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Biological control of strawberry crown rot, root rot and grey mould by the beneficial fungus *Aureobasidium pullulans*

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Abstract Utilization of biocontrol agents is a sustainable approach to reduce plant diseases caused by fungal pathogens. In the present study, we tested the effect of the candidate biocontrol fungus *Aureobasidium pullulans* (De Bary) G. Armaud on strawberry under in vitro and in vivo conditions to control crown rot, root rot and grey mould caused by *Phytophthora cactorum* (Lebert and Cohn) and *Botrytis cinerea* Pers, respectively. A dual plate confrontation assay showed that mycelial growth of *P. cactorum* and *B. cinerea* was reduced by 33–48% when challenged by *A. pullulans* as compared with control treatments. Likewise, detached leaf and fruit assays showed that *A. pullulans* significantly reduced necrotic lesion size on leaves and disease severity on fruits caused by *P. cactorum* and *B. cinerea*. In addition, greenhouse experiments with whole plants revealed enhanced biocontrol efficacy against root rot and grey mould when treated with *A. pullulans* either in combination with the pathogen or pre-treated with *A. pullulans* followed by inoculation of the pathogens. Our results demonstrate that *A. pullulans* is an effective biocontrol agent to control strawberry diseases caused by fungal pathogens and can be an effective alternative to chemical-based fungicides.

Keywords *Aureobasidium pullulans* · Biological control · *Botrytis cinerea* · *Fragaria* × *ananassa* · Garden strawberry · *Phytophthora cactorum*

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Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is one of the most fungicide-dependent crops, being highly susceptible to many pathogens (Garrido et al. 2011). This study investigates the potential to control two of the most detrimental strawberry pathogens using the candidate biocontrol agent *Aureobasidium pullulans*.

The hemibiotrophic oomycete *Phytophthora cactorum* (Lebert and Cohn) Schröeter, is a destructive pathogen that causes crown and root rot disease in strawberry (Ellis et al. 1998; Golzar et al. 2007; Nellist et al. 2019; Porras et al. 2007; Stensvand et al. 1999). Characteristic symptoms include wilting of plants and brown necrosis of vascular tissues of the crown, sometimes leading to complete yield loss. *P. cactorum* forms sexual oospores, which are a primary source of inoculum that remains persistent in the soil for several years, making management difficult (Nellist et al. 2019). When conditions are favourable, oospores germinate to produce sporangia which release more oospores, these give rise to sporangia that produce zoospores. Zoospores are the asexual motile stage and they are chemotactically attracted to roots, where they attach to the surface and penetrate the root epidermis. Subsequently, they start developing haustoria to acquire nutrients from the root for their growth and sporulation (Nellist et al. 2019).

The other devastating strawberry pathogen considered herein is the ascomycete necrotrophic fungus *Botrytis cinerea* Pers (perfect stage *Botryotinia fuckeliana*) which is the causative agent of grey mould on strawberry fruits (Petrasch et al. 2019). Grey mould is prevalent on all continents, mainly in cool, temperate, and warm-temperate zones (Jarvis 1977). The pathogen acts as a saprophyte in the absence of hosts and remains dormant under unfavourable conditions. Conidia from adjacent infected plants can be dispersed via the air and by water splashes, which facilitate primary infection, entering the plants through any injury or natural opening. Often an attack of the bottom of the receptacle occurs and the mycelium remains quiescent until ripening of fruits. Penetration can take place through open flowers (Bristow et al. 1986). Once environmental conditions become favourable, i.e., long periods of high humidity with temperatures around 25 °C, the pathogen starts causing rotting of flowers, fruits and leaves. If favourable conditions continue, *B. cinerea* keeps on sporulating, and this becomes the source of secondary inoculum. The infection severity of *B. cinerea* on strawberry plants is greatly increased under wet conditions and more than 80% of the flowers and fruit may die if fungicides are not applied in adequate time (Ries 1995).

Until now, the major approach to disease control in strawberry production has relied upon the use of synthetic fungicides. However, fungicide applications bring a range of issues such as accumulation of toxic residue on the fruits, development of resistance in the targeted pathogens, withdrawal of the chemical products from the market, and negative impact on the environment and human consumers (Dianez et al. 2002; Iqbal et al. 2019; Myresiotis et al. 2007; Rabølle et al. 2006; Yourman and Jeffers 1999). Moreover, application of fungicides in the flowering stage may reduce pollen viability and consequently hamper fruit formation (Kovach et al. 2000). In addition, application of fungicides is not allowed in organic production (Iqbal et al. 2018), hence the necessity for the development and implementation of alternative control strategies.

