

# Article

# Inclusion of Antifungal and Probiotic *Lactiplantibacillus plantarum* Strains in Edible Alginate Coating as a Promising Strategy to Produce Probiotic Table Grapes and Exploit Biocontrol Activity

Nicola De Simone <sup>1,2,\*</sup>, Angela Scauro <sup>1</sup>, Danial Fatchurrahman <sup>1</sup>, Pasquale Russo <sup>3</sup>, Vittorio Capozzi <sup>2</sup>, Giuseppe Spano <sup>1,\*</sup> and Mariagiovanna Fragasso <sup>1</sup>

- <sup>1</sup> Department of Agriculture, Food, Natural Science, Engineering, University of Foggia, Via Napoli 25, 71122 Foggia, Italy; angela.scauro@unifg.it (A.S.); danial.fatchurrahman@unifg.it (D.F.); mariagiovanna.fragasso@unifg.it (M.F.)
- <sup>2</sup> Institute of Sciences of Food Production, National Research Council (CNR), c/o CS-DAT, Via Michele Protano, 71121 Foggia, Italy; vittorio.capozzi@ispa.cnr.it
- <sup>3</sup> Department of Food, Environmental and Nutritional Sciences, University of Milan, Via Celoria 2, 20133 Milan, Italy; pasquale.russo1@unimi.it
- \* Correspondence: nicola.desimone@unifg.it (N.D.S.); giuseppe.spano@unifg.it (G.S.); Tel.: +39-(0)088-1589-303 (G.S.)

Abstract: The use of lactic acid bacteria (LAB) for the probiotic enrichment of minimally processed fruit is a well-established practice in the literature. In addition, several LAB demonstrated a strainspecific ability to control harmful microorganisms and decay agents, improving shelf life, maintaining quality, and promoting the safety of fruits and vegetables. Edible coatings can help modulate the phenomena of gas exchange and water loss by fruits, representing protection from physical damage and spoilage phenomena linked to oxidation and the development of undesired microorganisms. At the same time, the coating can represent an innovative delivery matrix for the LAB strains of potential interest to improve safety and quality in the postharvest management of fruits. In this work, five Lactiplantibacillus plantarum strains, previously characterised for their probiotic and antifungal activity, were incorporated into a sodium alginate coating to develop edible probiotic coatings with antifungal properties for table grapes cv. Italia. The bacterial transfer and their survival were evaluated by comparing coated and uncoated table grapes during 14 days of cold storage at 4 °C. The alginate edible coating increased the number of viable cells transferred to the surface of the berries from about 5 to more than 7 Log CFU/g, with a crucial impact on the potential functional attributes of the final product. The ability of the functionalised coatings to counteract the decay development was evaluated on table grape berries artificially contaminated with Aspergillus niger CECT 2805. A significant reduction in lesion diameter was observed in the alginate coating with L. plantarum 11-A, with a reduction from 15.40  $\pm$  1.14 mm of uncoated berries to 8.40  $\pm$  1.14 mm of berries coated with L. plantarum 11-A. The lesion diameter reduction was also accompanied by a reduction in the symptoms of infection, such as browning around the wound. These results suggest the application of selected strains of L. plantarum as promising bio-resources to enhance the overall value of ready-to-eat fruits and vegetables, particularly in combination with edible coating as a carrier matrix. While a strain-dependent effect was not detected with respect to the improvement in the number of cells in the edible coating, a variability depending on the biotype used was detected for the properties linked to biocontrol, suggesting that the inclusion in edible packaging may represent an innovative criterion in the selection of lactobacilli to be applied postharvest.

**Keywords:** lactic acid bacteria; lactobacilli; *Lactiplantibacillus plantarum*; table grapes; fruit; antimicrobial; probiotic; edible coating; postharvest; *Aspergillus niger*; spoilage



**Citation:** De Simone, N.; Scauro, A.; Fatchurrahman, D.; Russo, P.; Capozzi, V.; Spano, G.; Fragasso, M. Inclusion of Antifungal and Probiotic *Lactiplantibacillus plantarum* Strains in Edible Alginate Coating as a Promising Strategy to Produce Probiotic Table Grapes and Exploit Biocontrol Activity. *Horticulturae* **2024**, *10*, 419. https://doi.org/10.3390/ horticulturae10040419

Academic Editor: Luigi De Bellis

Received: 1 March 2024 Revised: 30 March 2024 Accepted: 18 April 2024 Published: 21 April 2024



**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/).

# 1. Introduction

Probiotics are live bacteria that, when administered in sufficient quantities, provide health benefits to the host [1,2]. Consumers typically ingest probiotics from dairy, pharmaceutical, and fermented foods. On the other hand, dairy products do not correspond to the dietary needs of specific customer categories, such as vegetarians and lactose-intolerant consumers [3]. As a result, the development of non-dairy alternatives based on fruit and vegetables, such as probiotic fresh-cut fruits, could serve as a preference vector for these consumers [4]. 'Minimally processed', 'ready-to-eat', 'fresh-cut', and some other designations indicate fruits ready for consumption [5], categories that have growing popularity attributable to their convenience, sensory quality, excellent nutritional value, and ability to retain freshness [6]. Recently, probiotic addition was proposed for a growing number of fresh-cut fruits, such as apples, pears, cantaloupes, pineapples, carrots, and blueberries [7–12]. However, several authors reported that specific features of the fruit matrices could affect the probiotic viability during shelf life and that the metabolic activity of the strain utilised could have a detrimental effect on the sensory characteristics of the fruit [8]. In this light, an edible coating based on biopolymers could act as an immobilising agent to limit the metabolic activity and the proliferation of the probiotic cells added [13,14], but also for extending the shelf life of fresh-cut fruits [15], as it could act as a barrier to water and gas and reduce the water loss and oxidative reactions of the fruits [16–18]. Among the most appreciated features of the probiotic strains selected for application in the fresh-cut sector, there is the ability to control harmful microorganisms and decay agents. In this context, the use of antagonistic microorganisms, such as lactic acid bacteria (LAB), has assumed international relevance as a promising eco-friendly alternative to chemical interventions, with the aim of sustainable improvement of the shelf life, quality, and safety of fruits and vegetables [19,20].

