horticulturae

MDPI

Article

Potential of *Debaryomyces hansenii* Strains on the Inhibition of *Botrytis cinerea* in Blueberry Fruits (*Vaccinium corymbosum* L.)

Surelys Ramos-Bell [1], Luis G. Hernández-Montiel [2], Rita M. Velázquez-Estrada [1], Juan A. Herrera-González [1,3] and Porfirio Gutiérrez-Martínez [1,*]

[1] Laboratorio Integral de Investigación en Alimentos, Tecnológico Nacional de México/Instituto Tecnológico de Tepic, Avenida Tecnológico #2595, Col. Lagos del Country, Tepic 63175, Nayarit, Mexico
[2] Centro de Investigaciones Biológicas del Noroeste, S.C., La Paz 23096, Baja California Sur, Mexico
[3] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental Uruapan, Av. Latinoamericana 1101, Col. Revolución, Uruapan 60150, Michoacán, Mexico
* Correspondence: pgutierrez@ittepic.edu.mx

Abstract: Blueberry (*Vaccinium corymbosum* L.) is a crop with great potential for exportation and is very important for its antioxidant properties. However, this fruit is susceptible to different factors of deterioration and attack by pathogens. *Botrytis cinerea* is the main cause of post-harvest losses in this crop. Antagonistic yeasts from the *Debaryomyces hansenii* species, which is of marine origin, have been proposed as an alternative method for fungal control. For this reason, this study evaluated the in vitro and in vivo antagonistic capacity of the yeast against *B. cinerea*. The in vitro growth of the fungus was inhibited by 90% and germination was reduced by 100%, and through the production of volatile organic compounds, the growth of the pathogen was inhibited by 32.5%. Photographic evidence by electron microscopy revealed the effects caused by *D. hansenii* on *B. cinerea*. The incidence study showed a 50% reduction in the incidence of the disease caused by *B. cinerea* in blueberries, while quality parameters—such as soluble solids, titratable acidity, and pH—presented stable values, delaying the maturation process of the fruits. Likewise, the rate of reduction in firmness and physiological weight loss was lower, and the blueberries preserved their quality for a longer time.

Keywords: *Debaryomyces hansenii*; blueberry; gray mold; postharvest quality

Citation: Ramos-Bell, S.; Hernández-Montiel, L.G.; Velázquez-Estrada, R.M.; Herrera-González, J.A.; Gutiérrez-Martínez, P. Potential of *Debaryomyces hansenii* Strains on the Inhibition of *Botrytis cinerea* in Blueberry Fruits (*Vaccinium corymbosum* L.). *Horticulturae* **2022**, *8*, 1125. https://doi.org/10.3390/horticulturae8121125

Academic Editor: Elazar Fallik

Received: 31 October 2022
Accepted: 25 November 2022
Published: 30 November 2022

1. Introduction

The blueberry fruit (*Vaccinium corymbosum* L.) has recently achieved great importance due to its content of anthocyanins, flavanols, procyanidins, and other phenolic compounds that directly impact human health [1]. This has led to a rise in demand, and as a result, producers have a greater interest in increasing the production of this crop. However, a problem persists that reduces production, and that is the attack of phytopathogenic agents, with the *Botrytis cinerea* species being particularly prevalent in blueberry cultivation [2]. Biological methods have been studied for its control. They seek to reduce the population of harmful organisms affecting blueberries through the use of living organisms—e.g., entomophagous insects (those that feed on insects), parasitoids (insects whose larvae develop inside the body of another), and entomopathogenic fungi—that are recognized as their natural enemies in order to help fight pests with techniques that are friendly to human health and the environment [3]. Beneficial microorganisms are used as a biological control to reduce the undesirable effects of postharvest pathogens. Due to the negative effects on health and the environment caused by the use of chemical and physical agents, other alternatives that delay fruit ripening and eliminate pathogens have been pursued. Antagonistic microorganisms, such as bacteria and yeasts, are among the biological control compounds being studied and used in the control of pathogenic fungi. These can be obtained from fruit surfaces, other surfaces such as soil, and extreme environments (marine environments). The use of microorganisms isolated from marine environments has shown excellent

results in the control of pathogenic fungi due to their resistance to changes in pH, temperature, oxidative stress, and humidity [4]. The mechanisms of action of these antagonistic microorganisms may vary depending on the host-pathogen-microorganism interaction. Through the mechanism of competition for space and nutrients, yeast can prevent the growth of fungal cells or the production of lytic enzymes, such as glucanase and chitinase that damage the cell membrane of the fungus. Likewise, yeast can produce volatile organic compounds that cause the loss of the homeostasis of the fungal cell [5]. As yeasts are safe for humans, they are ideal as an antifungal agent in food preservation. Yeasts have been demonstrated to be effective against pathogens, are simple to cultivate, do not produce antibiotics, are not toxic, and do not pollute the environment [6]. In this regard, yeasts such as *Debaryomyces hansenii*, *Meyerozyma caribbica*, *Rhodotorula glutinis*, and *Rhodotorula minuta*, among others, have been studied [7,8]. *Debaryomyces hansenii* is a halophilic or halotolerant yeast that can grow in NaCl levels greater than 25% concentration. This yeast has been isolated from marine environments, soil, and some foods, including cheese, wine, meat, and sugary products. Its biological control capacity has been reported in various pathogens, such as *Penicillium citrinum* in Persian lemon [9], *Colletotrichum gloeosporioides* in papaya [10], and *Pythium ultimum* in chili [11], among others. As a result of prior research on the biological action of *D. hansenii*, we proposed to study the in vitro and in vivo effect it has on the control of *Botrytis cinerea* in blueberry fruits.

2. Materials and Methods

2.1. Yeast Preparation

The marine yeasts were obtained from the collection of antagonistic microorganisms of the Phytopathology Laboratory of CIBNOR, previously isolated and identified [4,12]. Three *Debaryomyces hansenii* yeast strains (L1, IRIICB, and ECP4) were used. The inocula were prepared in a PDB liquid culture medium (Potato-Dextrose-Broth, Difco™, Issy-les-Moulineaux, France) at 25 °C under agitation (180 rpm) for 24 h. The concentration was adjusted to 10^8 cells/mL using a hematocytometer [10].

2.2. Pathogen and Fruit

Blueberry fruits were collected from an orchard located in Nayarit, Mexico, at physiological maturity. The fruits selected were without any apparent physical damage or decomposition by pathogens. The *Botrytis cinerea* fungus was previously isolated and identified at the Integrative Food Research Laboratory of the Technological Institute of Tepic. The strain was sown in Potato Dextrose Agar (PDA) (Difco™, France) and incubated at 25 °C for 7 days.

