agriculture

MDPI

Article

Screening for Antagonistic Yeasts to Manage *Alternaria* spp. in Organic Farming

Jolanta Kowalska, Joanna Krzymińska *, Kinga Matysiak and Magdalena Jakubowska

Institute of Plant Protection—National Research Institute, Wegorka 20, 60-318 Poznan, Poland
* Correspondence: j.krzyminska@iorpib.poznan.pl

Abstract: Early blight of potatoes when not controlled can lead to major yield loss. In organic farming, disease control methods using beneficial microorganisms are needed. This study aimed to use commercially available yeast strains to prevent early blight in organically grown potatoes. Six commercially yeast strains used in the food industry, mainly in baking, brewing and winemaking, were evaluated against *Alternaria alternata* and *A. solani*. An in vitro test was conducted to assess yeast antagonistic properties. Production of lytic exoenzymes by yeast strains was determined. In the greenhouse experiments, the abilities of yeast strains to colonize potato leaf surface and to minimize *Alternaria* symptoms on plants were assessed. *Saccharomyces cerevisiae* Coobra strain inhibited in vitro *Alternaria* mycelium growth and most effectively reduced *Alternaria* symptoms on inoculated plants (from approximately 60% to 9% for *A. solani* and 14% for *A. alternata*) after seven days. This strain produced the most enzymes, i.e., amylase, pectinase and protease. After eighteen days, only the *S. cerevisiae* Coobra population was isolated from the leaves. In conclusion, the Coobra strain shows antagonistic properties against *Alternaria* spp. and is promising for further field tests.

Keywords: commercially yeast; biocontrol; organic farming; *Alternaria*; early blight; potato

Citation: Kowalska, J.; Krzymińska, J.; Matysiak, K.; Jakubowska, M. Screening for Antagonistic Yeasts to Manage *Alternaria* spp. in Organic Farming. *Agriculture* **2022**, *12*, 1693. https://doi.org/10.3390/agriculture12101693

Academic Editor: Renata Bažok

Received: 18 August 2022
Accepted: 12 October 2022
Published: 14 October 2022

1. Introduction

Early blight of potatoes caused by *Alternaria* spp. is a global disease that, when not controlled, can cause a huge leaf loss and lead to yield loss of up to 30% [1,2]. The predominant species *Alternaria solani* Sorauer and a species that usually infects plants later in the plant growing season, *Alternaria alternata* (Fr.) Keissl., are soil-borne fungal pathogens that cause necrotic lesions on potato leaves. The lesions are spots with concentric rings, frequently surrounded by yellow chlorotic tissue created by the diffusion of fungal toxins [3]. Symptoms usually appear a few weeks after plant emergence on lower leaves as black or brown spots. The spots coalesce, which causes the leaves to die and disease to spread to other leaves [4]. Cultural methods to control early blight in potatoes are limited. It includes long crop rotation, good weed management, removing alternative hosts, appropriate fertilizer application and irrigation [5,6]. In organic farming, in which chemical pesticides are prohibited, other additional control methods, such as biological methods, need to be investigated [7].

Biological agents are a vital alternative to chemical pesticides [8,9]. To be successful, microorganisms antagonistic against plant pathogens can produce substances harmful to targeted pathogens. They also efficiently colonize plant surfaces such as leaves and fruit, effectively competing for available nutrients and space and surviving in shifting environments [10,11]. It is also crucial that they do not produce any harmful metabolites for animals and people, or that they negatively affect the final product and, therefore, cause any biosafety concerns. Among the microorganisms potentially antagonistic against plant pathogens, many yeast species meet these criteria [12].

Yeasts occur in every environment, including water, soil, plants and animals. These single-cell organisms have been used in the food industry and for direct human consumption as dietary supplements for millennia [13–15]. Usually, they do not produce toxic

substances such as mycotoxins, allergens or antibiotics; therefore, they are considered harmless for the health of humans and animals, even if directly digested. They also quickly adapt to the environment [16,17]. Many yeast species have been described as antagonists against various plant pathogens. They compete with plant pathogens for space and nutrients [18–20], and can produce lytic exoenzymes [21,22], toxins [23,24] and volatile organic compounds [25–28], which can damage plant pathogens and negatively influence their growth. They can also induce plant immunity [29–31].

Yeasts have been commonly used in the food industry, mainly in baking, brewing and winemaking. As such, many yeast strains are available on the market. Some of them may exhibit antifungal properties. Therefore, this study aims to explore the possibility of using strains of yeast, which are already commercially available and used in the food industry, to repurpose them for agriculture and prevent early blight in potatoes grown as organic crops.

2. Materials and Methods

2.1. Antagonistic Yeast and Pathogenic Fungi

Six commercial yeast strains were used. The names of the suppliers were substituted with capital letters (Table 1). The yeast strains were isolated from commercial products after rehydration and cultivation on a PDA medium (Potato Dextrose Agar, Difco, Thermo Fisher Scientific, Waltham, MA, USA) for 72 h at 23 °C.

Table 1. List of commercially available yeast strains used in the presented study.

Strain Code in Tests	Comercially Strain	Supplier	Species
01	Biodiva TD 291	A	*Torulaspora delbrueckii*
02	W 34/70	B	*Saccharomyces pastorianus*
03	V 116	C	*Saccharomyces cerevisiae*
04	EC 118	C	*Saccharomyces bayanus*
05	US 05	B	*Saccharomyces cerevisiae*
06	Coobra	D	*Saccharomyces cerevisiae*

Biodiva TD 291 is a pure culture of *Torulaspora delbrueckii* used to enhance wine complexity. This strain is tolerant to osmotic shock [32]. W 34/70 is the most popular yeast strain in brewing, used worldwide for its stability [33]. V 1116 is a killer yeast strain designed for the fermentation of red and white wine [34]. US 05 is a beer brewing strain active at lower temperatures such as 14–16 °C [35]. The strain EC 118, *Saccharomyces bayanus*, has low nutrient requirements [36] and Coobra is a highly productive distillery yeast strain.

Alternaria alternata (strain 12/124) and *Alternaria solani* (strain 1558) were obtained from the Institute of Plant Protection—National Research Institute Bank of Pathogens in Poznan, Poland.

2.2. Antagonism between Yeast and Alternaria spp.

In the in vitro experiment, each yeast culture was streaked onto a PDA medium (pH 6.5) as parallel lines (60 mm in length), each line 25 mm from the centre of the plate in five replications. Pathogenic fungi discs (*A. alternata* and *A. solani*), taken from the margin of a fresh colony, 5 mm in diameter, were placed in the middle of the plate (Figure 1). Plates with pathogenic fungi discs on the PDA medium were used as the control. The plates were incubated at a controlled temperature of 23 °C.

After six days, when any of the combinations were completely covered by pathogenic fungi mycelium., the diameter of the pathogenic fungi was measured. The percentage of growth inhibition was calculated: growth rate of control—growth rate of treated sample)/growth rate of control × 100%.