*Lactiplantibacillus plantarum* is a lactic acid bacterium belonging to the heterogenous class of lactobacilli. This bacterium is an extremely widespread species in the agro-food sector due to its outstanding biological versatility. Among the most interesting characteristics of this microorganism are probiotic features and antimicrobial properties, both of which are strain-related characteristics [21,22]. These are two interconnected attributes, as among the selection criteria of a probiotic microorganism, there are antimicrobial properties with respect to pathogenic bacteria, with the aim of supporting intestinal health. Different strains of *L. plantarum* have found applications in the postharvest of fruits and vegetables [19,23–25], with particular reference to the issues of (i) biocontrol against filamentous fungi such as *Botrytis cinerea*, *Aspergillus* spp., and *Penicillium* spp. and (ii) improvement in functional quality, supporting the interest in this microorganism as a model lactic acid bacterium for this kind of application.

Considering antimicrobial and probiotic applications, the inclusion of *L. plantarum* strains in coating formulations has recently shown encouraging results for application in fresh-cut produce [26–30]. In fact, LAB are susceptible to adverse effects from different physicochemical and biological variables as soon as they are applied to the fruit surface environment [31]. These variables, including osmotic shock, starvation, and drying as well as the storage environment, may affect the probiotic viability, resulting in a cell concentration unsuitable to confer health benefits. In this light, the use of coatings effectively protects biocontrol agents/probiotics from environmental stress, preventing bacterial cells from drying and starvation [31,32]. In addition, the biopolymeric layer of the coating can increase the number of cells transferred to the fruit surface, assuring the viability [29,30,33].

Table grapes are non-climacteric fruits, particularly perishable after harvest, which tend to deteriorate due to water loss, oxidation, and fungal colonisation. Several treatments were proposed to maintain the postharvest quality of table grapes [34] and to limit the decay induced by their most important inducer of fungal spoilage, *B. cinerea* [34,35]. However, colonisation by other fungal species was reported for table grapes [36]. Among those, *Aspergillus* genus is one of the most dangerous filamentous fungi because it can colonise a wide range of food commodities and can spread and propagate during the storage phase.

This genus is also responsible for safety concerns regarding allergenic reactions and the production of mycotoxins, such as aflatoxins and ochratoxin A [37].

In this work, five *Lactiplantibacillus plantarum* strains, previously characterised for their probiotic and antifungal activity, were incorporated into a sodium alginate coating to develop edible antifungal and probiotic coatings for table grapes cv. Italia. In addition, the ability of functionalised coatings to counteract the decay development was evaluated on table grape berries artificially contaminated with *Aspergillus niger*. *L. plantarum* has found applications in other production targets, but it is the first time that this species has been used in coatings for table grapes postharvest, evaluating, at the same time, antimicrobial and probiotic properties. In addition, this is one of the first studies that valorises *L. plantarum* strains in edible coatings and that assess the behaviour of a panel of different strains for this kind of application.

#### 2. Materials and Methods

# 2.1. Microbial Strains and Growth Conditions

Five LAB strains isolated from fruits and vegetables previously characterised for their probiotic and antimicrobial activity were selected for this study [38,39]. The strains were previously evaluated by 16S rRNA amplification and sequence and identified as *L. plantarum*. The 16S rRNA sequences were submitted to GenBank (https://submit.ncbi. nlm.nih.gov/, accessed on 22 May 2022) under the following accession numbers: ON584756, *L. plantarum* strain 10-A; ON584769, *L. plantarum* strain 11-A; ON585118, *L. plantarum* strain CB-56; ON585707, *L. plantarum* strain CZ-97; ON598622, *L. plantarum* strain CZ-103. Lactic acid bacteria were routinely cultured in MRS broth (Oxoid, Basingstoke, UK) at 30 °C.

The filamentous fungus *Aspergillus niger* CECT 2805 from cryopreserved cultures was propagated on Potato Dextrose Agar at 24 °C for five days. Fungal spores suspension was prepared by brushing the plate surface with saline solution (0.86% NaCl) supplemented with 0.01% Tween 80 using a sterile swab, storing the suspension at 4 °C for short-term uses. Fungal spores concentration was determined by plating serial dilution on PDA plates and adjusted to approximately  $1 \times 10^6$  spores/mL.

# 2.2. Preparation of the Antifungal and Probiotic Coating-Forming Solutions

The antifungal and probiotic coating-forming solutions were obtained as reported by Alvarez et al. [7]. The coating-forming solution consisted of 2% (w/v) alginate (Sigma-Aldrich, St. Louis, MO, USA) with 1.5% (w/v) glycerol as a plasticiser. After complete dissolving, the coating-forming solution was autoclaved at 121 °C for 15 min (Tecno-Gaz industries, Parma, Italy) and cooled down to room temperature (~24 °C). *L. plantarum* strains were grown overnight in MRS broth, washed twice, and resuspended in coating-forming solution to obtain a final concentration of ~10<sup>9</sup> CFU/mL. The inoculum concentration was checked by plating appropriate dilutions onto MRS agar.

# 2.3. Preparation of Antifungal and Probiotic-Coated Table Grape Berries

Healthy table grapes (cv. Italia) were purchased from a local retailer, sanitised by dipping for 1 min in 0.01% (w/v) sodium hypochlorite (NaOCl), rinsed twice with sterile demineralised water, and dried under a laminar flow hood. The berries were dipped into the coating solutions and then in the hardening solution (2% w/v CaCl<sub>2</sub>), both for 30 s. Uncoated grapes were dipped in saline solution containing the same amount of viable cells and then in hardening solution. After drying, the table grape berries were packed in plastic containers (five berries each) under a passive atmosphere and stored at 4 °C for 14 days to simulate commercial shelf life. Each treatment was performed in triplicate.