2.3. Mycelial Growth Inhibition

The PDA culture medium containing the different control agents was poured into Petri dishes with a diameter of 9 cm. As a control, some Petri dishes were inoculated with fungus without treatments, and others with fungus plus the fungicide chlorothalonil (Bravonil® 720 SC, Sifatec S.A, Mexico). Subsequently, 0.8 cm discs with 5-day-old *Botrytis cinerea* mycelium were placed in Petri dishes and incubated at 25 °C for 10 days under dark conditions (Jiang et al., 2016). The mycelial diameter was determined every 24 h for 10 days using the ImageJ® program. Five repetitions per treatment were performed and the experiment was performed in duplicate. The percentage of mycelial growth inhibition (%MGI) was calculated using Equation (1).

$$\%MGI = (1 - Da/Db) \times 100\% \quad (1)$$

where Da is the diameter of the growth zone in the test plates and Db is the diameter of the growth zone in the control plates.

2.4. Germination Percentage

The methodology described by [8] was used to determine the germination in the yeast treatments. In this context, 500 µL of suspension of the three yeast strains (1×10^8 cells/mL) were combined with 500 µL of a suspension of *B. cinerea* (1×10^4 spores/mL). The suspension of the fungus was placed as the control and then incubated at 25 °C for 48 h. After this time spore germination was counted. A spore was considered germinated when the length of the germinative tube was equal to or greater than the diameter of the spore. The germination percentage was estimated according to the number of germinated spores divided by the number of total spores.

2.5. Inhibition by Volatile Organic Compounds (VOCs)

Aliquots of 20 µL of each yeast concentration were deposited and streaked on plates with a PDA medium. The same method was used to deposit aliquots of 20 µL of the conidia suspension of the fungus on another batch of plates. The double culture method, in which yeast and fungus cultures are juxtaposed, was utilized [13]. Both plates were sealed with Kleen Pack plastic and incubated at 25 °C for 10 days. At the end of the experiment, the growth diameter (mm) of the pathogen was quantified, and the reduction in radial growth was calculated with the equation described by [13].

2.6. Scanning Electron Microscopy (SEM)

Petri dishes (PDA) with and without yeast were inoculated with 100 µL of adjusted spore suspension (1×10^6 mL^{-1}) of *B. cinerea* and then incubated for 10 days. SEM was performed according to the process reported by [14] with modifications. Agar discs were taken from each treatment with the fungus, immersed in 2.5% glutaraldehyde at 4 °C for 24 h, and then washed with ethanol at gradual concentrations (30–100% v/v) by 50 min, and for the 100% concentration three times for 20 min. The samples were dried in an oven at 30 °C for 24 h and then mounted on a plate sample holder and coated with gold for 3 min. The samples were examined using a SEC 3200 M model scanning electron microscope (SEMICOM, Seoul, Korea) with an accelerating voltage of 20 kV, a voltage of −5 kV, and a distance of −20 mm operating at 30 kV.

2.7. Debaryomyces hansenii In Vivo Evaluation

The blueberry fruits were washed with a 2% commercial chlorine solution (Allen S.A, N.L, Mexico City, Mexico) and then rinsed with water for one minute and allowed to dry at room temperature. A 1 × 1 cm wound was made in each fruit with a sterile needle and then immersed in a solution of the best in vitro strain of *D. hansenii* (1×10^8 cells/mL) for 1 min and allowed to dry for 2 h. Subsequently, the evaluation of the effect was carried out by inoculating the wounded fruits with 5 µL of the spore suspension (1×10^5 mL^{-1}) of *Botrytis cinerea* and allowing them to dry for 12 h at room temperature (25 °C). In addition, 30 fruits per treatment were stored in refrigeration (4 °C) and at room temperature (25 °C) for 9 days with and without treatments. The percentage of incidence of the disease was determined according to Equation (2) [9].

$$\%\text{Incidence} = (\text{Infected fruits}/\text{Total fruits}) \times 100\% \quad (2)$$

2.8. Effect of D. hansenii on the Postharvest Quality of the Fruit

The fruits were washed and disinfected with a 2% sodium hypochlorite solution (Allen S.A, N.L, Mexico City, Mexico) for 2 min. Subsequently, fruits were immersed in 1×10^8 cells/mL suspension of *D. hansenii*, left to dry at room temperature, and stored at 4 °C and 25 °C for 9 days. Each treatment consisted of 30 fruits, and the total soluble solids, titratable acidity, pH, color, firmness, and weight loss were measured every third day.

2.8.1. Soluble Solids

The content of soluble solids was determined according to the AOAC method (2000). In this context, 5 g of sample were homogenized and a few drops were placed in a digital refractometer (Hanna Instruments, Woonsocket, HI 96801, USA) and were reported as ºBrix. The analysis was performed in triplicate.

2.8.2. Titratable Acidity

The titratable acidity was determined with a solution of 0.1 N sodium hydroxide (Reagent grade, Proquisur S.A, Mexico) according to the AOAC method, (2000). Five grams of pulp from 10 fruits were homogenized with 25 mL of distilled water and 3 drops of phenolphthalein (Reagent, Proquisur S.A, Mexico) as an indicator.

2.8.3. pH

5 g of sample was homogenized and then quantified using a potentiometer (Sension^{TM}, Barcelona, Spain). The measurements were made in triplicate (AOAC, 2000).

2.8.4. Color

The color of the fruit surface was evaluated using a colorimeter (High-Quality Colorimeter, Shanghai, China), which expresses the measurements in coordinates L*, a*, and b*, where: L*, the luminosity; a*, the color red (positive values) or green (negative values); and b*, the color yellow (positive values) or blue (negative values).

2.8.5. Firmness

A texturometer (Stable Micro Systems, TA-XT Plus, Godalming, UK) equipped with a 2 mm diameter punch was used. Shear strength was recorded and results were expressed in Newton (N) (Dadzie and Orchard, 1997).

2.8.6. Physiological Weight Loss

The treated and untreated fruits were weighed on a digital scale (Ohaus Corporation, Parsippany, NJ, USA), expressing the results as a percentage of fresh weight loss based on the initial weight of the fruit.

2.9. Statistic Analysis

A complete factorial design was carried out, considering the different treatments as independent variables. The results were statistically analyzed by analysis of variance (ANOVA) and the LSD Fisher test ($p < 0.05$) was used to determine the comparisons of means using the statistical program Statistica v12.0 (StatSoft Inc., Tulsa, OK, USA, 2013).

3. Results and Discussion

3.1. Mycelial Growth Inhibition

Among the *D. hansenii* strains tested, the growth inhibition percentage of *B. cinerea* ranged from 88–90% (Table 1), with strain L1 achieving the highest level of pathogen inhibition. This is a very positive result, indicating that the yeasts have an antagonistic effect on the pathogen (Figure 1). This antagonism can occur due to the rapid consumption of carbon sources in the medium by the yeasts, which restricts the growth of *B. cinerea*, or due to the production of hydrolytic enzymes, such as β-1,3-glucanase and protease, by the *D. hansenii* yeasts, which interact directly with the cell wall of the pathogen [10].

