Lactic Acid Bacteria Incorporated into Edible Coatings to Control Fungal Growth and Maintain Postharvest Quality of Grapes

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Abstract. Lactic acid bacteria (LAB) have been shown to prevent the growth and activity of several postharvest pathogen fungi in fruit and vegetables because of their ability to produce antimicrobial metabolites. Edible coatings (ECs) can be used as carriers of LAB and could provide an alternative natural preservation method. The effectiveness of Lactobacillus plantarum against fungal decay on grapes applied together with EC was studied. Different formulations with or without L. plantarum were considered, using pregelatinized potato starch (PS) or sodium caseinate (NaC) as main components of the coating matrices. In some of the formulations, oleic acid (OA) was added as a surfactant. The population dynamics of the bacterium and its ability to control fungal decay were studied together with the assessment of fruit quality. NaC-based formulations improved survival of L. plantarum on fruit surface after 7 days of storage in comparison with a water control. On the other hand, L. plantarum in PS-based formulation without OA reduced Botrytis incidence more than when applied in NaC formulation or in water. Coatings had little effect on berry quality (weight, color, firmness, and soluble solids content) of grapes throughout storage, although some of the coated samples maintained acidity and maturity index during storage better than others. Therefore, LAB applied in ECs could provide a viable biocontrol method for postharvest disease in grapes.

Fruit spoilage from a variety of fungi is a major cause of economic losses after harvest and during transportation (Hodges et al., 2011). This may be due to environmental conditions in the field, which can be particularly conducive to fruit infection, but also to inadequate postharvest handling and storage where small wounds on fruit may favor the development of fungal decay (Janisiewicz and Korsten, 2002; Sharma et al., 2009; Spadaro and Gullino, 2004; Trias et al., 2008).

Grapes are one of the most consumed fruits in the world (Ghafoor et al., 2010). Due to their thin pericarp and relatively low pH, grapes are highly susceptible to injuries and colonization by fungi (Aloui et al., 2014). Among the different pathogenic fungi affecting grapes, Botrytis cinerea P. Micheli ex Pers., causal agent of gray mold, is one of the most important problems for grapevines worldwide (Reglinski et al., 2010). Field losses caused by B. cinerea can be particularly severe in climates with wet springs and warm and humid summers (Hartman and Kaiser, 2008). Currently, synthetic fungicides applied in the field before harvest, and sulfur dioxide fumigations after harvest remain the common methods to fight gray mold on grapes (Dean et al., 2012; Palou et al., 2010). However, commercial use of sulfur dioxide postharvest presents risks of phytotoxicity on the grape fruit and rachis, and also encounters an increase concern from consumers intolerant to sulfites (Palou et al., 2010). Legislative changes and the increasing concern over the use of synthetic pesticides by consumers, together with the emergence of resistance of some pathogens to fungicides and the high cost of developing new chemical products has promoted an intensive search for alternative methods before and after harvest (Seiber et al., 2014; Usall et al., 2016).

According to Schünr and Magnnusson (2005), biopreservation can be defined as the extension of shelf life and enhancement of safety of foods by using natural or added microflora and their antimicrobial products. In this sense, lactic acid bacteria (LAB) present a promising approach for several reasons: 1) they naturally occur in foods such as fresh vegetables and fruit, 2) are considered harmless to human health, 3) have a GRAS status (generally recognized as safe), 4) are widely used in food industry, and 5) and can act as biocontrol agents due to their ability to produce antimicrobial compounds and to colonize plant tissues vulnerable to infection (Lamont et al., 2017; Roselló et al., 2013; Sathe et al., 2007; Triaas et al., 2008). Different low-molecular-weight compounds produced by LAB have been reported as effective against several fungal pathogens in the literature: hydroxy derivatives of fatty acids (e.g., palmitic, stearic, oleic and linoleic acids), organic acids (e.g., phenylacetic acid, acetic and propionic acids), and cyclic dipeptides (diketopiperazines) (Gupta and Srivastava, 2014; Lamont et al., 2017; Prema et al., 2010; Sangmanee and Hongpattarakere, 2014; Schünr and Magnusson, 2005; Srom et al., 2002; Yang and Chang 2010).