The yeast like fungus *Aureobasidium pullulans* (De Bary) G. Armaud is a candidate biocontrol agent and is naturally present in the phyllosphere and carposphere of several fruits and vegetables, and accompanied with the endophyte population of various species of plant (Bozoudi and Tsaltas 2018). *A. pullulans* has potential to control fruit and vegetable diseases caused by fungal pathogens primarily during the post-harvest phase and can be used either alone or in combination with other sustainable physical methods (Di Francesco et al. 2018, 2020; Di Francesco and Mari 2014; Zhang et al. 2010). Certain *A. pullulans* strains have shown the ability to control fungal pathogens involved in causing post-harvest diseases, such as *B. cinerea* in grapes, *Monilinia* spp. in stone fruits and *Penicillium expansum* in apple (Di Francesco et al. 2018, 2020; Mari et al. 2012; Parafati et al. 2015, 2017). Many different biocontrol mechanisms have been described for *A. pullulans* including antibiosis through the production of antifungal compounds and enzymes (Parafati et al. 2015; Zhang et al. 2010), competition for space and nutrients (Di Francesco et al. 2018; Janisiewicz et al. 2000; Klein and Kupper 2018), mycoparasitism (Klein and Kupper 2018) and the induction of plant defence resistance (Di Francesco et al. 2017; Madhupani and Adikaram 2017).

To date, no studies have been conducted to investigate the biocontrol potential of *A. pullulans* for countering crown and root rot in strawberry. Therefore, this study was designed to explore the biocontrol efficacy of four different strains of *A. pullulans* against crown rot, root rot and grey mould on strawberry. We hypothesize that (1) *A. pullulans* can antagonize and inhibit the growth of *P. cactorum* and *B. cinerea*, (2) the antagonistic potential varies between different *A. pullulans* strains, and (3) application of *A. pullulans* leads to successful biocontrol of root rot and grey mould in strawberry. The results of this study suggest that all the tested *A. pullulans* strains significantly inhibited the mycelial growth of *P. cactorum* and *B. cinerea*. Additionally, either pre-application of *A. pullulans* or simultaneous application with the respective pathogens enhanced the protection against root rot and grey mould under greenhouse conditions. These results show that augmentation biological control using *A. pullulans* has great potential to combat the pathogens, and thus reduce fungicide dependency in strawberry production.

Materials and methods

Plant material

Plantlets of two different strawberry (*Fragaria* × *ananassa*) cultivars, Ostara and Honeoye, were obtained from Plantagen (Lund and Malmö, Sweden). The plants were grown in a greenhouse for ten weeks before being used in the experiment. Strawberry fruits (*cv*. Honeoye) were collected from Borgeby Jordgubbar farm, Bjärred, Sweden.

Fungal/oomycete strains and maintenance conditions

A. pullulans strains (Table 1) AP-30044, AP-30273, AP-53383 and AP-SLU6, *P. cactorum* strain RV4, and *B. cinerea* strain B05.10 were maintained on potato dextrose agar (PDA) medium (Oxoid; Basingstoke, Hampshire, England) at 25 °C under dark conditions. All fungal and oomycete strains were revived from stock culture preserved in 20% (wt/vol) glycerol at − 80 °C.

Inoculum preparation

The cultures of all *A. pullulans* strains, *P. cactorum* and *B. cinerea* were maintained on PDA plates at 25 °C for four weeks under dark conditions. The conidia produced by the *A. pullulans* strains and *B. cinerea* were harvested by adding 6–8 ml of sterile water to the fungal culture, followed by scraping the surface of the mycelium with a spreader. The zoospores produced by *P. cactorum* were obtained following the protocol of Toljamo et al. (2016). The concentrations of conidia and zoospores were determined using a haemocytometer (Hausser Scientific, Horsham, PA) under a light microscope (Laborlux12 Leitz, Germany).