Previous studies have demonstrated that *D. hansenii* isolated from terrestrial and marine environments can inhibit the growth of various pathogens due to mechanisms such as competition for space and nutrients, production of lytic enzymes and "killer" toxins, metal uptake, and others [11,15]. Other yeast strains, such as *Metschnikowia pulcherrima* and *Meyerozyma guilliermondii*, as well as *D. hansenii*, have also been reported as effective biocontrol agents on the in vitro growth of *B. cinerea*, with an inhibition rate of 100% [16]. Yeasts of marine origin can offer greater advantages in fungal control as they are adapted

to an environment of higher salinity and low temperature. In this regard, [17] applied the marine yeast *Sporidiobolus pararoseus* at a concentration of 1×10^8 cells/mL with an in vitro mycelial growth inhibition of *B. cinerea* greater than 80%. In this study, the fungicide chlorothalonil was used as a positive control, which completely inhibited the growth of *B. cinerea*. Although this effect is desired, some efforts are being made for the complete elimination of these chemicals, particularly in Europe, where the use of chlorothalonil is prohibited. This indicates that gradually the laws regarding synthetic fungicides will be changed to finally comply with the provisions of the FAO 2030 Agenda, hence, the importance of the yeast *D. hansenii* application as an alternative to fungal control.

Table 1. Inhibition of mycelial growth of *B. cinerea* under application of *D. hansenii* strains.

Treatments	Strains (10^8 cel/mL)	Growth (mm)	Inhibition (%)
D. hansenii	L1	15.86 ± 3.76 a	90.41 ± 4.59 d
	IRIICB	23.21 ± 2.36 c	81.44 ± 2.88 b
	ECP4	17.62 ± 4.75 b	88.26 ± 5.79 c
Untreated	-	90 ± 0 d	0.0 ± 0 a

Note: Values are expressed as mean ± standard deviation (n = 5). Different letters in each column indicate significant differences between treatments ($p < 0.05$).

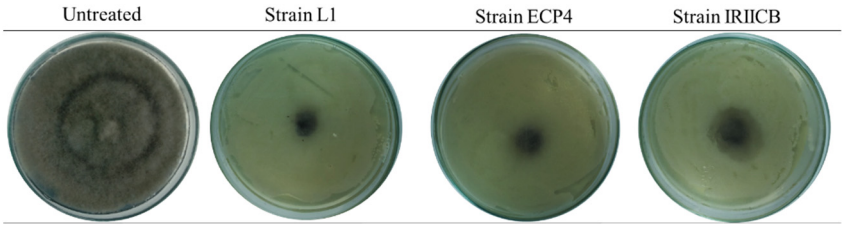

Figure 1. Effect of *D. hansenii* on the mycelial growth of *B. cinerea*.

3.2. Germination Percentage

All of the *D. hansenii* strains tested completely inhibited the germination of *B. cinerea*. This finding is consistent with reports of *D. hansenii* as antagonistic to *Botrytis cinerea*, where the germination of this pathogen's spores is completely inhibited [16]; this effect is attributed to the yeast's secretion of antifungal substances. As previously mentioned, yeasts consume carbon sources (glucose, sucrose, fructose, and others) at a faster rate than pathogens, which limits the latter's growth and prevents germination [18]. It has also been suggested that *Debaryomyces hansenii* can secrete "killer" protein toxins that can degrade the cell membrane function of pathogens, suppressing their growth and development [15].

3.3. Inhibition by Volatile Compounds

The strains challenged by dual culture against *B. cinerea* inhibited the growth of this pathogen (Figure 2), ranging from 13 to 32.5%. The L1 strain inhibited the growth of the pathogen the most. The growth inhibition of *Colletotrichum gloeosporioides* has been explained as an effect of the production of volatile organic compounds by the yeast *D. hansenii* [10].

The VOCs that have been identified include alcohols, esters, aldehydes, and others [19]. These can vary depending on the species and can be produced by microorganisms in very low concentrations. This inhibitory capacity of yeasts is attributed to the fact that the compounds produced are directly absorbed into the pathogen's cell membrane, causing the release of essential metabolites that inhibit the pathogen's normal development [20]. The ability of yeasts such as *S. cerevisiae* and *A. pullulans* to inhibit *B. cinerea* in vitro was attributed primarily to the production of volatile organic compounds [13].

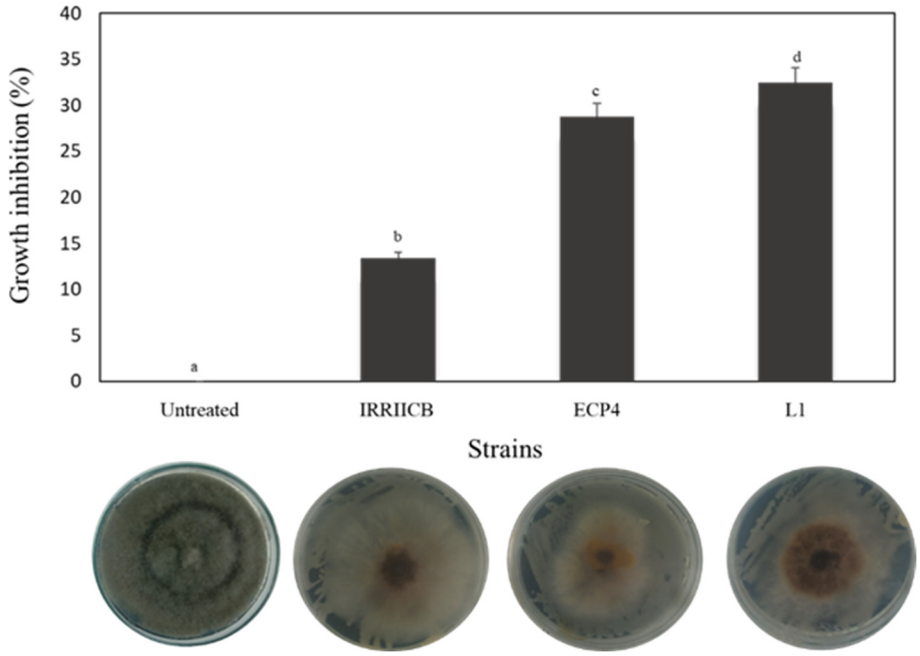

Figure 2. Effect of volatile organic compounds of *Debaryomyces hansenii* strains on the growth of *B. cinerea*. Different letters in the bars indicate significant differences between the strains a ($p < 0.05$).