After six days of cultivation, a pathogenic fungi disc from each plate was moved to a fresh PDA medium to assess whether it can resume growth. After the following three days the plates were checked for pathogenic fungi mycelium growth.

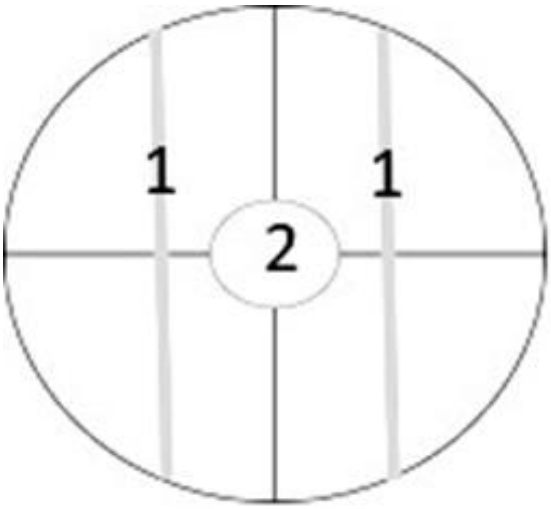

Figure 1. The layout of the plate experiment (1—antagonistic yeast; 2—pathogenic fungi).

Additionally, an in planta greenhouse experiment was conducted. For each combination, three unfertilized potato plants (Tajfun cultivar, FN Granum, Wodzierady, Poland) in the stage of development BBCH 19, in four replicates, were inoculated by conidial suspension of 10^5 conidia/mL of *A. alternata* and *A. solani* isolates grown on PDA (Potato Dextrose Agar, Sigma-Aldrich, St. Louis, MO, USA). The foliar spray inoculation method was performed with a hand sprayer. On average, 5 mL per plant was applied mainly directly to the leaves, incidentally coating stems. After inoculation, plants were covered with a plastic bag and kept at 25 °C and 12/12 photoperiod for 24 h to maintain a high level of humidity. Next, plants were transferred to a greenhouse and each plant was sprayed with an aqueous yeast suspension (McFarland concentration: 2 [37]). Inoculated plants, sprayed with distilled water were used as a control. After a week, when chlorotic and necrotic symptoms appeared, a disease severity assessment was conducted on 5 leaves per each plant selected at random. The following scale was used: grade 0—disease free, grade 1—1–10% of leaf area infected, grade 2—11–25% of leaf area infected, grade 3—26–50% of leaf area infected, grade 4—51–75% of leaf area infected, grade 5—over 76% of leaf area infected [38]. Next, the disease severity index (*DSI*) was calculated.

$$DSI\ (\%) = \frac{\Sigma(grade\ score\ x\ grade\ frequency)}{(total\ number\ of\ observations)x(maximal\ disease\ score)} \times 100$$

2.3. Production of Lytic Exoenzymes

The production of lytic exoenzymes by yeast strains was determined. Amylase, xylanase, lipase, pectinase and protease secretion were estimated according to the methods of Gabriel [39] and Strauss [40]. Yeast isolates were grown on specific media for each exoenzyme: base medium and starch for amylase secretion, base medium and 0.1% Remazol Brilliant Blue R−D-Xylan for xylanase secretion, Bacto Spirit Blue Agar with lipase reagent for lipase secretion, YNB medium with 1.25% polygalacturonic acid, 0.68% potassium phosphate (pH 3.5), 1% glucose and 2% agar for pectinase secretion and YPD medium with 2% casein for protease secretion. The observable transparent zones in specific media indicated the secretion of amylase, lipase, pectinase and protease by the tested yeast strains. The blue halo surrounding yeast colonies indicated their xylanase production.

2.4. Alternaria Mycelium Evaluation after Contact with Yeast Strains

Yeast strains' influence on *A. alternata* and *A. solani* mycelium was evaluated. Within 5 days, pathogens were co-cultured in a Petri dish on PDA medium with each tested yeast strain. The pathogen mycelium was evaluated under a light microscope at ×100 magnification for hyphal damage. Mycelium disks were taken from different parts of

the dish and excess media were removed. The mycelium morphology was compared to *A. alternata* and *A. solani* mycelium growing separately on PDA medium. The test was repeated.

2.5. Colonization of Yeasts on the Potato Leaf Surface

Each tested strain of the yeast was suspended in distilled water at 2×10^7 CFU/mL. Potted, unfertilized potato plants (Tajfun cultivar) at BBCH 19 were used. They were uniformly sprayed onto leaves with 5 mL of the suspension using a hand-held sprayer in a sterile controlled environment. Plants sprayed with distilled water were used as a control to inspect for contamination with non-target yeasts. The experiment was held in the greenhouse at 18–23 °C. There were three replicates of one pot holding one plant for each treatment. Every three days, starting on the third day and up to the eighteenth day, one leaf from each plant was carefully collected by clipping with sterile pliers and three round pieces measuring 1 cm^2 each were collected from each leaf. The fragments of the leaves were placed in test tubes in sterile water and shaken for 2 min at 6000 rotations/min in a mini centrifuge rotator. A total of 0.1 mL of the obtained suspension was collected and plated on a PDA medium. Plates were placed at 23 °C. Colonies were counted after 48 h. The result was expressed as CFU/1 cm^2 of a leaf surface and compared to the control sprayed with distilled water.

2.6. Statistical Analysis

Mean values were calculated. Final experimental data were represented as the mean. A one-way ANOVA was performed, and Tukey's test was used to identify whether differences among data were significant at the level of $p < 0.05$ using Statistica 12.

3. Results

3.1. Growth of Alternaria spp. Inhibited by Antagonistic Yeast Strains

Compared with the control, the growth of *A. solani* was significantly inhibited by four out of the six yeast strains ($p = 0.05$), i.e., strains number 03 (V 116), number 04 (EC 118), number 05 (US 05) and number 06 (Coobra). After six days of co-cultivation, mycelium growth was inhibited by 25.1% (mycelium measured 67.33 mm on the tested plate and 90 mm on the control plate) by strain number 04. The Coobra strain inhibited mycelium growth by 23.7% (mycelium measured 68.67 mm on the tested plate) and strains V 116 and US 05 by 20.7% (mycelium measured 71.33 mm on tested plates). Strain W 34/70 negatively influenced mycelium growth just by 5.9% (mycelium measured 84.67 mm on the tested plate) and the difference from the control plate was insignificant. The mycelium growth on the plate with strain number 01 was not inhibited. When the fungal discs were moved to fresh agar, the mycelium did not grow after being in contact with yeast strains number 04 (EC 118) and number 06 (Coobra) and it grew slower after being in contact with yeast strains number 02 (W 34/70), number 03 (V 116) and number 05 (US 05). Strain 01 (Biodiva TD 291), which belonged to the species *T. delbrueckii*, did not inhibit the mycelium growth of *A. solani* (Figure 2 and Table 2).