In vitro antagonistic assays

Fungal confrontation assay

The in vitro antagonistic ability of *A. pullulans* strains against *P. cactorum* and *B. cinerea* was determined by performing a dual-plate confrontation assay. A 9 cm PDA plate was inoculated with a 15 mm diameter mycelial agar plug of an *A. pullulans* strain on one side of the plate. After seven days of incubation at 25 °C,

Table 1 List of *Aureobasidium pullulans* strains used in this study including their origin

Strain ID	Other ID	Provided by	Origin	Isolated from
AP-30044	CCUG-30044 CBS 123.37	CCUG[a]	CBS[b], Baarn, The Netherlands 20-04-1992	Unknown
AP-30273	CCUG-30273 IHEM 5520	CCUG[a]	IHEM[c], Brussels, Belgium 19-06-1992	Environment of an asthmatic patient
AP-53383	CCUG-53383 A 2006322/3	CCUG[a]	Anonymous, Sweden 05-09-2006	Water, RO quality, industry
AP-SLU6	–	SLU[d]	SLU[d], Alnarp, Sweden	Wild woodland strawberry (*Fragaria vesca*)

[a]Culture Collection University of Göteborg

[b]Former Central Bureau of Fungal Cultures

[c]Former Scientific Institute of Public Health

[d]Swedish University of Agricultural Sciences (available upon request)

an agar plug of *B. cinerea* was inoculated at an equal distance on the opposite side of the plate to compensate for the growth difference. *P. cactorum*, however, was inoculated on the same day as *A. pullulans* and mycelial growth was measured daily for up to five days post-inoculation (DPI). The growth rates of *P. cactorum* and *B. cinerea* were compared with the control treatment in which the pathogenic fungi remained unchallenged by the *A. pullulans* strain. The assay was performed on six biological replicates. The experiment was performed in duplicate.

Detached leaf assay against P. cactorum

As all *A. pullulans* strains displayed similar patterns in the confrontation assay, we focused all consecutive experiments on one strain (AP-SLU6) only. In addition to being a root pathogen, *P. cactorum* also causes crown rot disease, which affects both crowns and leaves. Therefore, strawberry leaves (*cv*. Ostara) were detached and injuries were inflicted using a sterile razor blade by gently scraping the surface of the leaves. The conidial concentration of the *A. pullulans* strain AP-SLU6 and *P. cactorum* was maintained at 1.5×10^5 conidia or zoospores ml^{-1} followed by inoculation of 20 µl to the injured surface of each leaf and then incubation at 22 °C, 90% RH for one week. The lesion diameter was measured at a resolution of seven DPI using ImageJ software (version 1.52p). Five treatments were included: (1) leaves + water only, (2) leaves + *P. cactorum* (*Pc*) only, (3) leaves + AP-SLU6 only, (4) leaves + *Pc* + SLU6 (combined application), (5) leaves + *Pc* + AP-SLU6 (pre-application). In the fourth treatment, a mixture of the biocontrol and the pathogenic fungi was applied in a single delivery, while the fifth treatment involved pre-treated with *A. pullulans* strain AP-SLU6 followed by inoculation of *P. cactorum* after 24 h of incubation. The assay was performed on six biological replicates of each treatment. The experiments were performed in duplicate.

Trypan blue staining of leaves

The leaf tissues were washed with tap water for 10 min, then dipped in 70% ethanol for 2 s for disinfection. Subsequently, surface sterilization was performed with sodium hypochlorite (NaCLO) for 5 min, followed by washing 3–4 times with distilled water, as described previously (Munir et al. 2015). Thereafter, trypan blue was used to stain infected leaf tissue. The protocol was adapted from van Wees (2008). In brief, samples were submerged in trypan blue solution (1:1 mixture (v/v), 2.5 mg ml^{-1} trypan blue, 25% v/v lactic acid, 25% glycerol, 25% phenol, and water), boiled for 5 min, and incubated overnight at room temperature to stain. Leaves were de-stained with chloral hydrate solution (250 g in 100 ml H_2O) and left in the solution for two days. The chloral hydrate solution was replaced with 50% glycerol for the storage of samples. Images were acquired using an Epson Perfection V750 pro scanner.