3.4. Scanning Electron Microscopy

The possible damage caused to the fungal structures by the effect of the application of the antagonist can be observed through microscopy analysis. In this case, in the samples without treatment, septate, smooth, and undamaged *Botrytis cinerea* hyphae were observed (Figure 3A). In the case of the L1 strain, *D. hansenii* cells adhered to the hyphae of the pathogen (Figure 3B). This phenomenon of adhesion by the yeast *Pichia guilliermondii* and *D. hansenii* to *B. cinerea* was studied by [21].

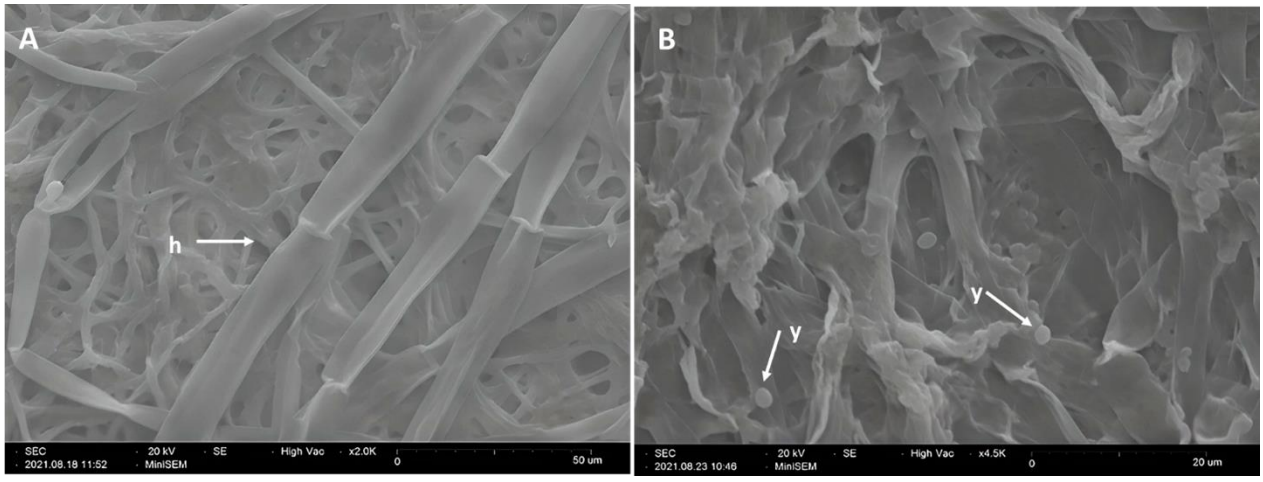

Figure 3. Effect of *Debaryomyces hansenii* on the structures of *B. cinerea*. (**A**) hyphae (h) of *B. cinerea* without treatment. (**B**) L1 cells of *Debaryomyces hansenii* (y) on hyphae of *B. cinerea*.

In this analysis, it is demonstrated that the union of the yeast cells with the fungal hyphae explains the yeasts′ antagonistic activity towards the pathogen and ability to block the diffusion of nutrients from the medium to the pathogen. Recent research suggests that one of the mechanisms of action of yeast is mycoparasitism, and it has been demonstrated that their adhesion to fungal structures can cause their collapse, which is also mediated by the secretion of hydrolytic enzymes, such as glucanase and chitinase [22].

3.5. Debaryomyces Hansenii In Vivo Evaluation

Preventive application and storage at room temperature of the L1 *Debaryomyces hansenii* strain significantly decreased the incidence of *B. cinerea* on blueberry fruits by 50%, compared to 95% of the untreated fruits (Figure 4). Regarding the refrigeration treatments, there was no disease development in the fruits during the days of storage. These results agree with previous studies in which fungal growth is delayed when applying low temperatures [23]. Regarding the antagonistic capacity of *Debaryomyces hansenii*, some authors have shown that the application of this microorganism is capable of reducing the incidence of diseases caused by pathogens, such as *P. italicum* and *Colletotrichum gloeosporoides*, in fruits [8,18]. Yeasts have a rapid metabolism, which allows them to exhaust carbon sources in the medium for growth and reproduce more quickly than pathogens [3]. This is one of the possible mechanisms involved in reducing the incidence of diseases in postharvest fruits. Similarly, the induction of fruit defense responses is influenced by the exogenous effect of yeasts, as shown by [24], a study in which there was an increase in the activities of the enzymes phenylalanine ammonia lyase, peroxidase, polyphenoloxidase, and others involved in the defense system of the fruit.

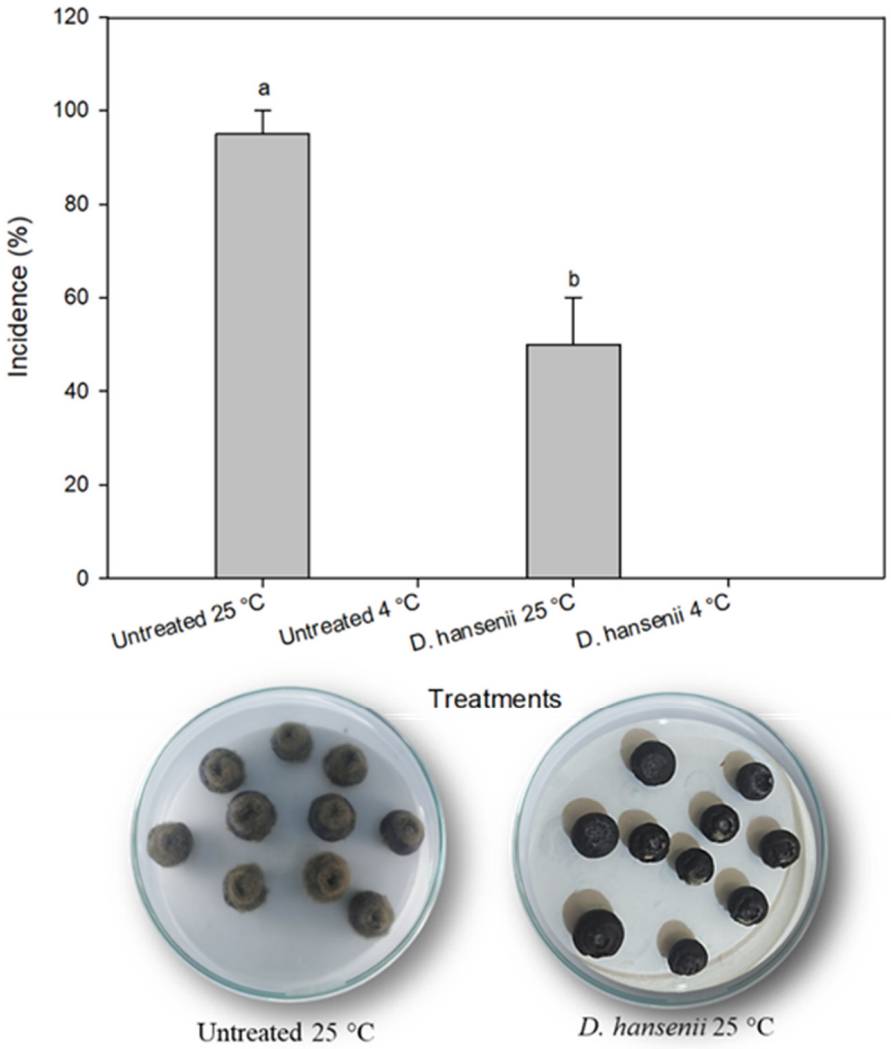

Figure 4. Incidence of *B. cinerea* on blueberries stored at 25 °C and 4 °C treated with *D. hansenii*. Different letters in the bars indicate significant differences between treatments at ($p < 0.05$).