The growth of *A. alternata* mycelium was significantly inhibited by the Coobra yeast strain (number 06) compared to the control ($p = 0.05$). The mycelium growth inhibition was noticeably weaker in comparison with *A. solani*. On the sixth day of the assay, the Coobra strain inhibited the mycelium growth by 14.44% (the mycelium measured 77 mm on the tested plate and 90 mm on the control plate). The growth of *Alternaria* mycelium grown together with strain US 05 (number 05) was inhibited by 8.14% (the mycelium measured 82.67 mm). Strains number 01–04 did not inhibit mycelium growth. After moving the fungal discs to the fresh medium, the growth of the mycelium was completely inhibited for the fungi that were in contact with the Coobra yeast strain (number 06) and visibly slowed down for the mycelium that was in contact with strain US 05 (number 05) (Figure 3 and Table 3).

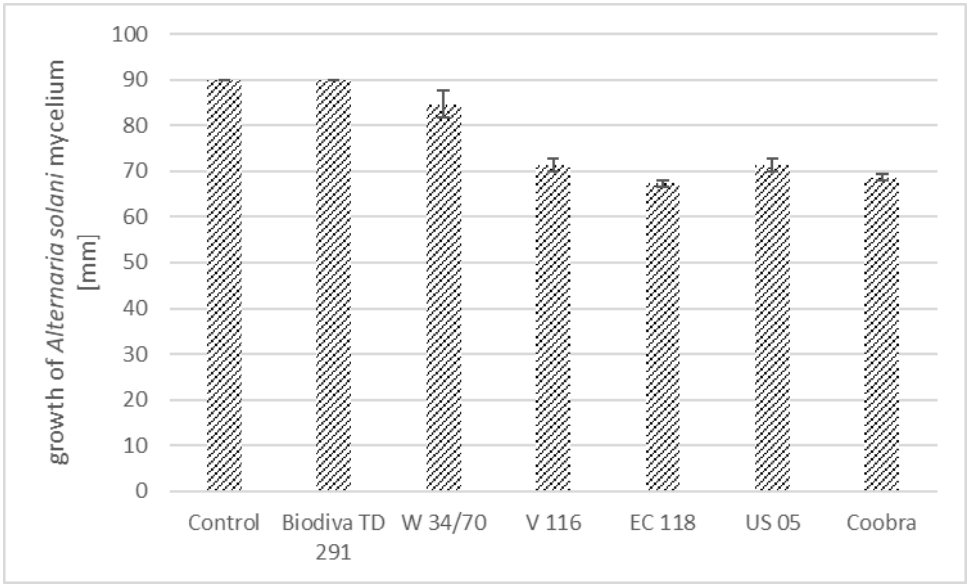

Figure 2. Inhibitory effect of antagonistic yeast strains on the growth of *Alternaria solani* mycelium after six days. Mycelium diameter in the cultures (mm) on Petri plates ± SD.

Table 2. Inhibitory effect of antagonistic yeast strains on the growth of *Alternaria solani* mycelium three days after moving the fungal disc to the fresh medium.

Strain	Control	Biodiva TD 291	W 34/70	V 116	EC 118	US 05	Coobra
Effect	0	0	+	+	++	+	++

0 no effect on mycelium; + growth of mycelium inhibited; ++ growth of mycelium completely inhibited.

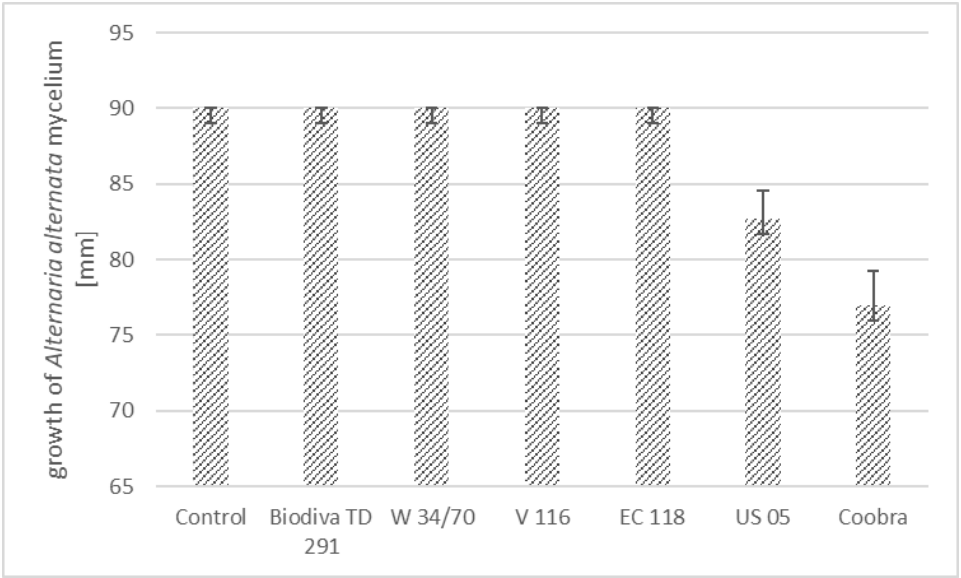

Figure 3. Inhibitory effect of antagonistic yeast strains on the growth of *Alternaria alternata* mycelium after six days. Mycelium diameter in the cultures (mm) on Petri plates ± SD.

Table 3. Inhibitory effect of antagonistic yeast strains on the growth of *Alternaria alternata* mycelium three days after moving the fungal disc to the fresh medium.

Strain	Control	Biodiva TD 291	W 34/70	V 116	EC 118	US 05	Coobra
Effect	0	0	+	+	++	+	++

0 no effect on mycelium; + growth of mycelium inhibited; ++ growth of mycelium completely inhibited.

3.2. Reducing Symptoms Caused by Alternaria spp. on Potato Plants in a Greenhouse Experiment

The disease severity index was significantly lower for strains number 03 (V 116), 04 (EC 118), 05 (US 05) and 06 (Coobra) of both *A. alternata* and *A. solani*. Strains number 05 and number 06 were the most effective. Strains number 01 (Biodiva TD 291) and number 02 (W 34/70) did not reduce symptom severity. All yeast strains were more effective against *A. solani* symptoms than against *A. alternata* (Table 4).

Table 4. Effect of antagonistic yeast strains spraying on the DSI (%) of *A. alternata* and *A. solani* on potted potato plants after seven days in greenhouse conditions.

Strain	Control	Biodiva TD 291	W 34/70	V 116	EC 118	US 05	Coobra
A. alternata	60.00 ± 3.27 a	54.67 ± 3.77 a	60.00 ± 3.27 a	41.33 ± 1.89 b	46.67 ± 1.89 b	17.33 ± 1.25 c	14.67 ± 1.89 c
A. solani	61.33 ± 4.99 a	49.33 ± 7.54 a	62.67 ± 1.89 a	25.33 ± 4.99 b	26.67 ± 5.56	26.67 ± 3.77 b	9.33 ± 2.87 c

Values in rows followed by the same letter do not differ significantly at $p = 0.05$.