Detached fruit assay against B. cinerea

Green strawberry fruits (*cv*. Honeoye) were harvested from the field, brought to the laboratory and then surface sterilized as described above. The fruits were injured by scraping with a sterile razor blade across the surface close to their neck. The conidial concentration of *A. pullulans* strain AP-SLU6 and *B. cinerea* was maintained at 1.5×10^5 conidia ml^{-1}, and 15 µl was inoculated onto the injured neck of each fruit before being sealed in a plastic tray, incubated at 22 °C and maintained at 70% RH for ten days. Four treatments were included: (1) fruits + *B. cinerea* (*Bc*) only, (2) fruits + AP-SLU6 only, (3) fruits + *Bc* + AP-SLU6 (combined application), (4) fruits + *Bc* + AP-SLU6 (pre-application). In the third treatment, a mixture of the biocontrol and the pathogenic fungi was applied in a single delivery, while treatment four involved pre-treated with *A. pullulans* AP-SLU6, followed by inoculation of *B. cinerea* after 48 h of incubation. The disease severity was scored at a resolution of 10 DPI as described previously (Adikaram et al. 2002). The assay was performed using ten biological replicates of each treatment. The experiments were performed twice. The following scale was used: 0: no fungal growth, 1: fungal growth only on the margin of the lesion, 2: even but slight fungal growth all over, and 3: dense fungal growth all over.

In vivo biocontrol assays

Biocontrol of root rot disease

Root rot assays on strawberry (*cv*. Ostara) plants were performed in a greenhouse, using a complete randomized experimental design. The strawberry plants were removed from their plastic pots, whereafter the roots were washed with tap water, and subsequently placed in glass jars for 3 h, these were filled with approximately 150 ml of *P. cactorum* zoospore suspension contained with 1.5×10^5 zoospores ml^{-1}. The assay was performed using five treatments: (1) plant + water only, (2) plant + *Pc* only, (3) plant + AP-SLU6 only, (4) plant + *Pc* + AP-SLU6 (combined application), (5) plant + *Pc* + AP-SLU6 (pre-application). Six biological replicates were used for each treatment. In the combined application, a mixture of the biocontrol and the pathogenic fungi was applied in a single delivery in the same jar, while the pre-treatment involved application of SLU6 followed by inoculation of *P. cactorum* after 24 h of incubation. Afterwards, the roots were removed from the jars and placed in plastic pots that were filled with 150 g of soil and incubated in a greenhouse at 23 ± 2 °C for four weeks. The disease scoring was performed by measuring the infected area of the roots using the following scale: 1:0–20%, 2:21–40%, 3:41–60%, 4:61–80%, 5:61–80% and 6:81–100%.

Biocontrol of grey mould disease

A grey mould assay on strawberry (*cv*. Honeoye) plants was performed in a greenhouse using a complete randomized experimental design. Plastic pots were filled with 150 g of soil and planted with strawberry plants, then maintained at 20 to 22 °C. The same conidial concentration was used as in the detached fruit assay against *B. cinerea*. The prepared formulation of *A. pullulans* strain AP-SLU6 was sprayed on flowers and fruits every seven days for the three weeks of the experiment, while *B. cinerea* was applied once. Five treatments were included in the assay: (1) plant + water only, (2) plant + *Bc*, (3) plant + AP-SLU6 only, (4) plant + *Bc* + AP-SLU6 (combined application), (5) plant + *Bc* + AP-SLU6 (pre-application). Six biological replicates were used for each treatment. Pre-treatment was performed with AP-SLU6 followed by inoculation of *B. cinerea* after 48 h of incubation, while in the combined application, a mixture of the biocontrol and the pathogenic fungi was applied in a single delivery. The inoculation was performed using a hand sprayer. After four weeks, the disease severity was scored by measuring the density of mycelial growth as described previously (Adikaram et al. 2002). In short, 0: no fungal growth, 1: fungal growth only on the margin of the lesion, 2: even but slight fungal growth all over, and 3: dense fungal growth all over.

Statistical analysis

Data on growth rates and lesion size were analysed using ANOVA in Minitab 18.1 (Minitab Inc., State College, PA, USA). Subsequent pairwise comparisons were carried out using Fisher's least significant difference at 95% significance level. Disease score data were not normally distributed and global comparisons were thus analysed using non-parametric Kruskal–Wallis tests. Pairwise comparisons were made using Dunn's test with Bonferroni correction for multiple comparisons. The mycelial growth of the *P. cactorum* and *B. cinerea* during the confrontation assay, lesion size on the leaves and severity of grey mould disease during in vitro or in vivo assays were used as dependent variables.