3.6. Effect of D. hansenii on the Postharvest Quality of the Fruit

3.6.1. Soluble Solids, pH, Titratable Acidity

The effect of the application of *Debaryomyces hansenii* on quality parameters such as soluble solids, pH, and titratable acidity is shown in Figure 4. Significant differences in the content of soluble solids at room temperature (25 °C) were found between the control and the antagonist, with values of 16.6 and 11.7 °Brix, respectively. During storage, no significant differences in refrigeration temperature (4 °C) were observed between treatments. However, on the last day of storage, the untreated fruits presented a higher content of soluble solids of 14.23 °Brix than the fruits with the *D. hansenii*, which showed values of 12.23 °Brix (Figure 5a). Soluble solids are compounds that include sugars, organic acids, and vitamins, which constitute the main substrates of respiration [25]. As a result, there is a greater requirement and increase in soluble solids content in ripening fruits. The application of *D. hansenii* yeast resulted in a lower concentration of soluble solids than the control. Hence, a reduction in the maturation process was observed. The titratable acidity content in all treatments tended to decrease; however, during storage at room temperature, there were no significant differences among them, and the values on day 9 ranged from 0.74 to 0.91% (Figure 5b). The refrigerated fruits showed a lower rate of decrease in the acidity content compared with the fruits stored at room temperature, with values of 0.47% in the control and 0.30% for *D. hansenii*. The reduction in titratable acidity values may be associated with the use of organic acids in the fruit respiration process [26]. Therefore, we can state that the treatments applied to the blueberry fruit reduce the consumption of organic acids, such as citric acid, which is found in greater proportion in blueberries. The pH values increased as the storage time passed at both temperatures; however, no significant differences between the control treatment and yeast application at 25 °C were found, showing values of 3.59 and 2.45, respectively (Figure 5c). Storage at 4 °C kept the pH values stable in blueberry fruit during the 9 days of storage. Similar results were reported by [27], a study that describes that by applying edible coatings, the pH values of the blueberry fruit decrease, which is due to the reduction rate of the metabolic processes that convert starch and fruit acids into sugar. The flavor is one of the main quality indicators of fruit, and the flavor involves the content of soluble solids, titratable acidity, and the pH of the fruit. In this sense, what is expected from the application of a treatment, whether physical, chemical, or biological, is that it can preserve these attributes for a longer time, extending the shelf life of the fruit. In this study, the treatments with the yeast *D. hansenii* maintained the levels of soluble solids and pH below the control, and there was a smaller decrease in the content of titratable acidity compared with the control. In addition, low storage temperature plays an important role in the preservation of soluble solids, pH, and titratable acidity in the blueberry fruit.

3.6.2. Firmness

The firmness reduction rate of the treated and untreated blueberry fruits during storage at both evaluated temperatures did not present large variations; however, the firmness decrease in the treatment with *D. hansenii* was less than the control (Figure 6). A similar result was obtained by [26], indicating that the applied control effectively prevented the increase in firmness loss in blueberries during cold storage.

Fruit firmness is an important quality parameter, which is affected by carbohydrate hydrolysis and degradation of pectin in the fruit cell wall [28]. The refrigeration temperature keeps the firmness of the fruits stable for longer, and this may be due to the decrease at low temperatures in the activity of hydrolytic enzymes, such as polygalacturonase, galactosidases, pectin methyl esterase, and β-1,4-glucanases [29]. In addition, the effect of yeasts as signaling response inducers and fruit cell wall reinforcement mechanisms is discussed [30].