#### 2.4. Probiotic Viability

The survival of the probiotic strains in coated and uncoated berries during the simulated shelf life was evaluated. Analysis was performed after 0, 7, and 14 days of storage at 4 °C. The samples were mixed (1/10 w/v) with sterile saline solution and homogenised for

three minutes in a stomacher blender. Then, serial 10-fold dilutions were plated on MRS agar. Viable counts expressed as log of colony forming units per gram (LogCFU/g) were determined after incubation of the plates at 30 °C for 48 h.

#### 2.5. Fruit Decay Assay

Coated table grape berries were prepared as described above. Then, artificial wounds were induced using a sterile needle to make 3 mm deep and 3 mm wide wounds (four wounds for each acinus) along the equatorial areas of the berries. Each wound was inoculated with 10  $\mu$ L of *A. niger* spore suspension (~10<sup>6</sup> spores/mL). After drying in a laminar flow hood for about 1 h, the table grape berries were packed as described above. Five berries were wound-inoculated for each antifungal treatment. The plastic containers were maintained at 24 °C for 3 days in order to create favourable conditions for the onset of postharvest pathology. The development of the fungus was monitored daily by visual analysis, and after 3 days of shelf life, the lesion diameter was measured starting from the wound.

#### 2.6. Sensorial Quality Analysis

A group of ten trained panellists performed the sensory evaluations of artificially contaminated table grape berries after 3 days of shelf life at 24 °C. The panellists received training in order to identify and rate the non-tasting quality attributes prior to evaluations. Positive descriptors, such as appearance, colour, freshness, firmness, and overall acceptance, were evaluated using a hedonic scale from 1 to 5, where 1 = not present/very low/not typical, and 5 = very pronounced/very typical of fresh fruits. Negative descriptors, such as off-odour and mould occurrence, were ranked from 1 = 0% mould presence/odour to 5 = 100% mould presence/odour. In both cases, a value of 3 was fixed as the limit of marketability.

# 2.7. Statistical Analysis

One-way analysis of variance (ANOVA) was performed by using the SAS statistical computer package version 3.81 (SAS Institute, Cary, NC, USA). Significant differences in bacterial viability were determined using Fisher's Least Significant Difference (LSD) test with p < 0.05 as the minimal level of significance. Significant differences in lesion diameter were determined by post hoc Tukey's Honestly Significant Difference (HSD) test with p < 0.05 as the minimal level of significance.

# 3. Results and Discussion

LAB belonging to the genus *Lactiplantibacillus* are typically found in association with fruits and vegetables [40], and they are considered natural competitors of the undesired microflora responsible for the spoilage and decay of fruits and vegetables, such as phytopathogenic bacteria and filamentous fungi [41]. In particular, the species Lactiplantibacillus *plantarum* also assumed a relevant role as a biocontrol agent because it is already adapted to fruit environments and their related stressors, thus simplifying its application for industrial purposes [41]. The competition between LAB and filamentous fungi is mediated by a plethora of mechanisms, including the competition for nutrients, the secretion of metabolic byproducts (i.e., organic acids), and the production of active compounds such as peptides and VOCs, but also for synergistic mechanisms as well [42,43]. In this study, five strains of Lactiplantibacillus plantarum isolated from wild plant matrices of the Mediterranean area (i.e., aloe, carob, blackthorn), considered as unconventional ecological niches due to their restricted use in food industries and already characterised for probiotic [38] and antimicrobial activity ([39]; De Simone et al.'s unpublished results), were selected for their ability to inhibit the growth of Aspergillus niger CECT 2805. Different modes of action and synergies among the antimicrobial features were previously identified as being responsible for the detected activity. In fact, the five strains are able to produce different organic acids, including lactic, acetic, and 3-phenyllactic acid, but also volatile organic compounds, such as high amounts of 2-undecanone and 2-nonanone, which are well known for their antifungal activity [39]. For these reasons, the strains were chosen for probiotic fortification and for applications as biopreservatives on ready-to-eat perishable fruits using table grapes cv. Italia berries as a model fruit. In this context, the use of coating was chosen as an immobilising agent with the aim of increasing the number of viable cells delivered by the berries and uniformly distributing the biocontrol strains on the surface of the fruits, but also as a combined treatment to ameliorate the effect of bioprotection. In this work, an edible coating based on sodium alginate and glycerol was chosen because these chemicals are already used as food additives, with the codes E401 and E422, respectively.

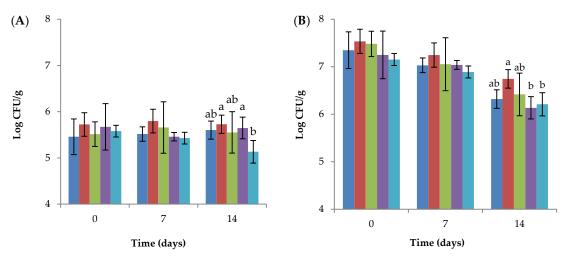
#### 3.1. Probiotic Viability on Table Grape Berries during Shelf Life

In our study, *L. plantarum* strains were separately resuspended in a coating-forming solution to obtain a final concentration of ~ $10^9$  CFU/mL. Several authors suggested different concentrations of strains belonging to this species to be added to the formulation of edible coatings [26–30]. Considering grape as a target fruit, Marin et al. [28] used a solution with a concentration of  $5 \times 10^7$  CFU/mL for *L. plantarum* applied as biocontrol agent against *B. cinerea*, reaching a maximum concentration of about 6 LogCFU/g in the final product. However, the purpose of the present study is to utilise *L. plantarum* strains not only as biocontrol agents, but also as probiotics. For this reason, with the aim of obtaining a higher bacterial consumption associated with a standard fruit serving, we decided to increase the concentration for the coating solution to ~ $10^9$  CFU/mL. In addition, this threshold allows for the maximisation of bacterial transfer without including a concentration phase of bacteria, which can be expensive in terms of industrial scale-up.

