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Lactic acid bacteria from fresh fruit and vegetables as biocontrol agents of phytopathogenic bacteria and fungi

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Summary. This study evaluated the efficacy of lactic acid bacteria (LAB) isolated from fresh fruits and vegetables as biocontrol agents against the phytopathogenic and spoilage bacteria and fungi, *Xanthomonas campestris, Erwinia carotovora, Penicillium expansum, Monilinia laxa*, and *Botrytis cinerea*. The antagonistic activity of 496 LAB strains was tested in vitro and all tested microorganisms except *P. expansum* were inhibited by at least one isolate. The 496 isolates were also analyzed for the inhibition of *P. expansum* infection in wounds of Golden Delicious apples. Four strains (TC97, AC318, TM319, and FF441) reduced the fungal rot diameter of the apples by 20%; only *Weissella cibaria* strain TM128 decreased infection levels by 50%. Cell-free supernatants of selected antagonistic bacteria were studied to determine the nature of the antimicrobial compounds produced. Organic acids were the preferred mediators of inhibition but hydrogen peroxide was also detected when strains BC48, TM128, PM141 and FF441 were tested against *E. carotovora*. While previous reports of antifungal activity by LAB are scarce, our results support the potential of LAB as biocontrol agents against postharvest rot. [**Int Microbiol** 2008; 11(4):231-236].

Key words: *Penicillium expansum* · lactic acid bacteria (LAB) · biocontrol · spoilage · fresh fruit

Introduction

During the processing of agricultural products, significant economic losses occur due to the action of deletereous microorganisms causing postharvest rot. Small wounds or cuts occurring during harvesting and transportation provide easy access for potential pathogens [14,29,34]. The blue mold rot, caused by fungi including various species of *Penicillium*, *Botrytis cinerea*, and *Monilinia laxa*, as well as other fungi that produce mycotoxins, and bacteria such as *Erwinia carotovora* and *Xanthomonas vesicatoria* have been described as common spoilage microorganisms of fresh fruits and vegetables [34]. The primary method of control of fruit fungal decay is the use of chemical fungicides. However, some of these are not authorized for postharvest treatment and several have been removed from the market due to possible toxicological risks (Directive 91/414/CEE of the EU). Moreover, growing public concern about the use of pesticides, together with the development of resistance to fungicides by several fungal pathogens and the high cost of the development of new chemicals, has promoted the search for alternative approaches [23]. Among the bacteria used as biocontrol agents, strains of the genera *Pantoea* [8,14], *Bacillus* [22,24], and *Pseudomonas* [9,18] have been described as suitable biocontrol agents [3].

In a previous study, we isolated lactic acid bacteria (LAB) from fresh vegetables and fruit and then tested their potential as bioprotective agents against food-borne human bacterial pathogens for Golden Delicious apples and Iceberg lettuce [37]. The aim of the present work was: (i) to test the in vitro capacity of indigenous LAB regarding the inhibition of spoilage and plant pathogenic microorganisms, (ii) to assess their ability to inhibit *P. expansum* infections in apple wounds, and (iii) to identify, characterize, and evaluate the production of antimicrobial compounds in the more efficient strains.

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Materials and methods

Sampling, quantification of lactic acid bacteria, and data analysis. LAB were isolated from 662 samples of fresh fruits and vegetables, either unprocessed or minimally processed, obtained from the field and markets. Samples consisted of three groups: group I, 13 types of fresh fruit (286 samples); group II, 18 types of raw whole vegetables (282 samples); and group III, packaged ready-to-eat salad vegetables (94 samples) including four types of individual ingredients. The samples were processed and plated as described [37].

In vitro assay of antagonistic activity. A number of 484 isolates of LAB strains previously isolated from fresh fruit and vegetables or dairy products [37] were tested for their antagonistic activity against phytopathogenic bacteria and fungi. Screening was done using the agar spot test in lactose-bromcresol purple agar (LBP) [16] and modified MRS agar (MRS.02) [32]. The tests were complemented with 12 strains obtained from dairy products.