3.3. Production of Lytic Exoenzymes—Results

The lytic exoenzymes secretion varied for tested strains. The Coobra yeast strain produced the most enzymes, i.e., amylase, pectinase and protease. Two strains produced two enzymes and one strain produced one enzyme. Two strains did not produce any tested enzymes (Table 5).

Table 5. Qualitative lytic exoenzyme production in antagonistic yeast strains.

Exoenzyme	Yeast Strain					
	Biodiva TD 291	W 34/70	V 116	EC 118	US 05	Coobra
Amylase	+	-	-	-	+	+
Xylanase	-	-	-	-	-	-
Lipase	-	-	-	-	+	-
Pectinase	-	-	-	+	-	+
Protease	+	-	-	-	-	+

- enzyme not produced, + enzyme produced.

3.4. Alternaria Mycelium Evaluation after Contact with Yeast Strains—Results

The presence of yeast strains number 05 and number 06 changed the cells of both *A. alternata* and *A. solani*. The mycelia analyzed under the microscope were partially deformed and ruptured and the loss of intracellular content was visible. The strains number 01–04 did not cause visible cell damage at all. The test is intended to be expanded in a future study to provide clarification of the mechanisms along with photographic evidence similar to that of Saleh Al-Maawali et al. [41].

3.5. Population Dynamics of the Antagonistic Yeast Strains on the Potato Leaf Surface

All six yeast strains colonized the surface of potato leaves under greenhouse conditions. After six days, four yeast strains i.e., Biodiva TD 291, W 34/70, US 05 and Coobra maintained a population over 20 CFU/ 1 cm^2 of the potato leaf surface: 26.89 for strain Biodiva TD 291, 23.67 for strain US 05, 22.44 for strain W 34/70 and 20.67 for the Coobra strain. After fifteen days, colonies of the yeast strains US 05 and Coobra were present on the leaf surface (1.22 and 5.00 CFU/ 1 cm^2 of the potato leaf surface) and after eighteen days only the Coobra yeast strain population was isolated from the leaves (2.67 CFU/ 1 cm^2 of the potato leaf surface) (Figure 4).

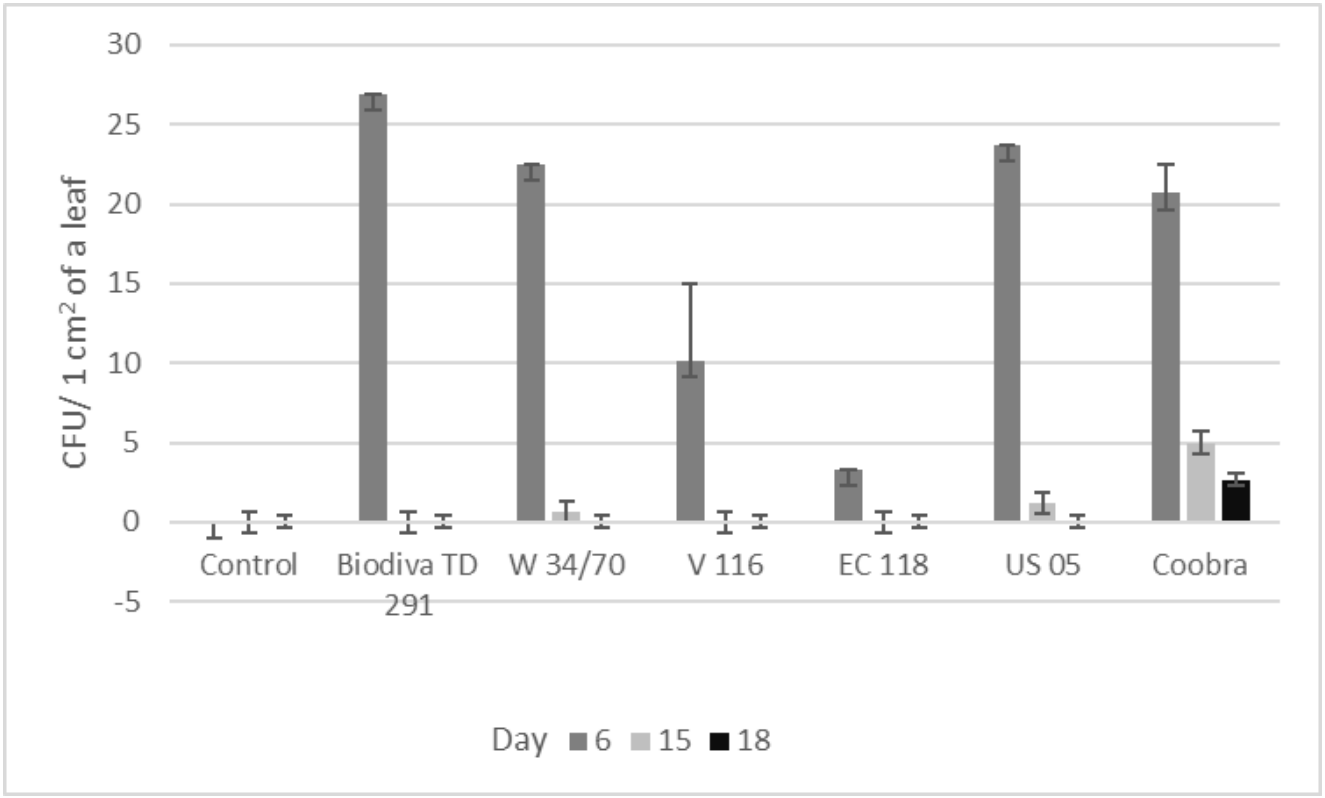

Figure 4. Survival of the population of antagonistic yeast strains on the surface of the potato leaves in greenhouse conditions [CFU/1 cm^2] ± SD.

4. Discussion

As biocontrol, the use of living organisms and natural products to control plant pests and diseases is benign for the environment and for human and animal health. It also allows for sustainable crop production, while being effective and target-specific with fewer environmental risks, and is an excellent alternative to chemical pesticides [42–45]. Yeast, among other microorganisms, are often used as biocontrol. Some of the researched yeast strains used in alcohol fermentation are promising as agents against the early blight of potatoes.

Yeasts have been reported to suppress *Alternaria* sp. growth both in in vitro and in planta studies. This study demonstrated that one of the tested *S. cerevisiae* strains, i.e., Coobra, inhibited *Alternaria* spp. mycelium growth when placed together on a Petri dish in an in vitro experiment. It also demonstrated that when a fungal disc was moved to a fresh medium and another strain, i.e., US 05, it showed antagonistic tendencies against mycelium growth. Similar results for other *S. cerevisiae* and other yeast species were described. *Alternaria* spp. Menolli Jr. et al. [46] noted that yeast *S. cerevisiae* NCYC1006 inhibited *Alternaria* sp. growth by 65% in in vitro experiments. In the in vitro study by Istifadah et al. [47], three yeast strains (SB 1, SB 2 and SB 10) inhibited *A. alternata* growth by between 42.8 and 67.4%. In an in vitro assay conducted by Sabaghian et al. [48] several different yeast species (i.e., *Starmerella bacillaris* FE08.05, *Metschnikowia pulcherrima* GP8, *Hanseniaspora uvarum* GM19, *Hanseniaspora opuntiae* GA22, *Hanseniaspora opuntiae* GM10, *Hanseniaspora guilliermondii* GA1, *Hanseniaspora lachancei* GM32, *Hansenaspora pseudoguilliermondii* GP14 and *Candida awuaii* GM3) significantly reduced *A. alternata* mycelium growth. In the test conducted on yeasts isolated from tomato fruit and leaf, *Meyerozyma guilliermondii* SQUCC-33Y inhibited *A. alternata* mycelial growth by 29.7% [41]. In an experiment conducted by Prendes et al. [49], 14 out of 15 yeast strains entirely prevented *A. alternata* infection. Li et al. [50] proved that infiltration and coating with yeast can inhibit *Alternaria* rot on tomatoes.