Results

In vitro antagonism assay against fungal pathogens

The antagonistic ability of the four *A. pullulans* strains was tested against *P. cactorum* and *B. cinerea* in a dual-plate confrontation assay. *P. cactorum* and *B. cinerea* showed significantly reduced growth rates during confrontation with all *A. pullulans* strains compared with their growth rates when grown alone (control) at a resolution of three and five DPI respectively (Fig. 1). *P. cactorum* showed a significantly reduced growth rate ($F_{4,25} = 8.28$; $p < 0.001$) when challenged with *A. pullulans* strains compared with the control treatment at five DPI (Fig. 1a). The highest reduction (42–44%) in the growth of *P. cactorum* was observed in the presence of AP-30273 and AP-SLU6 followed by AP-53383 and AP-30044, where mycelial growth was reduced by as much as 28–30% compared with the control treatment (Fig. 1a). Similarly, the AP-30044 strain significantly ($F_{4,25} = 26.75$; $p < 0.001$) reduced the growth (48%) of *B. cinerea* followed by AP-53383 (44%) while AP-30273 and AP-SLU6 reduced the growth of *B. cinerea* by 26–33% compared with the control treatment

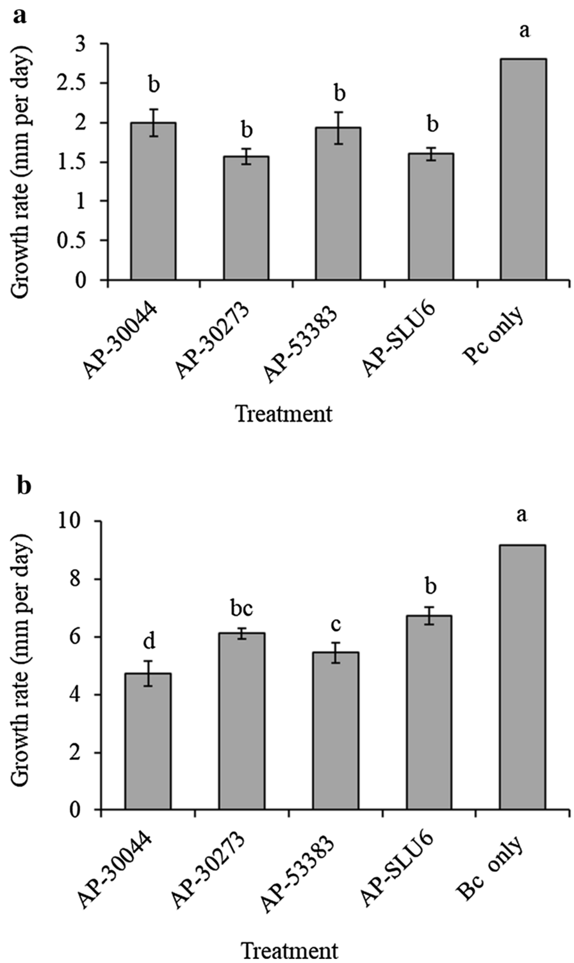

Fig. 1 Antagonism test of *Aureobasidium pullulans* strains (AP-30044, AP-30273, AP-53383 and AP-SLU6) against *Phytophthora cactorum* and *Botrytis cinerea* on potato dextrose agar (PDA) plates. **a** Growth rate of *P. cactorum* and **b** *B. cinerea* during interaction with *A. pullulans* strains. Error bars indicate SE of six biological replicates. Different letters indicate a statistically significant difference ($p < 0.001$) between treatments as determined by Fisher's least significant difference test. AP *Aureobasidium pullulans*, Bc *Botrytis cinerea*, Pc *Phytophthora cactorum*