The viability of probiotic *L. plantarum* strains on table grape berries included or not in the edible coating during 14 days of shelf life at 4 °C is shown in Figure 1. At day 0, the alginate edible coating increased the number of probiotic cells transferred to the surface of the berries, which generally increased from about 5 to more than 7 LogCFU/g for all strains used in this work. A greater number of viable cells for coated berries with respect to uncoated berries was recorded on all sampling days. However, in uncoated berries, the number of living cells remained stable during the 14 days, whereas a reduction of about 1 Log CFU/g was detected in the coated berries. No difference was found between the *L. plantarum* strains at sampling days 0 and 7 in either condition. At 14 days, *L. plantarum* 11-A and CZ-97 showed higher viability in uncoated grapes, with about 5.7 Log CFU/g, whereas, in coated berries, *L. plantarum* 11-A was found to be significantly different, with 6.7 Log CFU/g.

Without an edible coating, Lappa et al. [44] transferred a slightly lower quantity of L. plantarum than the amount we observed in uncoated grapes. In our earlier work, L. plantarum MEP3 had better adhesion ability on uncoated table grape berries, with approximately 7 Log CFU/g of viable cells transferred to the surface of the berries, and this remained stable during shelf life [45], indicating the need to develop new strategies to increase the adhesion of probiotic bacteria with limited ability to colonise the surface of the fruits. In addition, the LAB viability observed in ready-to-eat table grapes was slightly lower than in other fresh-cut fruits [8,9,45,46]. For this reason, it should be considered that the decreased ability to colonise the fruit's surface may be due to the structural and chemical characteristics of the cuticle of the grape berries, which may prevent microorganisms from adhering to and remaining on the fruit [45]. However, probiotic intake advantages can be gained from foods that contain at least 6-7 Log CFU of viable bacteria per gram of product [33]. Thus, the use of edible coatings could be considered an advantageous option to obtain probiotic ready-to-eat fruits. In fact, the content of viable probiotic cells in uncoated table grape berries does not reach the requirement limit to be considered as beneficial. From this perspective, the use of an edible coating gave the possibility of enhancing the number of viable bacteria transferred to the berries' surface by about 2 Log, thus fulfilling the requirement limit of 6–7 LogCFU/g at the end of the shelf life. In addition, assuming that the mean weight of one grape berry is between 8 and 12 g on average, and

considering that an estimated portion of fresh fruit in a single meal could be represented by 5–10 berries with a quantity of between 80 and 100 g of product, the concentrations of probiotic for a single portion of ready-to-eat table grape berries should be estimated at more than 8 LogCFU at the end of the shelf life, tailoring this matrix as a functional food.



**Figure 1.** Viability of probiotic *L. plantarum* strains in ready-to-eat table grape berries during 14 days of storage at 4 °C, applied through saline solution (**A**) or edible coating (**B**). Values are means and standard deviation of three biological replicates. Values with different letters are significantly different according to one-way ANOVA test (p < 0.05) followed by Fisher's Least Significant Difference (LSD) test. *L. plantarum* 10-A (blue); *L. plantarum* 11-A (red); *L. plantarum* CB-56 (green); *L. plantarum* CZ-97 (purple); *L. plantarum* CZ-103 (light blue).

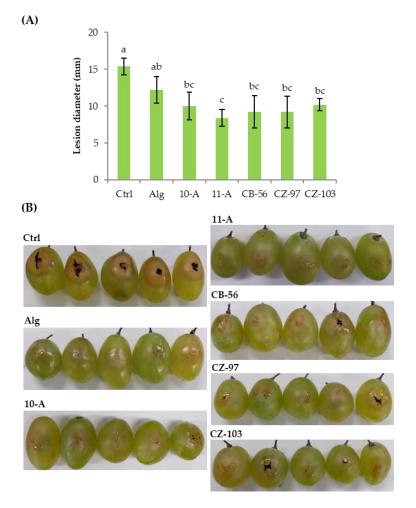
#### 3.2. Fruit Decay Assay

Based on the previous characterisation of antifungal activity against *Aspergillus niger* CECT 2805 [39], it was also hypothesised that *L. plantarum* strains could act as a preventive treatment against fungal contamination in table grape berries. For this reason, the bioprotective potential of the strains applied to edible coatings was further investigated against the same target when artificially wound-inoculated. The lesion diameter and the symptoms of infection of the table grape berries subjected to different antifungal and probiotic edible coating treatments are shown in Figure 2.

As reported in previous studies, the alginate coating treatments effectively reduced the postharvest deterioration and quality parameters, such as weight and firmness losses, total soluble solids, titratable acidity, and colour [47,48]. However, in previous work, these parameters were mainly evaluated in the absence of artificial inoculum. At the same time, the focus of this preliminary work was only to investigate the potential of antifungal and probiotic coatings to reduce the lesion diameter caused by fungal inoculation in artificially performed wounds, and, for this reason, the physicochemical parameters were not evaluated.

When alginate coating alone (without LAB strains) was compared to the uncoated control, a significant effect on lesion diameter reduction was observed, with a lesion diameter from  $15.40 \pm 1.14$  to  $12.20 \pm 1.79$  mm for uncoated and alginate-coated berries, respectively (Figure 2A). The reduction in lesion diameter was also accompanied by a reduction in the symptoms of infection, such as browning around the wound and sporulation (Figure 2B,C). This is consistent with previous literature on this topic and confirms the positive effect of edible coatings to limit the physiological damage of the berries. However, among the *L. plantarum* strains added to the coatings, most of them showed the same behaviour, and only slight differences were found between most of them and the alginate coating alone. In fact, four out of five strains showed the same statistical significance level, with values of lesion diameter ranging from  $10.00 \pm 1.87$  to  $9.20 \pm 2.05$  mm for *L. plantarum* strain 10-A and CZ-103, respectively. Differently, the coating treatment with *L. plantarum* 11-A