In the greenhouse experiment on the potted potato plants, the antagonistic properties of US 05 and Coobra strains, first demonstrated in laboratory conditions, were confirmed. The disease severity index was reduced from approximately 60% for both *A. alternata* and *A. solani* on the control plants (a week after an inoculation) to 17% and 26%, respectively, for the plants treated with the strain US 05, and 9% and 14%, respectively, for the plants treated with the Coobra yeast strain. Although the antifungal activity of yeasts on potatoes against early blight is not as well documented as other biocontrol agents such as bacteria, fungi and plant extracts [51–55], yeast activity against *Alternaria* symptoms on different plants was noted. In the study by Tumpa and Khokon [56], the severity of *Alternaria* incidence was significantly reduced on the chitosan and yeast elicitor-treated tomato and aubergine plants. Wang et al. [57] showed that marine yeast *Rhodosporidium paludigenum* after five days from inoculation reduced *A. alternata* symptoms on cherry tomato plants. The same team proved that this yeast was also effective against *A. alternata* in pears and Chinese winter jujubes [58]. Another study on the biocontrol of *Alternaria* on cherry tomatoes showed the efficacy of *Rhodotorula glutinis*, especially combined with rhamnolipids [59]. *Pichia guilliermondii* was proven to prevent post-harvest decay caused by *A. solani* on tomato fruit at three different stages of maturity [60].

To explore mechanisms behind pathogenic fungi inhibition by antagonistic yeast strains, the lytic exoenzyme production by yeast was evaluated. The production of amylase was produced by two strains, which inhibited the growth of *Alternaria* spp. the most. Additionally, other enzymes were also produced by these two strains. Both US 05 and Coobra strains, which produced the most kinds of exoenzymes, caused damage in *Alternaria* spp. Cells, and exoenzymes activity could contribute to this process. However, the presence of Biodiva TD 291, which produced two exoenzymes as well, did not damage *A. alternata* and *A. solani* cells, so other mechanisms are also involved. One of those mechanisms, as mentioned below, may be the competition for nutrients and space. The system for fungal cell lysis usually requires a complex of enzymes to lyse the cell wall [61,62]; therefore, it may be the presence of other untested enzymes that are the reason why these two strains proved effective. This demands further investigation. The inhibition mechanisms are complex, so further studies are needed, and enzyme participation cannot be clearly attributed. Biocontrol activity connected to enzyme secreting by yeasts was noted by other authors. Bar Shimon et al. described the inhibitory effect of exo-beta-1,3-glucanase secreted by C. olephila on spore germination [63]. Bae et al. [64] described the ability of *S. cerevisiae* to produce cellulose-degrading enzymes. The *Aureobasidium pullulans* strain of marine origin was reported to produce cellulase [65]. Cellulase secretion by biocontrol yeast was linked to post-harvest control of *B. cinerea* [66]. Protease activity in yeast strains was also reported [67–69]. Chen et al. [54] found an *A. pullulans* strain to produce protease and an *Aureobasidium* sp. strain to secrete chitinase. *Candida tropicalis* (strain MK-160) was reported to produce xylanase [70].

Yeast availability on plant leaves reduces colonization by plant pathogens [71]. The substantial issue in biocontrol is maintaining a high population of antagonistic microorganisms on the plant surface, since they are to a great extent influenced by conditions of the environment and by the relatively hostile environment of leaves. This restricts their establishment, survival and activity [72]. Microorganisms are affected by diurnal changes in nutrient availability, light and temperature [73–75]. In this assay, Biodiva TD 291, W 34/70, US 05 and Coobra strains remained at high population on the potato leaf surface for a week and the colonies of Coobra strain were present on the leaf after eighteen days under greenhouse conditions. The other strains did not adapt to plant surface conditions. However, it is possible to enhance the ability of microorganisms to colonize the plant surface. González-Estrada et al. [76] found that even though the population of marine yeast *Debaryomyces hansenii* decreased significantly over time, the addition of dextrose and Tween 80 to the suspension favoured the permanency of yeast on the fruit surface. In the study by Zheng et al. [77], the treatment with 10% maltose and lactose sugar solution increased the *Candida oleophila* survival rate on apple surface from 42% to approximately 60%. Therefore,

further study on yeast suspension enrichment might provide the solution to plant surface colonization by yeast.

5. Conclusions

1. The tested commercially yeast strains showed a diversified ability to maintain colonies on potato leaves (up to eighteen days for Coobra strain).
2. Two *Saccharomyces cerevisiae* strains US 05 and Coobra effectively minimized *Alternaria* mycelium growth in vitro and early blight symptoms on potato plants in a greenhouse experiment (from approximately 60% to 9% for *A. solani* and 14% for *A. alternata*).
3. The mechanisms underlying observed biocontrol need to be further investigated.
4. Two commercially available yeast strains show promise for the biological control of Alternaria. The selected yeast strains have the potential for practical use as biocontrol agents, and strategies will be developed for their use under habitat conditions.