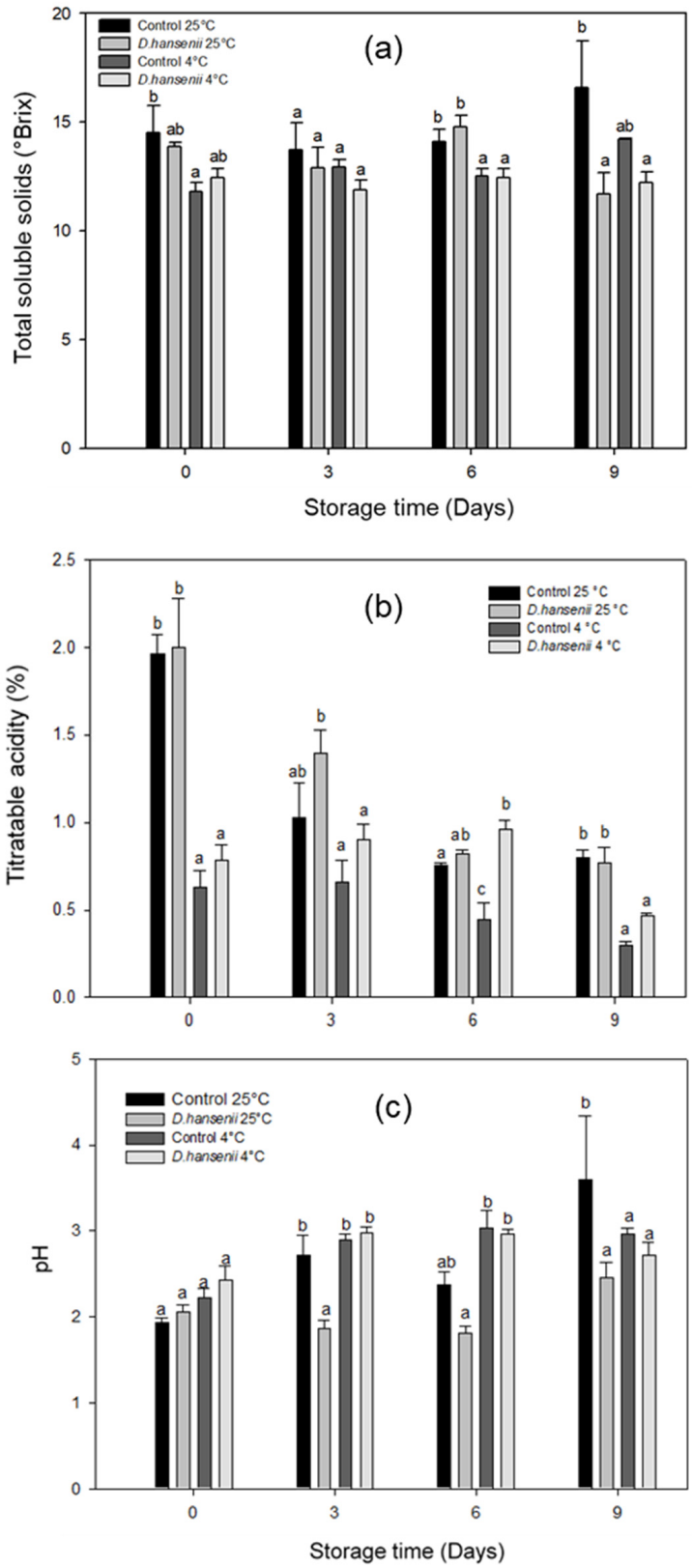

Figure 5. Total soluble solids (**a**), pH (**c**), and titratable acidity (**b**) of blueberries stored at 25 °C and 4 °C under the application of *D. hansenii*. Values are expressed as mean ± standard error. Different letters in the bars indicate significant differences between treatments at ($p < 0.05$).

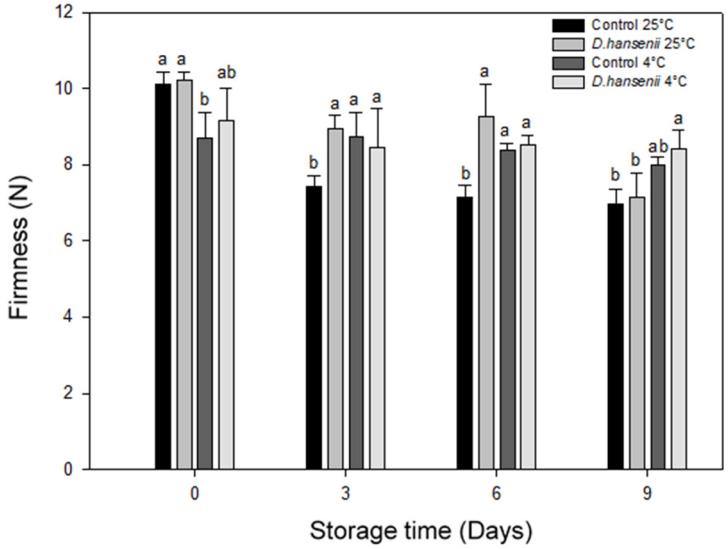

Figure 6. The firmness of blueberry fruits stored at 25 °C and 4 °C under the application of *D. hansenii*. Values are expressed as mean ± standard error. Different letters in the bars indicate significant differences between treatments at ($p < 0.05$).

3.6.3. Color

The L* parameter tended to decrease during storage at room temperature, showing no difference between treatments (Table 2). Similarly, at low temperatures, there was a slight decrease, and the luminosity (L*) generally shows a decreasing trend due to the ripening process of the fruit [31].

Table 2. Color parameters in blueberries treated with *D. hansenii* during storage at 25 and 4 °C.

Parameters	Treatments	Storage (25 °C)		Storage (4 °C)	
		Day 0	Day 9	Day 0	Day 9
L*	Control	35.01 ± 0.65 a	31.30 ± 0.70 a	35.24 ± 0.69 a	32.07 ± 0.70 a
	D. hansenii	35.23 ± 0.58 a	32.77 ± 0.67 a	32.24 ± 0.62 b	27.53 ± 0.66 a
a*	Control	−3.89 ± 0.12 a	−4.70 ± 0.07 a	−3.79 ± 0.11 a	−5.62 ± 0.18 a
	D. hansenii	−3.80 ± 0.10 a	−4.94 ± 0.11 a	−3.66 ± 0.16 a	−5.88 ± 0.41 a
b*	Control	−7.04 ± 0.23 a	−5.77 ± 0.21 a	−6.67 ± 0.23 a	−6.63 ± 0.23 a
	D. hansenii	−7.17 ± 0.19 a	−5.45 ± 0.14 a	−5.58 ± 0.31 a	−4.55 ± 0.25 b

Note: Values are expressed as mean ± standard deviation (n = 5). Different letters in each column indicate significant differences between treatments ($p < 0.05$).

The a* and b* parameters in the treatments did not show significant changes in these values. These results indicate that the yeast *D. hansenii* does not affect the color parameters in the blueberry fruit. This supports what has been reported in previous studies on Persian lime treated with *D. hansenii* [9]. An increase or variation in the color of blueberries can lead to the loss of the commercial value of the fruit [32]. As a result, it is critical to keep the color parameters of the fruits stable during a specific storage period.

3.6.4. Physiological Weight Loss

The weight loss of fruits stored at room temperature differed significantly between the control and yeast application (Figure 7), with the application of *D. hansenii* resulting in a 15% weight loss versus a 22% weight loss for the control. In contrast, storage under refrigeration kept the weight loss rate lower and without significant differences, with values of 5.30% and 6.45% for the treatment with yeast and the control, respectively.

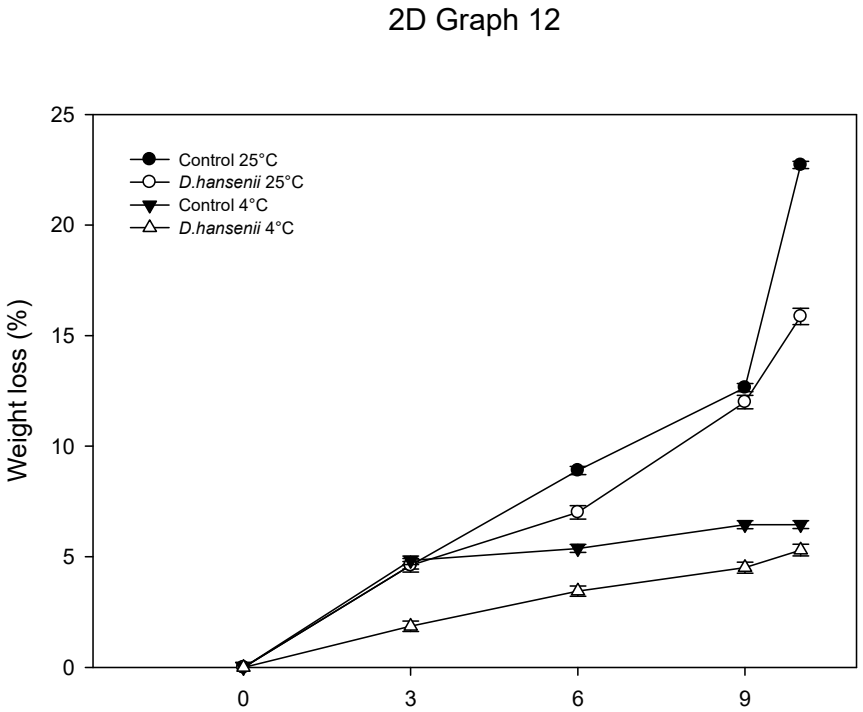

Figure 7. Physiological weight loss in blueberry fruits stored at 25 °C and 4 °C under the application of *D. hansenii*. Values are expressed as mean ± standard error.