Lactobacillus plantarum is one LAB species of particular interest and has been isolated in the context of screening antibacterial activity from large LAB collections (Dong et al., 2017; Magnusson et al., 2003). It is a gram-positive, facultative anaerobic and heterofermentative bacterium that can be isolated from a wide range of environmental niches, such as dairy, meat, fermented vegetables, and even oral cavity and gastrointestinal tract of humans and animals (de Vries et al., 2006; Smetanková et al., 2012). Like other Lactobacilli, it is able to grow under aerobic conditions, producing hydrogen peroxide as the final metabolite of its respiratory metabolism (Watanabe et al., 2012). Although the genetic basis for the production of antifungal compounds by this bacterium has not been clearly characterized, several studies have demonstrated its ability to produce hydrogen peroxide, organic acids, phenylacetic acid, cyclic dipeptides, and fatty acids (Crowley et al., 2013; Gajbhiye and Kapadnis, 2016; Srivastava, 2014; Lamont et al., 2017; Prema et al., 2010; Srom et al., 2002; Yang and Chang 2010).

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may explain the broad spectrum of inhibition of this LAB and suggests synergy between compounds.

The use of biopolymer-based coatings to prevent fungal decay of fruit has been extensively reviewed (Marín et al., 2017a; Palou et al., 2015). One of the multiple advantages of ECs is their role in acting as carriers of beneficial organisms producing bioactive compounds, such as LAB. In this context, several studies exploring the incorporation of LAB in coatings and biopolymer-based films have been published (Ebrahim et al., 2018; Pereira et al., 2016; Sánchez-González et al., 2013). Nevertheless, to our knowledge, no studies deal with the application of biopolymer coatings containing LAB on grapes with the purpose of controlling fungal decay, although some studies with antagonistic yeasts have been recently reported (Jiwanit et al., 2018; Marín et al., 2016; Parafari et al., 2016).

The objectives of the present work were the following: 1) to study the adherence of L. plantarum incorporated to biopolymeric coatings on the fruit surface and its survival over storage under controlled conditions, 2) to test the antifungal activity of L. plantarum subsp. plantarum against B. cinerea on grapes, and 3) to assess the effect of such coatings on grape quality.

Material and Methods

Materials. Table grapes (Vitis vinifera L. cv. Red Globe) were purchased in a local store and selected without signs of mechanical damage or fungal decay. Glycerol was purchased from Panreac Quimica, S.L.U. (Barcelona, Spain). Pregelatinized PS was provided by Roquette Laia España S.A. (Valencia, Spain). Tween 85°, OA, and NaC were supplied by Sigma-Aldrich (Madrid, Spain). Man Rosoga and Sharpe (MRS) broth and agar, Potato Dextrose Agar (PDA), and buffered peptone water (BPW) were purchased from Scharlab (Barcelona, Spain).

Microbial strains and culture conditions. The strain ATCC 14917 of L. plantarum subsp. plantarum, originally isolated from pickled cabbage, was obtained from the Spanish Type Culture Collection (CECT; Universitat de Valéncia, Burjasot, Valencia, Spain). For long-term storage, the culture was kept in MRS broth containing 30% glycerol at −80 °C. The culture was activated by transferring it to MRS broth, until optimal bacterial growth was achieved, as determined by visual broth turbidity and plating serial dilutions in MRS agar. The incubation took place at 37 °C for 48 h under anaerobic conditions. Anaerobiosis was created by placing samples at 37 °C for 10 min in erlenmeyer flasks containing 100 mL sterile BPW, which were shaken at 150 rpm for 20 min. Afterward they were sonicated in an ultrasonic bath to achieve the maximum detachment of the microorganism from the fruit surface. Serial dilutions of the washes were carried out in duplicate and plated on MRS agar. The plates were incubated at 37 °C for 48 h under anaerobic conditions. Results were expressed as CFU per g or grape.

Effectiveness of L. plantarum against B. cinerea. Before the application of the CFDs, each berry was lightly rubbed with sand paper to weaken its skin and to favor pathogen infection. Formulations containing L. plantarum were applied as described previously. An additional control consisting of sterile deionized water (C-W) was used. After the application of CFDs, the fungal dispersion of B. cinerea at 10° spores/mL was sprayed with an airbrush for 5 s. Samples were left dry again before incubation at the aforementioned conditions. The antifungal effectiveness of the CFDs containing L. plantarum was visually evaluated in terms of incidence (number of berries presenting visible growth of fungal mycelium) and severity (percentage of decayed berry surface in the affected samples) after 7 d of incubation, as described by Cañamás et al., (2011). Results were expressed as the percentage of reduction of the incidence and severity with respect to that observed in the control sample C-W.

Grape quality parameters. To evaluate the impact of the different CFDs on fruit quality parameters, weight loss and color were measured before application and after 9 d of storage at 20 °C and 85% RH. Firmness, soluble solids content (SSC), and titratable acidity (TA) were measured before the application of the formulations and after 9 d of storage. In all cases, uncoated grapes were used as a control (C-W).