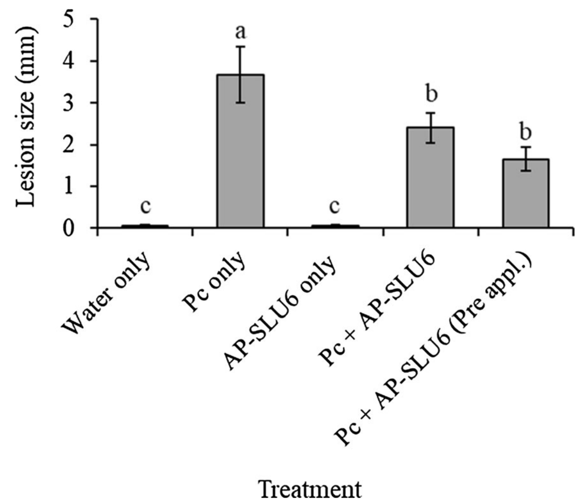

Fig. 2 Antagonism test of *Aureobasidium pullulans* strain AP-SLU6 on detached strawberry leaves against crown rot disease. Error bars indicate SE of six biological replicates. Different letters indicate a statistically significant difference ($p < 0.001$) between treatments as determined by Fisher's least significant difference test. AP *Aureobasidium pullulans*, Pc *Phytophthora cactorum*

(Fig. 1b; Supplementary Figure S1). However, *A. pullulans* strains and both pathogenic fungi *B. cinerea*, and *P. cactorum* had overgrown each other after seven and ten DPI, respectively.

Detached leaf assay against *P. cactorum*

The necrotic lesion size on strawberry leaves was significantly reduced ($F_{4,25} = 18.6$; $p < 0.001$) in treatments where AP-SLU6 was applied either directly in combination with *P. cactorum* or as a pre-treatment compared with the control treatment in which only *P. cactorum* was applied (Fig. 2). However, pre-application of AP-SLU6 produced a more pronounced antagonistic effect against *P. cactorum*, which suggests that *A. pullulans* had enough time to colonize the surface of the leaves. No lesions were observed when AP-SLU6 was applied alone, while hardly any lesions developed in the control water treatment (Fig. 2). This is further evidenced by the trypan blue staining of the leaves where the reduced necrotic lesions were visualized after AP-SLU6 treatment, as shown in Fig. 3.

Detached fruit assay against *B. cinerea*

B. cinerea on fruits showed significantly ($\chi^2_3 = 25.15$; $p < 0.001$) reduced disease severity when the AP-SLU6 strain conidial suspension was applied in direct combination with *B. cinerea* inoculum (Fig. 4). Likewise, disease severity was significantly reduced on fruits pre-treated with the AP-SLU6 strain followed by *B. cinerea* compared with the treatment where only *B. cinerea* was applied, i.e. the control (Fig. 4). No

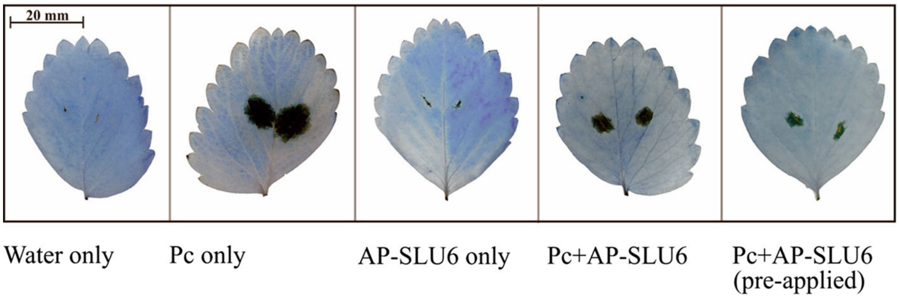

Fig. 3 Trypan blue staining of detached strawberry leaves during antagonism test of *Aureobasidium pullulans* strain AP-SLU6 against crown rot disease. Necrotic lesion sizes were recorded using an Epson perfection V750 pro scanner. AP *Aureobasidium pullulans*, Pc *Phytophthora cactorum*