Author Contributions: Conceptualization, J.K. (Jolanta Kowalska) and J.K. (Joanna Krzymińska); methodology, J.K. (Joanna Krzymińska); validation, M.J.; formal analysis, J.K. (Jolanta Kowalska); investigation, M.J. and K.M.; resources, J.K. (Jolanta Kowalska) and J.K. (Joanna Krzymińska); data curation, J.K. (Joanna Krzymińska) and J.K. (Jolanta Kowalska); writing—original draft preparation, J.K. (Joanna Krzymińska); writing—review and editing, J.K. (Jolanta Kowalska) and J.K. (Joanna Krzymińska); visualization, K.M.; supervision, J.K. (Jolanta Kowalska); project administration, J.K. (Jolanta Kowalska) and K.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Budde-Rodriguez, S.; Pasche, J.S.; Mallik, I.; Gudmestad, N.C. Sensitivity of *Alternaria* spp. from potato to pyrimethanil, cyprodinil, and fludioxonil. *Crop Prot.* **2022**, *152*, 105855. [CrossRef]
2. Jindo, K.; Evenhuis, A.; Kempenaar, C.; Pombo Sudré, C.; Zhan, X.; Goitom Teklu, M.; Kessel, G. Holistic pest management against early blight disease towards sustainable agriculture. *Pest Manag. Sci.* **2021**, *77*, 3871–3880. [CrossRef]
3. Saharan, G.S.; Mehta, N.; Meena, P.D.; Dayal, P. *Alternaria Diseases of Crucifers: Biology, Ecology and Disease Management*, 1st ed.; Springer: Singapore, 2016; pp. 17–51.
4. Nowicki, M.; Nowakowska, M.; Niezgoda, A.; Kozik, E. *Alternaria* black spot of crucifers: Symptoms, the importance of the disease, and perspectives of resistance breeding. *Veg. Crops Res. Bull.* **2012**, *76*, 5–19. [CrossRef]
5. Tsedaley, B. Review on early blight (*Alternaria* spp.) of potato disease and its management options. *J. Biol. Agric. Healthc.* **2014**, *4*, 191–199.
6. Rashmi, T.; Vishunavat, K. Management of early blight (*Alternaria solani*) in tomato by integration of fungicides and cultural practices. *Int. J. Plant Prot.* **2012**, *5*, 201–206.
7. Van Bruggen, A.H.C.; Finckh, M.R. Plant diseases and management approaches in organic farming systems. *Annu. Rev. Phytopathol.* **2016**, *54*, 25–54. [CrossRef] [PubMed]
8. Singh, J.; Yadav, A.N. *Natural Bioactive Products in Sustainable Agriculture*, 1st ed.; Springer Nature: Singapore, 2020; p. 319.
9. Damalas, C.A.; Koutroubas, S.D. Current status and recent developments in biopesticide use. *Agriculture* **2018**, *8*, 13. [CrossRef]
10. Pal, K.K.; Gardener, B.M. Biological Control of Plant Pathogens. The Plant Health Instructor. Available online: https://www.apsnet.org/edcenter/disimpactmngmnt/topc/Documents/PHIBiologicalControl.pdf (accessed on 21 April 2022).
11. Trivedi, P.; Leach, J.E.; Tringe, S.G.; Sa, T.; Singh, B.K. Plant–microbiome interactions: From community assembly to plant health. *Nat. Rev. Microbiol.* **2020**, *18*, 607–621. [CrossRef] [PubMed]
12. Kowalska, J.; Krzymińska, J.; Tyburski, J. Yeasts as a Potential Biological Agent in Plant Disease Protection and Yield Improvement—A Short Review. *Agriculture* **2022**, *12*, 1404. [CrossRef]
13. Caplice, E.; Fitzgerald, G.F. Food fermentations: Role of microorganisms in food production and preservation. *Int. J. Food Microbiol.* **1999**, *50*, 131–149. [CrossRef]
14. Chambers, P.J.; Pretorius, I.S. Fermenting knowledge: The history of winemaking, science and yeast research. *EMBO Rep.* **2010**, *11*, 914–920. [CrossRef] [PubMed]

15. Jach, M.E.; Serefko, A.; Sajnaga, E.; Kozak, E.; Poleszak, E.; Malm, A. Dietary supplements based on the yeast biomass. *Curr. Top. Nutraceutical Res.* **2015**, *13*, 83–88.
16. Kurtzman, C.P.; Fell, J.W.; Boekhout, T. (Eds.) *The Yeasts: A Taxonomic Study*, 5th ed.; Elsevier: Amsterdam, The Netherlands, 2011; p. 2354.
17. Gasch, A.P.; Werner-Washburne, M. The genomics of yeast responses to environmental stress and starvation. *Funct. Integr. Genom.* **2002**, *2*, 181–192. [CrossRef] [PubMed]
18. Bencheqroun, S.K.; Bajji, M.; Massart, S.; Labhilili, M.; El Jaafari, S.; Jijakli, M.H. In vitro and in situ study of postharvest apple blue mould biocontrol by *Aureobasidium pullulans*: Evidence for the involvement of competition for nutrients. *Postharvest Biol. Technol.* **2007**, *46*, 128–135. [CrossRef]
19. Spadaro, D.; Droby, S. Development of biocontrol products for postharvest diseases of fruit: The importance of elucidating the mechanisms of action of yeast antagonists. *Trends Food Sci. Technol.* **2016**, *47*, 39–49. [CrossRef]
20. Di Francesco, A.; Ugolini, L.; D'Aquino, S.; Pagnotta, E.; Mari, M. Biocontrol of *Monilinia laxa* by *Aureobasidium pullulans* strains: Insights on competition for nutrients and space. *Int. J. Food Microbiol.* **2017**, *248*, 32–38. [CrossRef] [PubMed]
21. Castoria, R.; Wright, S.A.I. Host Responses to Biological Control Agents. In *Post-Harvest Pathology*; Springer: Berlin/Heidelberg, Germany, 2009; Volume 2, pp. 171–181.
22. Pretscher, J.; Fischkal, T.; Branscheidt, S.; Jäger, L.; Kahl, S.; Schlander, M.; Claus, H. Yeasts from different habitats and their potential as biocontrol agents. *Fermentation* **2018**, *4*, 31. [CrossRef]
23. Mimee, B.; Labbe, C.; Belanger, R.R. Catabolism of flocculosin, an antimicrobial metabolite produced by *Pseudozyma flocculosa*. *Glycobiology* **2009**, *19*, 995–1001. [CrossRef]
24. Mannazzu, I.; Domizio, P.; Carboni, G.; Zara, S.; Zara, G.; Comitini, F.; Ciani, M. Yeast killer toxins: From ecological significance to application. *Crit. Rev. Biotechnol.* **2019**, *39*, 603–617. [CrossRef] [PubMed]
25. Mari, M.; Bautista-Baños, S.; Sivakumar, D. Decay control in the postharvest system: Role of microbial and plant volatile organic compounds. *Postharvest Biol. Technol.* **2016**, *122*, 70–81. [CrossRef]
26. Perez, M.F.; Contreras, L.; Garnica, N.M.; Fernández-Zenoff, M.V.; Farías, M.E.; Sepulveda, M.; Dib, J.R. Native killer yeasts as biocontrol agents of postharvest fungal diseases in lemons. *PLoS ONE* **2016**, *11*, 0165590. [CrossRef]
27. Belda, I.; Ruiz, J.; Alonso, A.; Marquina, D.; Santos, A. The biology of *Pichia membranifaciens* killer toxins. *Toxins* **2017**, *9*, 112. [CrossRef] [PubMed]
28. Cai, S.T.; Chiu, M.C.; Chou, J.Y. Broad-spectrum activity of volatile organic compounds from three yeast-like fungi of the *Galactomyces* genus against diverse plant pathogens. *Mycobiology* **2021**, *49*, 69–77. [CrossRef] [PubMed]
29. Pérez-Montaño, F.; Alías-Villegas, C.; Bellogín, R.A.; Del Cerro, P.; Espuny, M.R.; Jiménez-Guerrero, I.; López-Baena, F.J.; Ollero, F.J.; Cubo, T. Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. *Microbiol. Res.* **2014**, *169*, 325–336. [CrossRef] [PubMed]
30. Nafady, N.A.; Hashem, M.; Hassan, E.A.; Ahmed, H.A.; Alamri, S.A. The combined effect of arbuscular mycorrhizae and plant-growth-promoting yeast improves sunflower defense against *Macrophomina phaseolina* diseases. *Biol. Control* **2019**, *138*, 104049. [CrossRef]
31. Czarnecka, M.; Żarowska, B.; Połomska, X.; Restuccia, C.; Cirvilleri, G. Role of biocontrol yeasts *Debaryomyces hansenii* and *Wickerhamomyces anomalus* in plants' defence mechanisms against *Monilinia fructicola* in apple fruits. *Food Microbiol.* **2019**, *83*, 1–8. [CrossRef]
32. Mbuyane, L.L. Investigating Polyol and Acetic Acid Metabolism in Wine-Related Non-Saccharomyces Yeasts. Ph.D. Thesis, Stellenbosch University, Stellenbosch, South Africa, 2017.
33. Bruner, J.; Marcus, A.; Fox, G. Brewing Efficacy of Non-Conventional *Saccharomyces* Non-*cerevisiae* Yeasts. *Beverages* **2021**, *7*, 68. [CrossRef]
34. León-Teutli, A.K.; Hernández-Iturriaga, M.; Miranda-Castilleja, D.E.; Aldrete-Tapia, J.A.; Soto-Muñoz, L.; Martínez-Peniche, R.Á. Selection of native yeasts for the production of sparkling ciders from apple varieties established in Querétaro, Mexico. *Rev. Chapingo Ser. Hortic.* **2020**, *26*, 29–47. [CrossRef]
35. Tyrawa, C.; Preiss, R.; Armstrong, M.; van der Merwe, G. The temperature dependent functionality of *Brettanomyces bruxellensis* strains in wort fermentations. *J. Inst. Brew.* **2019**, *125*, 315–325. [CrossRef]
36. Wang, X.D.; Bohlscheid, J.C.; Edwards, C.G. Fermentative activity and production of volatile compounds by Saccharomyces grown in synthetic grape juice media deficient in assimilable nitrogen and/or pantothenic acid. *J. Appl. Microbiol.* **2003**, *94*, 349–359. [CrossRef] [PubMed]
37. McFarland, J. The nephelometer: An instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *J. Am. Med. Assoc.* **1907**, *49*, 1176–1178. [CrossRef]
38. Qazi, N.; Nayeema, J.; Qaisar, A.; Aadil, A. Status and symptomatology of early blight (*Alternaria solani*) of potato (*Solanum tuberosum* L.) in Kashmir valley. *Afr. J. Agric. Res.* **2013**, *8*, 5104–5115. [CrossRef]
39. Gabriel, B.P. Enzymatic activities of some entomophthorous fungi. *J. Invertebr. Pathol.* **1968**, *11*, 70–81. [CrossRef]
40. Strauss, M.L.A.; Jolly, N.P.; Lambrechts, M.G.; Van Rensburg, P. Screening for the production of extracellular hydrolytic enzymes by non-*Saccharomyces* wine yeasts. *J. Appl. Microbiol.* **2001**, *91*, 182–190. [CrossRef] [PubMed]