Weight loss was determined following a gravimetric method and expressed as the percentage loss of the initial weight. Color was measured using a CM-3600d colorimeter (Minolta Co, Tokyo, Japan) with a 10-mm-diameter window. To avoid the effect of the fruit surface heterogeneity, measurements were always taken in the same two marked zones of each fruit. CIE-L*a*b* coordinates, hue (h°ab), and chroma (C°ab) were obtained from the reflection spectra of the samples using D65 illuminant/10° observer. Firmness was assessed with a TA-XT-plus Texture Analyzer (Stable Micro Systems, Surrey, UK), with a 500 N load cell, using a 10-mm-diameter cylindrical probe. The samples were cut longitudinally and 50% compressed at a 0.2 mm/s deformation rate. Maximum force (N) was recorded as firmness parameter.
After firmness measurements, seeds were removed and berries were homogenized at 3500 rpm for 1 min and the SSC was measured using a 3T ABBE refractometer (Atago Co. Ltd., Tokyo, Japan) at 22 °C. Results were expressed as % of SSC. For TA, a 10-mL sample was titrated with 0.1 mol L−1 to a pH endpoint of 8.1 using an 800 Dosino titrator (Metrohm, Herisau, Switzerland). Results were expressed as g citric acid per 100 g of grapes. Maturity index (MI) was calculated as the quotient between SSC and TA. According to several authors, an MI of ≈30 could be considered as optimum for commercial harvest (Nicolosi et al., 2018; Sortino et al., 2017).

Statistical analysis. Analysis of variance (ANOVA) was performed using Statgraphics Centurion XVI version 16.1.17 (Manugistics Corp., Rockville, MD). CFU data were logarithmically transformed before ANOVA to improve the homogeneity of variances. Significant differences were determined using the least significant difference test (P < 0.05).

Results and Discussion

As can be observed in Fig. 1, 1 day after the application of the formulations, counts of L. plantarum were ≈5 log CFU/g. These data agreed with those previously published for the biocontrol agent Candida sake applied on grapes (Caamás et al., 2011; Marin et al., 2016). The population of L. plantarum after 1 d of application was significantly higher (P < 0.05) than the control (C-LAB) when applied in NaC CFD, with and without OA (Log CFU: 5.7 and 5.8, respectively), whereas there were no significant differences in LAB counts between formulations based on PS and C-LAB. These results suggest a positive effect of protein coatings on LAB cell viability when applied on fruit.

After 7 d of storage, the survival of L. plantarum showed a significant decrease when applied without coating-forming agents (C-LAB) and in the case of PS formulation without OA (log reduction of 0.6 and 0.9, respectively). Both, NaC with and without OA and PS with OA formulations showed a better performance at maintaining and/or increasing of L. plantarum population on fruit surface. The best result was achieved with NaC, with a final population of 6.2 CFU/g.

Surfactants, such as OA, are usually incorporated in biopolymer coatings with the purpose of improving their adherence to fruit surface. Thus, a higher adherence of the dispersion, and consequently of the microorganism, when OA was present in CFDs could be expected. No clear effect of OA was observed in protein-based formulations, whereas it seemed to have a positive effect in the starch coatings, through the enhancement of L. plantarum population after 7 storage days. Marin et al. (2016, 2017b) also observed little effect of several surfactants on the CFD retention on the grapes surface or improved cell adherence of C. sake. On the other hand, Hayek and Ibrahim (2013) reported that OA enhanced the growth of some LAB due to its incorporation into cell membranes, which improves their fluidity and offers protection from environmental conditions. The preceding data confirm the ability of L. plantarum to first colonize fruit, which is a key factor to ensure a high population of the antagonist to fight against pathogenic fungi (McGuire and Dimitroglou, 1999), and to establish itself. Other authors have reported similar results studying the antifungal performance of L. plantarum. For instance, Trias et al. (2008) reported that ATCC 14917 strain population remained relatively stable after 142 d of postharvest storage at 0.5 to 1 °C in inoculated apple wounds. Likewise, Roselló et al. (2013) described good results with different strains of this species used for biopreservation of apples and pears against Erwinia amylovora for at least 1 week at 25 °C.

In general, it could be said that coating-forming agents promoted the ability of L. plantarum to colonize the grape surface, especially when NaC was used as their main compound. This confirms the good compatibility between L. plantarum and the tested coating-forming agents, suggesting that proteins could be a more suitable source of nutrients, as previously reported by Marin et al. (2016).