This behavior of lower losses in fruits stored at 4 °C is because refrigeration delays the ripening of the fruit since the respiration processes are dependent on temperature [30]. The weight loss of the fruit is associated with the loss of water through their respiration and transpiration processes. This loss slows down or is less when yeast is applied to the fruit, as this microorganism has the ability to form biofilms. This biofilm formed on the surface of the fruit acts as a physical barrier with respect to the atmosphere that surrounds blueberries. Consequently, this could be the mechanism by which the yeast slows down the weight loss of the fruit [33]. It has been mentioned that the yeast *D. hansenii* reduces weight loss in lime fruits by improving the effectiveness of the coating as a barrier against water loss [9].

4. Conclusions

The L1 *Debaryomyces hansenii* strain showed the best in vivo results in the control of *Botrytis cinerea*, with a great reduction of the mycelial growth of the fungus and inhibition by the production of volatile organic compounds. The in vivo study also demonstrated the antagonistic capacity of *D. hansenii*, because it significantly reduced the percentage of incidence of the disease caused by *B. cinerea*. Regarding the evaluated quality parameters, *D. hansenii* delayed the ripening of blueberries by maintaining stable total soluble solids, titratable acidity, and pH. The control agent reduced the firmness and weight loss of the fruits, which is why it is proposed that the marine yeast *D. hansenii* is an alternative to fungal control because it manages to reduce the fungal attack of *B. cinerea* in blueberries and preserve the physicochemical quality of this cultivar for a longer time compared to untreated fruits.

Author Contributions: Conceptualization, S.R.-B., L.G.H.-M. and P.G.-M.; investigation, S.R.-B., L.G.H.-M., R.M.V.-E., J.A.H.-G. and P.G.-M.; writing—original draft preparation, S.R.-B.; writing—review and editing, S.R.-B., L.G.H.-M., R.M.V.-E., J.A.H.-G. and P.G.-M.; funding acquisition, P.G.-M. and L.G.H.-M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by "Programas Nacionales Estratégicos de Ciencia, Tecnología y Vinculación con los Sectores Social, Público y Privado" CONACyT-Mexico.

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 authors.

Acknowledgments: The authors thank CONACYT (Mexico) for the scholarship granted to Surelys Ramos Bell.

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

References

1. Liu, B.; Wang, K.; Shu, X.; Liang, J.; Fan, X.; Sun, L. Changes in fruit firmness, quality traits and cell wall constituents of two highbush blueberries (*Vaccinium corymbosum* L.) during postharvest cold storage. *Sci. Hortic.* **2019**, *246*, 557–562. [CrossRef]
2. Ramos-Bell, S.; Hernández-Montiel, L.G.; González-Estrada, R.R.; Gutiérrez-Martínez, P. Main diseases in postharvest blueberries, conventional and eco-friendly control methods: A review. *LWT—Food Sci. Technol.* **2021**, *149*, 7–12. [CrossRef]
3. 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]
4. Hernandez-Montiel, L.G.; Zulueta-Rodriguez, R.; Angulo, C.; Rueda-Puente, E.O.; Quiñonez-Aguilar, E.E.; Galicia, R. Marine yeasts and bacteria as biological control agents against anthracnose on mango. *J. Phytopathol.* **2017**, *165*, 833–840. [CrossRef]
5. Medina-Córdova, N.; López-Aguilar, R.; Ascencio, F.; Castellanos, T.; Campa-Córdova, A.I.; Angulo, C. Biocontrol activity of the marine yeast *Debaryomyces hansenii* against phytopathogenic fungi and its ability to inhibit mycotoxins production in maize grain (*Zea mays* L.). *Biol. Control* **2016**, *97*, 70–79. [CrossRef]
6. Mari, M.; Martini, C.; Guidarelli, M.; Neri, F. Postharvest biocontrol of *Monilinia laxa*, *Monilinia fructicola* and *Monilinia fructigena* on stone fruit by two *Aureobasidium pullulans* strains. *Biol. Control* **2012**, *60*, 132–140. [CrossRef]
7. Palou, L.; Smilanick, J.L.; Droby, S. Alternatives to conventional fungicides for the control of citrus postharvest green and blue moulds. *Stewart Postharvest Rev.* **2008**, *4*, 1–16. [CrossRef]
8. Hernández-Montiel, L.G.; Rivas-García, T.; Romero-Bastidas, M.C.; Chiquito-Contreras, J.; Ruiz-Espinoza, F.H.; Chiquito-Contreras, R.G. Potencial antagónico de bacterias y levaduras marinas para el control de hongos fitopatógenos. *Rev. Mex. Cienc. Agrícolas* **2018**, *1*, 4311–4321. [CrossRef]
9. González-Estrada, R.R.; Carvajal-Millán, E.; Ragazzo-Sánchez, J.A.; Bautista-Rosales, P.U.; Calderón-Santoyo, M. Control of blue mold decay on *Persian lime*: Application of covalently cross-linked arabinoxylans bioactive coatings with antagonistic yeast entrapped. *LWT—Food Sci. Technol.* **2017**, *85*, 187–196. [CrossRef]
10. Hernandez-Montiel, L.G.; Gutierrez-Perez, E.D.; Murillo-Amador, B.; Vero, S.; Chiquito-Contreras, R.G.; Rincon-Enriquez, G. Mechanisms employed by *Debaryomyces hansenii* in biological control of anthracnose disease on papaya fruit. *Postharvest Biol. Technol.* **2018**, *139*, 31–37. [CrossRef]
11. Lara-Capistran, L.; Zulueta-Rodriguez, R.; Castellanos-Cervantes, T.; Reyes-Perez, J.J.; Preciado-Rangel, P.; Hernandez-Montiel, L.G. Efficiency of marine bacteria and yeasts on the biocontrol activity of *pythium ultimum* in ancho-type pepper seedlings. *Agronomy* **2020**, *10*, 408. [CrossRef]
12. Hernández-Montiel, L.G.; Holguín-Peña, R.J.; Larralde-Corona, C.P.; Zulueta-Rodríguez, R.; Rueda-Puente, E.; Moreno-Legorreta, M. Effect of inoculum size of yeast Debaryomyces hansenii to control Penicillium italicum on Mexican lime (*Citrus aurantiifolia*) during storage. *J. Food* **2012**, *10*, 235–242. [CrossRef]
13. Parafati, L.; Vitale, A.; Restuccia, C.; Cirvilleri, G. Biocontrol ability and action mechanism of food-isolated yeast strains against *Botrytis cinerea* causing post-harvest bunch rot of table grape. *Food Microbiol.* **2015**, *47*, 85–92. [CrossRef] [PubMed]
14. Ramos-Guerrero, A.; González-Estrada, R.R.; Hanako-Rosas, G.; Bautista-Baños, S.; Acevedo-Hernández, G.; Tiznado-Hernández, M.E.; Gutiérrez-Martínez, P. Use of inductors in the control of *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* isolated from soursop fruits: In *vitro* tests. *Food Sci. Biotechnol.* **2018**, *27*, 755–763. [CrossRef] [PubMed]
15. Çorbacı, C.; Uçar, F.B. Purification, characterization and in vivo biocontrol efficiency of killer toxins from *Debaryomyces hansenii* strains. *Int. J. Biol. Macromol.* **2018**, *119*, 1077–1082. [CrossRef]
16. Fernandez-San Millan, A.; Larraya, L.; Farran, I.; Ancin, M.; Veramendi, J. Successful biocontrol of major postharvest and soil-borne plant pathogenic fungi by antagonistic yeasts. *Biol. Control.* **2021**, *160*, 104683. [CrossRef]
17. Shen, H.; Wei, Y.; Wang, X.; Xu, C.; Shao, X. The marine yeast *Sporidiobolus pararoseus* ZMY-1 has antagonistic properties against *Botrytis cinerea in vitro* and in strawberry fruit. *Postharvest Biol. Technol.* **2019**, *150*, 1–8. [CrossRef]
18. Vázquez-Vázquez, M.L.; Hernández-Montiel, L.G.; Sanchez-Viveros, G.; Reyes-Pérez, J.; Martinez-Hernandez, M.; Chiquito-Contreras, R. Efecto de levaduras de origen marino y ulvan en el control poscosecha de *Penicillium italicum* agente causal del moho azul en limón persa. *Biotecnia* **2021**, *23*, 78–88. [CrossRef]
19. Di Francesco, A.; Ugolini, L.; Lazzeri, L.; Mari, M. Production of volatile organic compounds by *Aureobasidium pullulans* as a potential mechanism of action against postharvest fruit pathogens. *Biol. Control.* **2015**, *81*, 8–14. [CrossRef]
20. Ferreira, E.M.S.; Resende, D.A.; Vero, S.; Pimenta, R.S. The Use of Psychrophilic Antarctic Yeast in the Biological Control of Post-harvest Diseases of Fruits Stored at Low Temperatures. *Fungi Antarct.* **2019**, 243–263. [CrossRef]