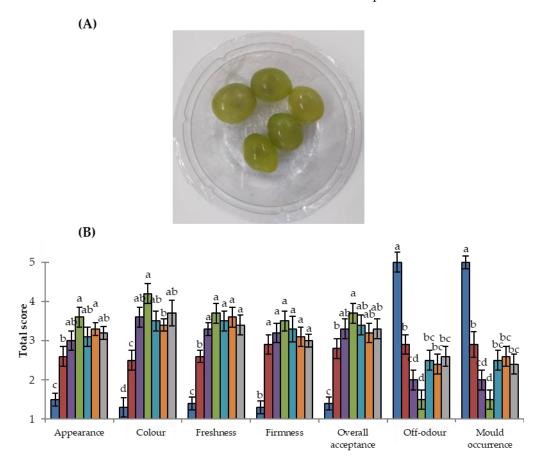
showed a higher reduction in lesion diameter, with values of  $8.40 \pm 1.14$  mm, which was significantly different from the control and from the alginate coating alone. In addition, the symptoms of infection were also lower, with the absence of sporulation and only a limited browning around the wound, which partially healed (Figure 2B–D). The detected effect is partially consistent with previous observations regarding the strains utilised. In fact, the strains are able to produce different antifungal metabolites such as organic acid (mainly lactic, acetic, and 3-phenyllactic) and volatile organic compounds (2-undecanone and 2nonanone) [39]. Differences in the values of lesion diameter detected could be attributed to the metabolic activity and to the different ability to produce these metabolites when the strains are included in the coated matrices. Different authors have previously reported the protective effect of living cells of L. plantarum against fungal decay caused by Aspergillus species [44,45]. At the same time, the inhibitory effect of the CFS of *L. plantarum* strains was assessed in table grapes against Pseudomonas syringae pv. syringae and Botrytis cinerea [49]. This suggests the high potential of this LAB species as a broad-spectrum biocontrol agent for table grapes. However, to the best of our knowledge, the biocontrol effect of edible coatings functionalised with antifungal strains of L. plantarum against fungal decay has not been previously investigated.



**Figure 2.** Lesion diameters of table grape berries artificially contaminated with *A. niger* CECT 2805 after 3 days of shelf life at 24 °C. Data are the means  $\pm$  SD of five replicates. Values with different letters are significantly different according to one-way ANOVA test (p < 0.05) followed by Tukey's multiple comparison test. (**A**) Table grapes cv. Italia berries artificially contaminated with *A. niger* CECT 2805 (**B**). Ctrl: Untreated control. Alg: Alginate coating. 10-A: *L. plantarum* 10-A/Alginate coating. 11-A: *L. plantarum* 11-A/Alginate coating. CB-56: *L. plantarum* CB-56/Alginate coating. CZ-97: *L. plantarum* CZ-97/Alginate coating. CZ-103: *L. plantarum* CZ-103/Alginate coating.

# 3.3. Sensorial Analysis

Figure 3 represents variations in the sensory characteristics of table grape berries at 3 days of shelf life at 24 °C. As expected, the artificial contamination of berries was found to negatively impact the product's quality significantly. In fact, among the parameters evaluated, those related to positive features, such as appearance, colour, freshness, firmness, and overall acceptance (Figure 3B, left side), were all ranked significantly lower than the limit of marketability for uncoated berries. At the same time, mould occurrence and off-odour (Figure 3B, right side), which were both related to fungal contamination, fermentations, and fruit tissue necrosis, were ranked at the top for uncoated berries.



**Figure 3.** Example of probiotic table grapes cv. Italia portion (**A**). Sensorial evaluation of table grapes cv. Italia berries with different treatments artificially contaminated with *A. niger* CECT 2805 after 3 days of shelf life at 24 °C (**B**). Values with different letters are significantly different according to one-way ANOVA test (p < 0.05) followed by Tukey's multiple comparison test. Untreated control (blue); Alginate coating (red); *L. plantarum* 10-A/Alginate coating (purple); *L. plantarum* 11-A/Alginate coating (green); *L. plantarum* CB-56/Alginate coating (light blue); *L. plantarum* CZ-97/Alginate coating (orange); *L. plantarum* CZ-103/Alginate coating (gray).

Alginate-coated berries showed intermediate values of all of the descriptors evaluated, with those related to positive features being slightly below the limit of marketability. On the contrary, mould occurrence and off-odour were ranked higher than the limit and considered unacceptable for marketing, whereas, among the Table grape berries coated with *L. plantarum* as probiotic/antimicrobial, the strain 11-A showed the best performance, with positive feature values still higher than the limit of marketability and with low impact of mould occurrence and off-odour even after fungal artificial contamination. In addition, as above, *L. plantarum* 11-A was the only strain which showed a clear significant difference, with respect to alginate coating alone, in almost all of the sensorial parameters evaluated.

For these reasons, table grape berries coated with the probiotic strain *L. plantarum* 11-A were considered to be of sufficient quality for marketing.

# 4. Conclusions

Fresh fruit and vegetables represent crucial factors in a balanced diet aimed at maintaining a healthy state. Due to their water and nutritional content, fruit and vegetables are very perishable foods, particularly due to the development of mould. In light of lifestyle changes, in order to maximise the presence of fruit and vegetables in the diet, it becomes crucial to optimise postharvest management to improve the shelf life, quality, and safety of ready-to-eat products. There is growing interest in postharvest applications of LAB for the production of ready-to-eat fruit and vegetables with selected LAB strains. In particular, these solutions enhance the antimicrobial and probiotic properties of selected strains to improve hygienic quality and functional characteristics. Edible packaging solutions represent further useful solutions to improve the characteristics of the finished product appreciated by the market, protecting the product from a physical, chemical, and biological point of view. This scientific work proposes a synergy between these two categories of innovations, using L. plantarum, an alginate coating, and table grapes as model factors for the experimental design. An alginate coating was used to improve the transfer of viable cells on table grape berry surfaces. With this strategy, the probiotic cell transfer increased by about 2 Log CFU/g, and higher viability was maintained until the end of shelf life. Significant differences in terms of decay prevention were also found in the alginate coating containing the probiotic strain L. plantarum 11-A. All of the strains displayed the same behaviour after inclusion in alginate in terms of the improvement in the number of cells. Conversely, a strain-dependent effect was underlined for the properties linked to biocontrol properties after inclusion in the alginate matrix, suggesting that the application of antimicrobial microbes as bio-tools in edible packaging may represent an innovative criterion in the selection of lactobacilli to be exploited postharvest.