The antifungal capacity of L. plantarum against B. cinerea on grapes is reflected in Fig. 2, which shows the percentages of reduction of the incidence and severity of the infection achieved with the bioactive coatings, as compared with the control sample (without LAB cells). In contrast with the cell population data, the best inhibition of the fungal action was reached with the starch coating without OA, which led to a 100% reduction of infection and significantly (P < 0.05) improved the results obtained with LAB without coating (C-LAB). No significant differences were observed for NaC-OA and OA-PG coatings.
and PS-OA formulations when compared with the control, whereas the formulation based on NaC without surfactant showed the lowest inhibitory effect. In this sense, Sánchez-González et al. (2013, 2014) reported a greater production of bacteriocins by some strains of LAB in polysaccharide than in protein media. In both studies, greater bacteriocin production implied a better antilisterial effect and could also have some antifungal action. With respect to the reduction of infection severity, formulations containing L. plantarum achieved values that ranged between 92% and 100%. All combinations of CFDs significantly reduced percentage of fruit surface affected by Botrytis infection in comparison with control. No coherent role of OA on the antifungal activity of L. plantarum was observed for starch and protein systems, probably due to the differences in antifungal metabolites induced by the components in PS and NaC matrices. Likewise, the coating components also can affect fungal growth to different extents. Therefore, the interactions of the coating compounds both with the antagonist and the pathogen, might have influence in the resulting antifungal activity.

In every case, the levels of infection incidence and severity, achieved when L. plantarum was incorporated into the coatings or water suspensions, were lower than those reached in the C-W control samples, which confirmed the potential of the ATCC 14917 L. plantarum strain to control B. cinerea by its ability to produce antifungal metabolites on the fruit surface. Other studies also reported the effectiveness of L. plantarum strains against fruit fungal pathogens. Sathe et al. (2007) showed a strong inhibitory effect against B. cinerea in cucumber wounds inoculated with L. plantarum CUK501. Trías et al. (2008) observed that different L. plantarum strains were effective against B. cinerea, among other pathogens. These authors reported that, in in vitro tests, the production of organic acids was the main mechanism of action against the spoilage fungi. Organic acids might cause an acidification of the fruit surface that could negatively affect the fungal growth and development. Production of lactic, acetic, and succinic acids or ethanol were measured in several strains of L. plantarum grown under aerobic and anaerobic conditions, and showed little differences in production of these acids between growing conditions (Smetanková et al., 2012; Valan Arasu et al., 2013). In our study, even though initial growth of L. plantarum was done under anaerobic conditions, because the film layer on the fruit surface is so thin, it was assumed that the environment became aerobic, providing the bacterium multiple opportunities to produce antimicrobial compounds, and also perhaps different types of compounds.

Fig. 2 shows weight loss of grapes coated with the different CFD containing or not L. plantarum, after 9 d of storage at 20 °C and 85% RH. The fruit weight loss observed during this period is a physical process caused by the migration of water from the plant tissues to the environment (Sánchez-González et al., 2011), with great influence of RH and postharvest biological processes (Kader, 2002). After 9 storage days, weight loss ranged from 1.5% to 3.0% and, in general, there were no remarkable differences (P > 0.05) with respect to uncoated control samples. This suggests that the applied coatings, hydrophilic in nature, only exerted a light barrier on the fruit surface. The presence of OA in the formulations did not reduce sample weight loss despite an enhancement in coating hydrophobicity (Pasquali et al., 2008). In this sense, some authors have observed an improvement in water vapor barrier properties of starch films when fatty acids were incorporated (Jiménez...
et al., 2013; Marín et al., 2017b). Vargas et al. (2006) also reported that the incorporation of OA to chitosan coatings increased the water vapor resistance of strawberries. However, there were no significant differences (P > 0.05) in weight loss between samples coated with NaC or PS formulations with and without surfactant.

Adding LAB to coatings had a variable effect on grape weight loss depending on the biopolymer type. Although in the case of protein-based coatings those formulations containing L. plantarum gave rise to higher (but not significant) weight losses, coatings based on starch showed significantly lower values when the bacteria was incorporated.

In practical terms, the observed weight losses could be considered as low because in no treatment was more than 5%, which, according to Deng et al. (2006) and Valverde et al. (2005) is considered the acceptable limit in table grapes for retail purposes. Likewise, data are in line with those reported by other authors who previously applied polysaccharide-based coatings on grapes (Aloui et al., 2014; Pastor et al., 2011). Because few differences between coated and uncoated grapes were observed, it could be concluded that the formed coatings were too thin to seriously affect the fruit’s water vapor exchanges.