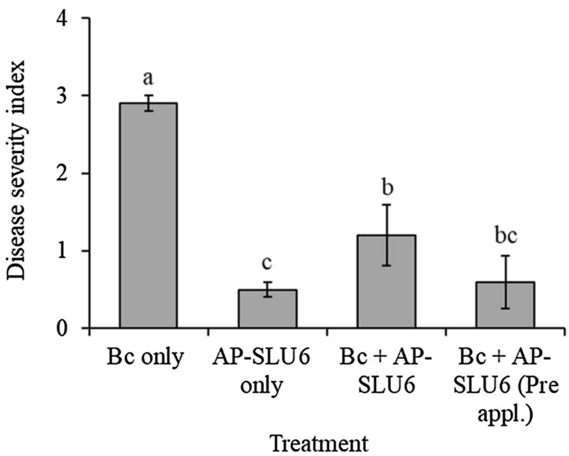

Fig. 4 Antagonism test of *Aureobasidium pullulans* strain AP-SLU6 on detached strawberry fruits against grey mould disease. Error bars indicate SE of ten biological replicates. Different letters indicate a statistically significant difference ($p < 0.05$) between treatments as determined by Dunn's test with Bonferroni correction. AP *Aureobasidium pullulans*, Bc *Botrytis cinerea*

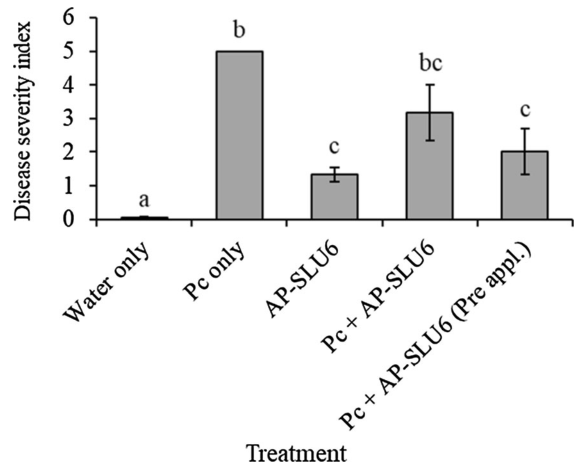

Fig. 5 In vivo bioassay to test the biocontrol efficacy of *Aureobasidium pullulans* strain AP-SLU6 against root rot disease on strawberry. Error bars indicate SE of six biological replicates. Different letters indicate a statistically significant difference ($p < 0.05$) between treatments as determined by Dunn's test with Bonferroni correction. AP *Aureobasidium pullulans*, Pc *Phytophthora cactorum*

significant difference in symptom development was found between fruits pre-treated with AP-SLU6 followed by *B. cinerea* as compared to when AP-SLU6 was applied alone (Fig. 4).

Biocontrol of root rot disease

Application of AP-SLU6 significantly ($\chi^2_4 = 22.27$; $p < 0.001$) reduced the severity (approximately 73%) of root rot disease compared with the control treatment where only *P. cactorum* was applied (Fig. 5). Likewise, application of AP-SLU6 in combination with *P. cactorum* reduced the disease severity (37%). However, this reduction was not statistically significant. Similarly, significantly enhanced biocontrol efficacy was observed compared with the control treatment when AP-SLU6 was applied before *P. cactorum* (Fig. 5).

Biocontrol of grey mould

Spray application of AP-SLU6 to intact plants in any form, directly combined or in a pre-treatment application followed by *B. cinerea,* reduced the disease severity of grey mould on fruits compared with the control treatment (Supplementary Figure S2).

However, this reduction was not statistically significant ($\chi^2_4 = 6.84$; $p = 0.144$).

Discussion

The current study was designed to investigate the biocontrol potential of *A. pullulans* against two important strawberry pathogens, i.e., *P. cactorum* and *B. cinerea*. Importantly, this is the first time that *A. pullulans* has been tested against the pathogen *P. cactorum*. The results are promising, showing that *A. pullulans* has potential to inhibit both of these pathogens, possibly reducing the need for synthetic fungicides in strawberry production.