Author Contributions: N.D.S.: writing—original draft, methodology, investigation, data curation, conceptualisation. A.S.: methodology, investigation, data curation, conceptualisation. D.F.: investigation, data curation. P.R.: writing—review and editing, supervision, conceptualisation. V.C.: writing—review and editing, supervision, resources, conceptualisation. G.S.: writing—review and editing, supervision, resources, funding acquisition, conceptualisation. M.F.: writing—review and editing, resources, conceptualisation. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was partially supported by PON project 'Conservabilità, qualità e sicurezza dei prodotti ortofrutticoli ad alto contenuto di servizio'—POFACS-CUP B74I20000120005. Mariagiovanna Fragasso is supported by the European Union Next-Generation EU [Piano Nazionale di Ripresa e Resilienza (PNRR)—Missione 4 Componente 2, Investimento 1.4—D.D. 1032 17/06/2022, CN00000022] within the Agritech National Research Centre for Agricultural Technologies. Vittorio Capozzi is supported by the Next-Generation EU [PNRR], in the framework of the Mission 4 Component 2 Investment 1.3 Award Number: Project code PE00000003, Project title: "ON Foods—Research and innovation network on food and nutrition Sustainability, Safety and Security—Working ON Foods".

**Data Availability Statement:** The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

**Acknowledgments:** The authors acknowledge Massimo Franchi of the Institute of Sciences of Food Production—CNR for the skilled technical support provided during the realisation of this work.

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

# References

- Guo, Z.; Liu, X.M.; Zhang, Q.X.; Shen, Z.; Tian, F.W.; Zhang, H.; Sun, Z.H.; Zhang, H.P.; Chen, W. Influence of Consumption of Probiotics on the Plasma Lipid Profile: A Meta-Analysis of Randomised Controlled Trials. *Nutr. Metab. Cardiovasc. Dis.* 2011, 21, 844–850. [CrossRef]
- 2. Gerritsen, J.; Smidt, H.; Rijkers, G.T.; de Vos, W.M. Intestinal Microbiota in Human Health and Disease: The Impact of Probiotics. *Genes Nutr.* **2011**, *6*, 209–240. [CrossRef]
- 3. Vijaya Kumar, B.; Vijayendra, S.V.N.; Reddy, O.V.S. Trends in Dairy and Non-Dairy Probiotic Products—A Review. J. Food Sci. Technol. 2015, 52, 6112–6124. [CrossRef]
- 4. Martins, E.M.F.; Ramos, A.M.; Vanzela, E.S.L.; Stringheta, P.C.; de Oliveira Pinto, C.L.; Martins, J.M. Products of Vegetable Origin: A New Alternative for the Consumption of Probiotic Bacteria. *Food Res. Int.* **2013**, *51*, 764–770. [CrossRef]
- Gross, K.C.; Wang, C.Y.; Saltveit, M.E. The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks. In United States Department of Agriculture (USDA)-Agricultural Research Service-Agriculture Handbook No. 66; Gross, K.C., Wang, C.Y., Saltveit, M.E., Eds.; U.S. Department of Agriculture: Washington, DC, USA, 2016; p. 780.
- 6. Remize, F.; Garcia, C. Fresh-Cut Vegetables and Fruits: Do They Really Meet Sustainability and Nutritional Benefits? *Curr. Food Sci. Technol. Rep.* **2024**, *2*, 37–44. [CrossRef]
- 7. Alvarez, M.V.; Bambace, M.F.; Quintana, G.; Gomez-Zavaglia, A.; del Rosario Moreira, M. Prebiotic-Alginate Edible Coating on Fresh-Cut Apple as a New Carrier for Probiotic Lactobacilli and Bifidobacteria. *LWT* **2021**, *137*, 110483. [CrossRef]
- 8. Russo, P.; Peña, N.; de Chiara, M.L.V.; Amodio, M.L.; Colelli, G.; Spano, G. Probiotic Lactic Acid Bacteria for the Production of Multifunctional Fresh-Cut Cantaloupe. *Food Res. Int.* 2015, *77*, 762–772. [CrossRef]
- 9. Russo, P.; de Chiara, M.L.V.; Vernile, A.; Amodio, M.L.; Arena, M.P.; Capozzi, V.; Massa, S.; Spano, G. Fresh-Cut Pineapple as a New Carrier of Probiotic Lactic Acid Bacteria. *BioMed Res. Int.* **2014**, 2014, 309183. [CrossRef]
- 10. Bambace, M.F.; Alvarez, M.V.; del Rosario Moreira, M. Novel Functional Blueberries: Fructo-Oligosaccharides and Probiotic Lactobacilli Incorporated into Alginate Edible Coatings. *Food Res. Int.* **2019**, *122*, 653–660. [CrossRef]