21. Widyastuti, S.R.I. Physical Interactions between Yeast *Pichia guilliermondii* and Post-Harvest Fruit Pathogen *Penicillium expansum*. *HAYATI J. Biosci.* **2008**, *15*, 27–31. [CrossRef]
22. Freimoser, F.M.; Rueda-Mejia, M.P.; Tilocca, B.; Migheli, Q. Biocontrol yeasts: Mechanisms and applications. *World J. Microbiol. Biotechnol.* **2019**, *35*, 1–19. [CrossRef] [PubMed]
23. Chiabrando, V.; Peano, C.; Giacalone, G. The efficacy of different postharvest treatments on physico-chemical characteristics, bioactive components and microbiological quality of fresh blueberries during storage period. *Food Res.* **2017**, *1*, 240–248. [CrossRef]
24. Ahima, J.; Zhang, X.; Yang, Q.; Zhao, L.; Tibiru, A.M.; Zhang, H. Biocontrol activity of *Rhodotorula mucilaginosa* combined with salicylic acid against *Penicillium digitatum* infection in oranges. *Biol. Control.* **2019**, *135*, 23–32. [CrossRef]
25. Li, Y.; Rokayya, S.; Jia, F.; Nie, X.; Xu, J.; Elhakem, A.; Almatrafi, M.; Benajiba, N.; Helal, M. Shelf-life, quality, safety evaluations of blueberry fruits coated with chitosan nano-material films. *Sci. Rep.* **2021**, *11*, 55. [CrossRef]
26. Vieira, J.M.; Flores-lópez, M.L.; Jasso, D.; Rodríguez, D.; Sousa, M.C.; Vicente, A.A.; Martins, J.T. Effect of chitosan—Aloe vera coating on postharvest quality of blueberry (*Vaccinium corymbosum*) fruit. *Postharvest Biol. Technol.* **2016**, *116*, 88–97. [CrossRef]
27. Mannozzi, C.; Cecchini, J.P.; Tylewicz, U.; Siroli, L.; Patrignani, F.; Lanciotti, R.; Rocculi, P.; Dalla Rosa, M.; Romani, S. Study on the efficacy of edible coatings on quality of blueberry fruits during shelf-life. *LWT—Food Sci. Technol.* **2017**, *85*, 440–444. [CrossRef]
28. Jiang, H.; Sun, Z.; Jia, R.; Wang, X.; Huang, J. Effect of Chitosan as an Antifungal and Preservative Agent on Postharvest Blueberry. *J. Food Qual.* **2016**, *39*, 516–523. [CrossRef]
29. Djioua, T.; Charles, F.; Lopez-Lauri, F.; Filgueiras, H.; Coudret, A.; Freire, M., Jr.; Ducamp-Collin, M.N.; Sallanon, H. Improving the storage of minimally processed mangoes (*Mangifera indica* L.) by hot water treatments. *Postharvest Biol. Technol.* **2009**, *52*, 221–226. [CrossRef]
30. Zhi Shao, Y.; Ke Zeng, J.; Tang, H.; Zhou, Y.; Li, W. The chemical treatments combined with antagonistic yeast control anthracnose and maintain the quality of postharvest mango fruit. *J. Integr. Agric.* **2019**, *18*, 1159–1169. [CrossRef]
31. Chiabrando, V.; Giacalone, G. Quality evaluation of blueberries coated with chitosan and sodium alginate during postharvest storage. *Int. Food Res. J.* **2017**, *24*, 1553–1561.
32. Xu, F.; Wang, S.; Xu, J.; Liu, S.; Li, G. Effects of combined aqueous chlorine dioxide and UV-C on shelf-life quality of blueberries. *Postharvest Biol. Technol.* **2016**, *117*, 125–131. [CrossRef]
33. Kumari, P.; Barman, K.; Patel, V.B.; Siddiqui, M.W.; Kole, B. Reducing postharvest pericarp browning and preserving health promoting compounds of litchi fruit by combination treatment of salicylic acid and chitosan. *Sci. Hortic.* **2015**, *197*, 555–563. [CrossRef]