The reduced growth rate of *P. cactorum* and *B. cinerea* during confrontation with *A. pullulans* strains compared with the control suggests that the biocontrol fungus *A. pullulans* has an inhibitory effect on the growth of the pathogenic oomycete/fungi. The mechanism behind the observed inhibitory impact on the growth of *P. cactorum* and *B. cinerea* remains unknown. However, it could be that *A. pullulans* produces antifungal compounds, proteases or enzymes or secretes volatile compounds that inhibit the pathogens. Previously, it has been shown that *A. pullulans* produces a broad range of extracellular enzymes (Molnárová et al. 2014) and antifungal peptides, for instance, aurebasidin A (Takesako et al. 1991). It has also been reported that *A. pullulans* is involved in degrading *B. cinerea* cell walls by secreting chitinase and protease in potato (Chen et al. 2018). Gostinčar et al. (2014) showed that different strains of *A. pullulans* vary considerably in their production of extracellular enzymes and sugar transporters. They suggested that these major differences between strains reflect ecological or evolutionary adaptations to their respective environment. Thus, strains isolated from different plants or other biotic or abiotic substrates may have very different lifestyles, and differ in their suitability for biocontrol use. Hence, we included several strains in our initial assays and studied the antagonistic activity of *A. pullulans* on both strawberry fruits and leaves. Our results revealed similar patterns in reducing necrotic lesions of *P. cactorum* on leaves and growth of *B. cinerea* on strawberry fruits when inoculation of pathogens was performed together with the biocontrol fungus AP-SLU6, either on leaves or fruit. However, pre-application of AP-SLU6 resulted in higher antagonism against *P. cactorum*, which could be explained by *A. pullulans* having enough time to colonize the surface of leaves and thus deliver greater protection against the development of necrotic lesions in the detached leaf assay. Previously, Adikaram et al. (2002) also showed that pre-treatment application of *A. pullulans* suppressed the growth of grey mould more efficiently on wound sites of green fruits of strawberries.

Our in vivo bioassay showed that AP-SLU6 is effective in controlling root rot disease of strawberry. It has previously been shown that *A. pullulans* can produce a compound called pullulan as well as polysaccharides which improve biofilm formation and are therefore helpful in the adhesion mechanism, thus explaining the increased level of antagonism and biocontrol efficacy against *P. cactorum* during in vitro or in vivo bioassays (Bozoudi and Tsaltas 2018; Freimoser et al. 2019).

Unexpectedly, our experiment to investigate the ability of *A. pullulans* to control grey mould under greenhouse conditions revealed no significant differences between the different treatments. However, application of AP-SLU6 alone or in combination with *B. cinerea* delayed the development of the disease on plants. The fact that our results on biological control of grey mould are in contrast with the observed in vitro antagonism might not be surprising given the complexity of biological control mechanisms, including not only competition for nutrients and space, but also antibiosis and direct or indirect parasitism via induction of plant defence reactions (Harman et al. 2004; Iqbal et al. 2020; Jensen et al. 2017). Another reason could be that poor colonization of *A. pullulans* on the fruit surface made it unable to cope with the high pathogenicity of *B. cinerea*. It has previously been shown that *A. pullulans* has the potential to control grey mould on strawberry and rotting of cherries, grapes and kiwi fruit (Ippolito et al. 1997; Schena et al. 1999). According to Jersch et al. (1989), reduction in the disease was attributed to quiescent pathogens and the presence of green fruits (*cv*. Senga Sengana). An extract of green fruits contains an antifungal compound called proanthocyanidin and its higher concentration in the receptacle reduces colonization of *B. cinerea*, which is involved in controlling grey mould. Recently, Di Francesco et al. (2020) showed that *A. pullulans* plays an important role in reducing the disease incidence of grey mould in tomato in vivo.

Surprisingly, our results showed grey mould symptoms on plants treated with only water. This could be explained by the dispersal mechanism of the *B. cinerea*, which is possibly spread through the air and via water splashes.

In summary, this study showed that *A. pullulans* is capable of reducing the severity of crown rot, root rot and grey mould in strawberry. This approach is probably a more environmentally and evolutionarily sustainable way to control these diseases than chemically based fungicides. The results are important because biocontrol is a cornerstone in Integrated Pest Management which in turn is globally endorsed as the future paradigm of crop protection (Stenberg 2017). However, more investigations are required to test commercially important parameters such as yield, fruit quality and as well as the mechanisms involved in order to understand the exact role of the biocontrol fungus *A. pullulans* and to improve biocontrol efficacy against the strawberry diseases.

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Author contributions MI, MJ, RRV, EA and JAS conceived and designed the experiments. MJ performed the experiments. MI and MJ analysed the data. MAZ performed the trypan blue staining of strawberry leaves. MI and MJ wrote the manuscript with critical input from all authors. All authors read and approved the manuscript.

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Data availability All data generated or analysed during this study are included in this manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest related to funding or otherwise.

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Consent for publication All authors consent to publication.

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