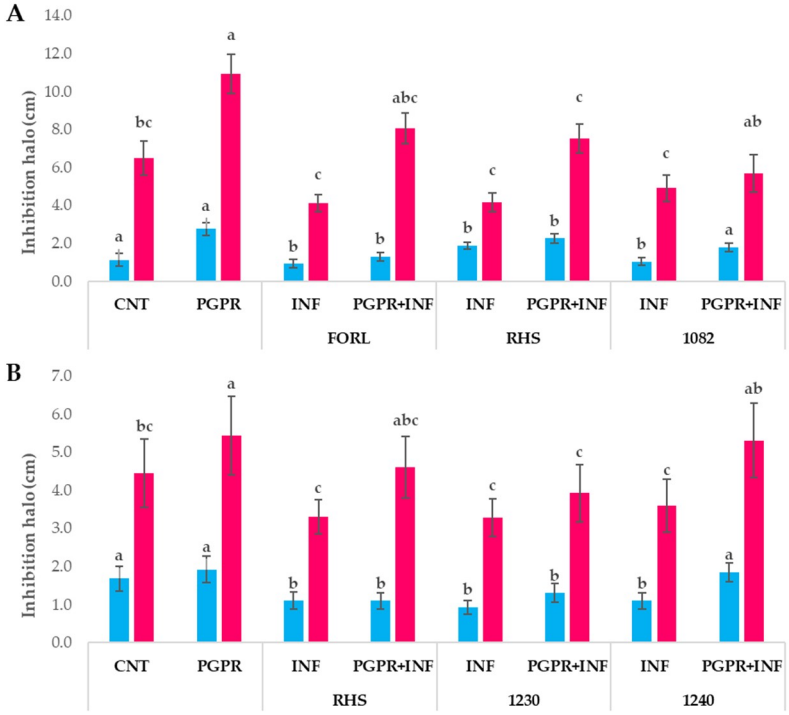

Figure 2. Shoot and root lengths (cm) of S. *lycopersicum* (**A**), *and D. carota* (**B**). Results followed by the same case letter not differed significantly according to Tukey's HSD post-hoc test ($p > 0.05$). In the Figure: 1082, *P. syringae*; 1501, *P. corrugata;* 1230, *P. syringae* pv. *actinidiae*; 1240 *P. carotovorum* subsp. *carotovorum.*

Infection decreased germination rates, growth parameters, and total chlorophyll content, and caused leaves damages with extension up to > 20mm in uninoculated plants (INF). Treatment with the consortium improved germination in infected plants (PGPB + INF) up to 15–54% for tomato, and up to 30–100% for carrots. The presence of PGPB also alleviated infection simpthoms; PGPB + INF plants showed less damages, and better chlorophyll content than the control ($p < 0.05$).

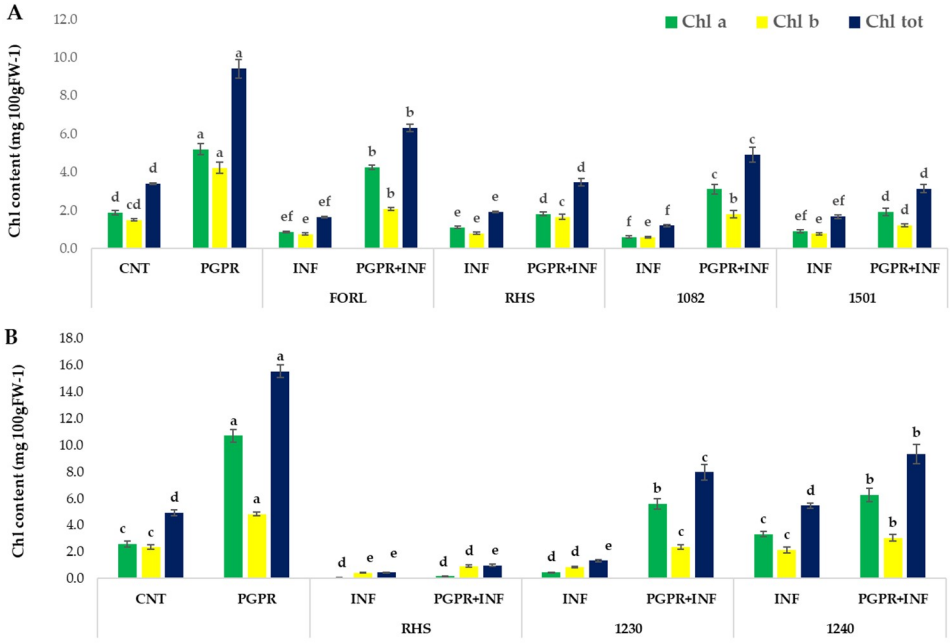

Figure 3. Chlorophyll b (chl a), chlorophyll b (chl b), and total chlorophyll (chl tot) content of tomato (**A**), and carrots (**B**) fresh leaves. Results followed by same case letter are not significantly different

according to Tukey's HSD posthoc test ($p > 0.05$). In the Figure: 1082, *P. syringae*; 1501, *P. corrugata;* 1230, *P. syringae* pv. *actinidiae*; 1240 *P. carotovorum* subsp. *carotovorum.*

4. Discussion

The different abilities to promote plant-growth by actinomycetes strains, such as nutrient solubilization, nitrogen fixation, and phytohormones production act indirectly in the control of plant diseases [5]. The use of combined bacteria is a strategy for plants protection against pathogens attack [7]. Actinobacteria are well known for their ability to produce various bioactive compounds [9]. They are biological agents for their antagonistic activities and plants protection against several soil borne pathogens [10]. The bio-control activity of actinomycetes is linked to antibiosis, lysis mechanisms, and host defenses induction [5]. The actinomycetes strains investigated in this study also have different plant growth-promoting traits [11]. These bacteria enhance plant physiological status and offer an additional advantage to their use as biological control agents for sustainable agriculture.

5. Conclusion

Nowadays, bacterial and fungal plant diseases are controlled almost exclusively by agrochemicals. These chemical products entail serious consequences for human's and ecosystems' health. The use of biocontrol agents should be encouraged to counteract this problem. Our findings show that actinomycetes could be considered a valid biocontrol agents. Further experiments are needed to determine their effectiveness on other plants, against other pathogens and under different cultivation conditions. However, these preliminary results underline that actinomycetes, and in particular *Streptomyces* and *Nocardiopsis* genera, can be biological alternatives for plants disease management.

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