41. Al-Maawali, S.; Al-Sadi, A.M.; Ali Khalifa Alsheriqi, S.; Al-Sabahi, J.N.; Velazhahan, R. The potential of antagonistic yeasts and bacteria from tomato phyllosphere and fructoplane in the control of *Alternaria* fruit rot of tomato. *All Life* **2021**, *14*, 34–48. [CrossRef]
42. Samada, L.H.; Tambunan, U.S.F. Biopesticides as promising alternatives to chemical pesticides: A review of their current and future status. *Online J. Biol. Sci.* **2020**, *20*, 66–76. [CrossRef]
43. Ab Rahman, S.F.S.; Singh, E.; Pieterse, C.M.; Schenk, P.M. Emerging microbial biocontrol strategies for plant pathogens. *Plant Sci.* **2018**, *267*, 102–111. [CrossRef]
44. Droby, S.; Wisniewski, M.; Teixidó, N.; Spadaro, D.; Jijakli, M.H. The science, development, and commercialization of postharvest biocontrol products. *Postharvest Biol. Technol.* **2016**, *122*, 22–29. [CrossRef]
45. Raymaekers, K.; Ponet, L.; Holtappels, D.; Berckmans, B.; Cammue, B.P. Screening for novel biocontrol agents applicable in plant disease management—A review. *Biol. Control* **2020**, *144*, 104240. [CrossRef]
46. Menolli, N., Jr.; Marques, V.; Paccola, L.; Andrade-Nóbrega, G. Inhibitory activity of yeasts against soybean pathogenic fungi. *Agrotrópica* **2020**, *32*, 75–80. [CrossRef]
47. Istifadah, N.; Putri, R.A.; Hartati, S. The abilities of bacteria and yeast isolated from vermicompost water extract to inhibit *Alternaria solani* in vitro and early blight disease on tomato. *Cropsaver J. Plant Prot.* **2021**, *4*, 73–79. [CrossRef]
48. Sabaghian, S.; Braschi, G.; Vannini, L.; Patrignani, F.; Samsulrizal, N.H.; Lanciotti, R. Isolation and identification of wild yeast from Malaysian grapevine and evaluation of their potential antimicrobial activity against grapevine fungal pathogens. *Microorganisms* **2021**, *9*, 2582. [CrossRef] [PubMed]
49. Prendes, L.P.; Merín, M.G.; Fontana, A.R.; Bottini, R.A.; Ramirez, M.L.; de Ambrosini, V.I.M. Isolation, identification and selection of antagonistic yeast against *Alternaria alternata* infection and tenuazonic acid production in wine grapes from Argentina. *Int. J. Food Microbiol.* **2018**, *266*, 14–20. [CrossRef] [PubMed]
50. Li, Q.; Xie, F.; Zhao, Y.; Cao, J. Inhibitory effect of postharvest yeast mannan treatment on *Alternaria* rot of tomato fruit involving the enhancement of hemicellulose polysaccharides and antioxidant metabolism. *Sci. Hortic.* **2021**, *277*, 109798. [CrossRef]
51. Cwalina-Ambroziak, B.; Damszel, M.M.; Glosek-Sobieraj, M. The effect of biological and chemical control agents on the health status of the very early potato cultivar Rosara. *J. Plant Prot. Res.* **2015**, *55*, 389–395. [CrossRef]
52. Jyoti, C.; Jain, D.K. Exploring the potentiality of biocontrol agents against *Alternaria solani*: A causal agent of early blight of potato and tomato crops. *Ann. Biol.* **2020**, *36*, 67–70.
53. Gorai, P.S.; Ghosh, R.; Konra, S.; Mandal, N.C. Biological control of early blight disease of potato caused by *Alternaria alternata* EBP3 by an endophytic bacterial strain *Bacillus velezensis* SEB1. *Biol. Control* **2021**, *156*, 104551. [CrossRef]
54. Da Silva, H.A.O.; Teixeira, W.D.; Borges, Á.V.; Silva Junior, A.L.; Alves, K.S.; Rodrigues Junior, O.M.; de Abreu, L.M. Biocontrol of potato early blight and suppression of *Alternaria grandis* sporulation by *Clonostachys* spp. *Plant Pathol.* **2021**, *70*, 1677–1685. [CrossRef]
55. Meena, B.R.; Yadav, J.; Chittora, D.; Meena, S.R.; Kumar, S.; Jain, T.; Sharma, K. Biocontrol Efficacy of Thevetia Peruviana Bioformulations Against Early Blight Disease of Potato. *Thevetia Peruviana* **2022**, *submitted*. [CrossRef]
56. Tumpa, F.H.; Khokom, M.A.R. Foliar application of chitosan and yeast elicitor fa-cilitate reducing incidence and severity of *Alternaria* leaf blight of tomato and brinjal. *Res. J. Plant Pathol.* **2020**, *3*, 4.
57. Wang, Y.; Bao, Y.; Shen, D.; Feng, W.; Yu, T.; Zhang, J.; Zheng, X.D. Biocontrol of *Alternaria alternata* on cherry tomato fruit by use of marine yeast *Rhodosporidium paludigenum* Fell & Tallman. *Int. J. Food Microbiol.* **2008**, *123*, 234–239. [CrossRef] [PubMed]
58. Wang, Y.; Wang, P.; Xia, J.; Yu, T.; Lou, B.; Wang, J.; Zheng, X.D. Effect of water activity on stress tolerance and biocontrol activity in antagonistic yeast *Rhodosporidium paludigenum*. *Int. J. Food Microbiol.* **2010**, *143*, 103–108. [CrossRef] [PubMed]
59. Yan, F.; Xu, S.; Chen, Y.; Zheng, X. Effect of rhamnolipids on *Rhodotorula glutinis* biocontrol of *Alternaria alternata* infection in cherry tomato fruit. *Postharvest Biol. Technol.* **2014**, *97*, 32–35. [CrossRef]
60. Zhao, Y.; Tu, K.L.; Shao, X.F.; Jing, W.; Yang, J.L.; Su, Z.P. Biological control of the post-harvest pathogens *Alternaria solani*, *Rhizopus stolonifer*, and *Botrytis cinerea* on tomato fruit by *Pichia guilliermondii*. *J. Hortic. Sci. Biotechnol.* **2008**, *83*, 132–136. [CrossRef]
61. Salazar, O.; Asenjo, J.A. Enzymatic lysis of microbial cells. *Biotechnol. Lett.* **2007**, *29*, 985–994. [CrossRef]
62. Peberdy, J.F. Mycolytic enzymes. In *Fungal Protoplasts*; CRC Press: Boca Raton, FL, USA, 2020; pp. 31–44.
63. Bar-Shimon, M.; Yehuda, H.; Cohen, L.; Weiss, B.; Kobeshnikov, A.; Daus, A.; Droby, S. Characterization of extracellular lytic enzymes produced by the yeast biocontrol agent *Candida oleophila*. *Curr. Genet.* **2004**, *45*, 140–148. [CrossRef]
64. Bae, J.; Kuroda, K.; Ueda, M. Proximity effect among cellulose-degrading enzymes displayed on the *Saccharomyces cerevisiae* cell surface. *Appl. Environ. Microbiol.* **2015**, *81*, 59–66. [CrossRef]
65. Zhang, L.; Chi, Z.M. Screening and identification of a cellulase producing marine yeast and optimization of medium and cultivation conditions for cellulase production. *J. Ocean Univ. China* **2007**, *37*, 101–108.
66. Saravanakumar, D.; Spadaro, D.; Garibaldi, A.; Gullino, M.L. Detection of enzymatic activity and partial sequence of a chitinase gene in *Metschnikowia pulcherrima* strain MACH1 used as post-harvest biocontrol agent. *Eur. J. Plant Pathol.* **2009**, *123*, 183–193. [CrossRef]
67. Hesham, A.; Sulaiman, A.; Mona, A.; Al-Dayel, M.A.; Salah, H.A. Screening and genetic identification of acidic and neutral protease-producing yeasts strains by 26S rRNA gene sequencing. *Genet. Cytol.* **2017**, *51*, 221–229. [CrossRef]
68. Barzkar, N. Marine microbial alkaline protease: An efficient and essential tool for various industrial applications. *Int. J. Biol. Macromol.* **2020**, *161*, 1216–1229. [CrossRef] [PubMed]