Texture is a key factor to maintain fruit postharvest quality and has a strong impact on acceptability by consumers (Aloui et al., 2014). The initial firmness value of the grapes was of 32 ± 13 N. A trend toward a decrease in firmness was observed in both uncoated and coated grapes after 9 d in storage (Table 2). Loss of firmness can be explained by the breakdown of pectins and other polysaccharides causing a progressive softening of the tissues, together with the water loss (Aloui et al., 2014; Kader, 2002). The statistical analysis revealed no significant differences (P > 0.05) between uncoated and coated samples. Grapes treated with PS-OA, showed significantly higher firmness than...
grapes coated with NaC, NaC-OA, and PS-OA+LAB (Table 2). Similar results were obtained by de Oliveira et al. (2014), who did not observe firmness differences between non-coated and chitosan-coated grapes stored at room temperature. Likewise, Pastor et al. (2011) applied hydroxypropylmethylcellulose-based coatings to grapes and they did not find differences in the firmness of uncoated and coated grapes after cold storage. In contrast, other authors found greater loss of firmness in uncoated grapes compared with the coated ones (Aloui et al., 2014; Sánchez-González et al., 2011; Valverde et al., 2005). These different behaviors might be due to different environmental conditions during storage, the nature of biopolymers used as coatings, and even the grape cultivar. Moreover, contrary to that reported by Aloui et al. (2014), no clear coherence between weight loss and firmness loss was observed, because samples coated with PS-OA+LAB formulation exhibited the lowest weight loss and present the lowest firmness by the end of storage.

Initial grape SSC was 18.6 ± 0.3 and, as shown in Table 2, an increase in SSC content, a decrease in the acidity from the initial value (0.390 ± 0.004) was observed when the samples coated with NaC and NaC-OA+LAB (Table 2). Similar results were observed in samples coated with NaC and NaC-OA+LAB (Table 2).

Table 2. Firmness, soluble solids content (SSC), titratable acidity (TA), and maturity index (MI) of uncoated grapes or coated with coating-forming dispersions containing or not Lactobacillus plantarum after 9 d of storage.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Firmness (N)</th>
<th>SSC (%)</th>
<th>TA (%)</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-W</td>
<td>23 (7) ab</td>
<td>19.7 (1.3)</td>
<td>0.309 (0.004)</td>
<td>68 (5) ab</td>
</tr>
<tr>
<td>NaC</td>
<td>20 (5) ab</td>
<td>19.93 (0.15)</td>
<td>0.343 (0.007)</td>
<td>58.3 (1.2)</td>
</tr>
<tr>
<td>NaC-OA</td>
<td>21 (6) ab</td>
<td>19.6 (0.6)</td>
<td>0.314 (0.012)</td>
<td>62.6 (1.7)</td>
</tr>
<tr>
<td>NaC + LAB</td>
<td>24 (8) ab</td>
<td>20.07 (0.06)</td>
<td>0.297 (0.017)</td>
<td>67 (4)</td>
</tr>
<tr>
<td>NaC-OA + LAB</td>
<td>22 (5) ab</td>
<td>19.6 (0.10)</td>
<td>0.331 (0.005)</td>
<td>59.2 (0.8)</td>
</tr>
<tr>
<td>PS</td>
<td>23 (7) ab</td>
<td>19.33 (0.14)</td>
<td>0.318 (0.007)</td>
<td>60.8 (1.0)</td>
</tr>
<tr>
<td>PS-OA</td>
<td>27 (12) ab</td>
<td>19.33 (0.15)</td>
<td>0.323 (0.013)</td>
<td>60 (3)</td>
</tr>
<tr>
<td>PS + LAB</td>
<td>26 (11) ab</td>
<td>19.43 (0.12)</td>
<td>0.324 (0.018)</td>
<td>60 (4)</td>
</tr>
<tr>
<td>PS-OA + LAB</td>
<td>20 (8) ab</td>
<td>20.0 (0.10)</td>
<td>0.333 (0.005)</td>
<td>60.1 (0.8)</td>
</tr>
</tbody>
</table>

* Different superscripts (a, b) within the same column indicate significant differences determined using least significant difference test ($p < 0.05$) between formulations. Values are means of three replicates (with standard deviations).

* g citric acid per 100 g of grapes.

This strategy has the advantage that the bioactive (L. plantarum) complies with all recommendations for food products and could provide a basis for further studies aimed at deepening the mechanisms whereby L. plantarum exerts its antifungal activity, testing their inhibitory effect under different storage conditions and optimizing the coating formulations to improve its performance to a higher extent.

**Literature Cited**