- 11. Iglesias, M.B.; Abadias, M.; Anguera, M.; Sabata, J.; Viñas, I. Antagonistic Effect of Probiotic Bacteria against Foodborne Pathogens on Fresh-Cut Pear. *LWT-Food Sci. Technol.* 2017, *81*, 243–249. [CrossRef]
- 12. Shigematsu, E.; Dorta, C.; Rodrigues, F.J.; Cedran, M.F.; Giannoni, J.A.; Oshiiwa, M.; Mauro, M.A. Edible Coating with Probiotic as a Quality Factor for Minimally Processed Carrots. *J. Food Sci. Technol.* **2018**, *55*, 3712–3720. [CrossRef]
- 13. Tapia, M.S.; Rojas-Graü, M.A.; Rodríguez, F.J.; Ramírez, J.; Carmona, A.; Martin-Belloso, O. Alginate- and Gellan-Based Edible Films for Probiotic Coatings on Fresh-Cut Fruits. *J. Food Sci.* **2007**, *72*, E190–E196. [CrossRef]
- 14. Temiz, N.N.; Özdemir, K.S. Microbiological and Physicochemical Quality of Strawberries (Fragaria × Ananassa) Coated with *Lactobacillus rhamnosus* and Inulin Enriched Gelatin Films. *Postharvest Biol. Technol.* **2021**, *173*, 111433. [CrossRef]
- 15. Yousuf, B.; Qadri, O.S.; Srivastava, A.K. Recent Developments in Shelf-Life Extension of Fresh-Cut Fruits and Vegetables by Application of Different Edible Coatings: A Review. *LWT* **2018**, *89*, 198–209. [CrossRef]
- 16. Rojas-Graü, M.A.; Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso, O. The Use of Packaging Techniques to Maintain Freshness in Fresh-Cut Fruits and Vegetables: A Review. *Int. J. Food Sci. Technol.* **2009**, *44*, 875–889. [CrossRef]
- 17. Senturk Parreidt, T.; Lindner, M.; Rothkopf, I.; Schmid, M.; Müller, K. The Development of a Uniform Alginate-Based Coating for Cantaloupe and Strawberries and the Characterization of Water Barrier Properties. *Foods* **2019**, *8*, 203. [CrossRef]
- 18. Jin, T.Z.; Chen, W.; Gurtler, J.B.; Fan, X. Effectiveness of Edible Coatings to Inhibit Browning and Inactivate Foodborne Pathogens on Fresh-Cut Apples. J. Food Saf. 2020, 40, e12802. [CrossRef]
- 19. De Simone, N.; Capozzi, V.; de Chiara, M.L.V.; Amodio, M.L.; Brahimi, S.; Colelli, G.; Drider, D.; Spano, G.; Russo, P. Screening of Lactic Acid Bacteria for the Bio-Control of *Botrytis cinerea* and the Potential of *Lactiplantibacillus plantarum* for Eco-Friendly Preservation of Fresh-Cut Kiwifruit. *Microorganisms* **2021**, *9*, 773. [CrossRef]
- Agriopoulou, S.; Stamatelopoulou, E.; Sachadyn-Król, M.; Varzakas, T. Lactic Acid Bacteria as Antibacterial Agents to Extend the Shelf Life of Fresh and Minimally Processed Fruits and Vegetables: Quality and Safety Aspects. *Microorganisms* 2020, *8*, 952. [CrossRef]
- 21. Behera, S.S.; Ray, R.C.; Zdolec, N. *Lactobacillus plantarum* with Functional Properties: An Approach to Increase Safety and Shelf-Life of Fermented Foods. *BioMed Res. Int.* **2018**, 2018, e9361614. [CrossRef]
- 22. Li, Q.; Zeng, X.; Fu, H.; Wang, X.; Guo, X.; Wang, M. *Lactiplantibacillus plantarum*: A Comprehensive Review of Its Antifungal and Anti-Mycotoxic Effects. *Trends Food Sci. Technol.* **2023**, *136*, 224–238. [CrossRef]
- 23. Zhao, Q.; Shi, M.; Jiang, Y.; Hu, B.; Guo, X.; Gong, D.; Zhang, Y. Compositional Shifts in Fresh-Cut Apples Microbiome in Response to Application of *Lactiplantibacillus plantarum* Assessed by Next-Generation Sequencing. *LWT* **2024**, *191*, 115627. [CrossRef]
- 24. Li, K.; Zhang, W.; Kwok, L.-Y.; Menghe, B. Screening of *Lactobacillus plantarum* with Broad-Spectrum Antifungal Activity and Its Application in Preservation of Golden-Red Apples. *Czech J. Food Sci.* **2020**, *38*, 5. [CrossRef]
- Islam, S.; Biswas, S.; Jabin, T.; Moniruzzaman, M.; Biswas, J.; Uddin, M.S.; Akhtar-E-Ekram, M.; Elgorban, A.M.; Ghodake, G.; Syed, A. Probiotic Potential of *Lactobacillus plantarum* DMR14 for Preserving and Extending Shelf Life of Fruits and Fruit Juice. *Heliyon* 2023, 9, 6. [CrossRef]
- Zhao, Q.; Tang, S.; Fang, X.; Wang, Z.; Jiang, Y.; Guo, X.; Zhu, J.; Zhang, Y. The Effect of *Lactiplantibacillus plantarum* BX62 Alone or in Combination with Chitosan on the Qualitative Characteristics of Fresh-Cut Apples during Cold Storage. *Microorganisms* 2021, 9, 2404. [CrossRef]