69. Duarte, A.W.F.; Dos Santos, J.A.; Vianna, M.V.; Vieira, J.M.F.; Mallagutti, V.H.; Inforsato, F.J.; Durães Sette, L. Cold-adapted enzymes produced by fungi from terrestrial and marine Antarctic environments. *Crit. Rev. Biotechnol.* **2018**, *38*, 600–619. [CrossRef] [PubMed]
70. Chen, P.H.; Chen, R.Y.; Chou, J.Y. Screening and evaluation of yeast antagonists for biological control of *Botrytis cinerea* on strawberry fruits. *Mycobiology* **2018**, *46*, 33–46. [CrossRef] [PubMed]
71. Kapoor, D.; Karnwal, A. Yeast as a plant growth promoter and biocontrol agent. In *Fungi Bio-Prospects in Sustainable Agriculture, Environment and Nano-Technology*; Academic Press: Cambridge, MA, USA, 2021; pp. 429–457.
72. Shariq, M.; Sohail, M. Application of *Candida* tropicalis MK-160 for the production of xylanase and ethanol. *J. King Saud Univ.—Sci.* **2019**, *31*, 1189–1194. [CrossRef]
73. Van Driesche, R.; Bellows, T.S., Jr. *Biological Control*, 1st ed.; Springer Science & Business Media: Norwell, MA, USA, 2012; p. 539.
74. Cheng, Y.T.; Zhang, L.; He, S.Y. Plant-microbe interactions facing environmental challenge. *Cell Host Microbe* **2019**, *26*, 183–192. [CrossRef] [PubMed]
75. Carvalho, S.D.; Castillo, J.A. Influence of light on plant–phyllosphere interaction. *Front. Plant Sci.* **2018**, *9*, 1482. [CrossRef]
76. González-Estrada, R.R.; de Jesus Ascencio-Valle, F.; Ragazzo-Sánchez, J.A.; Santoyo, M.C. Use of a marine yeast as a biocontrol agent of the novel pathogen *Penicillium citrinum* on Persian lime. *Emir. J. Food Agric.* **2017**, *29*, 114–122. [CrossRef]
77. Zheng, F.; Zhang, W.; Sui, Y.; Ding, R.; Yi, W.; Hu, Y.; Zhu, C. Sugar protectants improve the thermotolerance and biocontrol efficacy of the biocontrol yeast, *Candida oleophila*. *Front. Microbiol.* **2019**, *10*, 187. [CrossRef]