- 27. Álvarez, A.; Manjarres, J.J.; Ramírez, C.; Bolívar, G. Use of an Exopolysaccharide-Based Edible Coating and Lactic Acid Bacteria with Antifungal Activity to Preserve the Postharvest Quality of Cherry Tomato. *LWT* **2021**, *151*, 112225. [CrossRef]
- Marín, A.; Plotto, A.; Atarés, L.; Chiralt, A. Lactic Acid Bacteria Incorporated into Edible Coatings to Control Fungal Growth and Maintain Postharvest Quality of Grapes. *HortScience* 2019, 54, 337–343. [CrossRef]
- 29. Khodaei, D.; Hamidi-Esfahani, Z. Influence of Bioactive Edible Coatings Loaded with *Lactobacillus plantarum* on Physicochemical Properties of Fresh Strawberries. *Postharvest Biol. Technol.* **2019**, *156*, 110944. [CrossRef]
- 30. Speranza, B.; Campaniello, D.; Bevilacqua, A.; Altieri, C.; Sinigaglia, M.; Corbo, M.R. Viability of *Lactobacillus plantarum* on Fresh-Cut Chitosan and Alginate-Coated Apple and Melon Pieces. *Front. Microbiol.* **2018**, *9*. [CrossRef]
- Chang, S.; Guo, Q.; Du, G.; Tang, J.; Liu, B.; Shao, K.; Zhao, X. Probiotic-Loaded Edible Films Made from Proteins, Polysaccharides, and Prebiotics as a Quality Factor for Minimally Processed Fruits and Vegetables: A Review. *Int. J. Biol. Macromol.* 2023, 253, 127226. [CrossRef]
- 32. Guo, Q.; Tang, J.; Li, S.; Qiang, L.; Chang, S.; Du, G.; Yue, T.; Yuan, Y. *Lactobacillus plantarum* 21805 Encapsulated by Whey Protein Isolate and Dextran Conjugate for Enhanced Viability. *Int. J. Biol. Macromol.* **2022**, *216*, 124–131. [CrossRef]
- Romano, N.; Tavera-Quiroz, M.J.; Bertola, N.; Mobili, P.; Pinotti, A.; Gómez-Zavaglia, A. Edible Methylcellulose-Based Films Containing Fructo-Oligosaccharides as Vehicles for Lactic Acid Bacteria. *Food Res. Int.* 2014, 64, 560–566. [CrossRef]
- De Simone, N.; Pace, B.; Grieco, F.; Chimienti, M.; Tyibilika, V.; Santoro, V.; Capozzi, V.; Colelli, G.; Spano, G.; Russo, P. *Botrytis cinerea* and Table Grapes: A Review of the Main Physical, Chemical, and Bio-Based Control Treatments in Post-Harvest. *Foods* 2020, *9*, 1138. [CrossRef]
- 35. De Simone, N.; Capozzi, V.; Amodio, M.L.; Colelli, G.; Spano, G.; Russo, P. Microbial-Based Biocontrol Solutions for Fruits and Vegetables: Recent Insight, Patents, and Innovative Trends. *Recent Pat. Food Nutr. Agric.* **2021**, *12*, 3–18. [CrossRef]
- 36. Serra, R.; Lourenço, A.; Alípio, P.; Venâncio, A. Influence of the Region of Origin on the Mycobiota of Grapes with Emphasis on *Aspergillus* and *Penicillium* Species. *Mycol. Res.* **2006**, *110*, 971–978. [CrossRef]
- 37. Lasram, S.; Oueslati, S.; Mliki, A.; Ghorbel, A.; Silar, P.; Chebil, S. Ochratoxin A and Ochratoxigenic Black *Aspergillus* Species in Tunisian Grapes Cultivated in Different Geographic Areas. *Food Control* **2012**, *25*, 75–80. [CrossRef]
- Rocchetti, M.T.; Russo, P.; De Simone, N.; Capozzi, V.; Spano, G.; Fiocco, D. Immunomodulatory Activity on Human Macrophages by Cell-Free Supernatants to Explore the Probiotic and Postbiotic Potential of *Lactiplantibacillus plantarum* Strains of Plant Origin. *Probiotics Antimicrob. Proteins* 2023. [CrossRef]
- De Simone, N.; López, L.; Ciudad, C.S.; Scauro, A.; Russo, P.; Rodríguez, J.; Spano, G.; Martínez, B. Antifungal Activity of Lactiplantibacillus plantarum Isolated from Fruit and Vegetables and Detection of Novel Antifungal VOCs from Fungal-LAB Co-Cultures. Food Biosci. 2024, 58, 103824. [CrossRef]
- 40. Plessas, S. Advancements in the Use of Fermented Fruit Juices by Lactic Acid Bacteria as Functional Foods: Prospects and Challenges of *Lactiplantibacillus* (Lpb.) *plantarum* subsp. *plantarum* Application. *Fermentation* **2022**, *8*, 6. [CrossRef]
- Shi, C.; Chen, Y.; Li, C.; Al-Asmari, F.; Cui, H.; Lin, L. Potential Application of *Lactiplantibacillus plantarum* in Food Bio-Preservation—A Comprehensive Review with a Focus on the Antibacterial and Anti-Virulence Effects on Foodborne Pathogens. *Food Rev. Int.* 2024, 1–27. [CrossRef]
- 42. Ren, Q.; Zhang, M.; Xue, R.; Liu, T.; Yang, Z.; Yang, Z. Purification and Characterization of a Novel Low-Molecular-Weight Antimicrobial Peptide Produced by *Lactiplantibacillus plantarum* NMGL2. *Int. J. Biol. Macromol.* **2023**, 248, 125932. [CrossRef]
- 43. Li, Y.; Yu, S.; Weng, P.; Wu, Z.; Liu, Y. Purification and Antimicrobial Mechanism of a Novel Bacteriocin Produced by *Lactiplantibacillus plantarum* FB-2. *LWT* **2023**, *185*, 115123. [CrossRef]
- 44. Lappa, I.K.; Mparampouti, S.; Lanza, B.; Panagou, E.Z. Control of Aspergillus Carbonarius in Grape Berries by *Lactobacillus plantarum*: A Phenotypic and Gene Transcription Study. *Int. J. Food Microbiol.* **2018**, 275, 56–65. [CrossRef]
- De Simone, N.; Rocchetti, M.T.; la Gatta, B.; Spano, G.; Drider, D.; Capozzi, V.; Russo, P.; Fiocco, D. Antimicrobial Properties, Functional Characterisation and Application of *Fructobacillus fructosus* and *Lactiplantibacillus plantarum* Isolated from Artisanal Honey. *Probiotics Antimicrob. Proteins* 2022, 15, 1406–1423. [CrossRef]
- 46. Alegre, I.; Viñas, I.; Usall, J.; Anguera, M.; Abadias, M. Microbiological and Physicochemical Quality of Fresh-Cut Apple Enriched with the Probiotic Strain *Lactobacillus rhamnosus* GG. *Food Microbiol.* **2011**, *28*, 59–66. [CrossRef]
- 47. Konuk Takma, D.; Korel, F. Impact of Preharvest and Postharvest Alginate Treatments Enriched with Vanillin on Postharvest Decay, Biochemical Properties, Quality and Sensory Attributes of Table Grapes. *Food Chem.* **2017**, 221, 187–195. [CrossRef]
- 48. Aloui, H.; Khwaldia, K.; Sánchez-González, L.; Muneret, L.; Jeandel, C.; Hamdi, M.; Desobry, S. Alginate Coatings Containing Grapefruit Essential Oil or Grapefruit Seed Extract for Grapes Preservation. *Int. J. Food Sci. Technol.* **2014**, *49*, 952–959. [CrossRef]
- Petkova, M.; Gotcheva, V.; Dimova, M.; Bartkiene, E.; Rocha, J.M.; Angelov, A. Screening of *Lactiplantibacillus plantarum* Strains from Sourdoughs for Biosuppression of *Pseudomonas syringae* Pv. *syringae* and *Botrytis cinerea* in Table Grapes. *Microorganisms* 2022, 10, 2094. [CrossRef]

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.