Antagonistic activity was assayed against the phytopathogens *Erwinia carotovora* ATCC 15713, *Xanthomonas campestris* pv *vesicatoria* 2133.2 (IVIA, Instituto Valenciano de Investigaciones Agrarias, Valencia, Spain), *P. expansum* EPS (Escola Politècnica Superior, University of Girona, Spain), *Monilinia laxa* ISCI 334 (Istituto Sperimentale per le Colture Industriali, Bologna, Italy; presently, Centro di Ricerca per le Colture Industriali, Bologna, Italy), and *Botrytis cinerea* CECT 2100 (Colección Española de Cultivos Tipo, Valencia, Spain). The assay was performed as described [37]. In the case of *X. campestris* and *M. laxa*, only LBP agar was used, because these microorganisms are not able to grow on MRS.02.

To test the activity against the fungi *B. cinerea* and *M. laxa*, four 20-µl spots of a LAB suspension prepared at 10^7 colony-forming units (CFU)/ml were orthogonally inoculated 25 mm from the center of a 90 mm Petri dish containing LBP or MRS.02 agar. After 48 h of incubation at 25°C, the fungus was spotted at the center of the plate [21]. Fungal growth inhibition was estimated using measurements of the radius from the center of the colony towards the edge in the direction of the grown LAB colonies. Controls of fungal growth in the selected media were performed in the absence of LAB. The growth proportion of the fungus was calculated by dividing each measurement by the radius of the fungus in control agar plates and setting the highest proportion to 1.0. All tests were run in duplicates.

Inhibition of *Penicillium expansum* infections in apple wounds. The inhibition of *P. expansum* infection was tested in Golden Delicious apple wounds. Fruits surfaces were disinfected and inoculated as described [37], with the following differences: nine wounds per fruit were made, and LAB were applied in three replicates using 20 µl of a suspension consisting of 10^8 CFU/ml. Each apple was used to test two LAB strains and three wounds were kept as positive controls, where no LAB was inoculated. Fruits were left for 1 h at room temperature to remove excess water after which the wounds were inoculated with 10 µl of the fungal suspension at 5×10^4 spores/ml. Fruits were placed in polystyrene tray packs in boxes and then sealed with plastic bags to maintain a high humidity. In each box, a negative non-inoculated control was added. The boxes were incubated for 5 days at 20°C at 85% relative humidity in a controlled environment chamber Conviron PGR 15 (Conviron, Manitoba, Canada). The diameters of the lesions produced by *P. expansum* were measured and compared with the respective control. All assays were run in triplicates consisting of a series of nine wounds in three different fruits.

Identification and characterization of selected lactic acid bacteria strains. Isolates showing moderate to high antagonistic activity towards at least one of the pathogens or displaying activity against at least three microorganisms were selected for their enhanced in vitro and ex vivo antagonistic activity. Strains were routinely grown in MRS medium at 23°C (Oxoid, Hampshire, UK) and stored at –80°C with 20% glycerol. Dextran production from sucrose was tested using the methods described by Schillinger and Lüke [31]. Acetoin production was detected by the Voges-Proskauer test. Temperature sensitivity was evaluated by inoculating strains at 8, 15, 25, 37, and 45°C on MRS broth for 48 h. Determination of the hypersensitive response in tobacco (*Nicotiana tabacum*) plants was carried out with the 19 selected LAB strains; the positive control consisted of the pathogenic strain *Pseudomonas syringae* EPS94 and the negative control contained sterile distilled water [20]. Isolates PM456, MC6, FF441, TC110, and XM360 were identified by the partial sequences of their 16S rDNA genes as previously described [37]. Sequences were deposited in GenBank (accession nos. EU074849, EU074827, EU074844, EU074833, and EU074847).

Assessment of neutralization and selected enzyme treatments. Cell-free culture supernatants of the selected LAB isolates grown in MRS.02 or LBP for 48 h at 23°C were obtained by removing the cells following centrifugation at 5000 rpm for 15 min (5810 R, Eppendorf, Hamburg, Germany) and sterilizing the supernatants by filtration through 0.45-µm pore filter (Schleicher & Schuell, Dassel, Germany). These supernatants were treated as separate fractions and assayed in microplate cultures against *E. carotovora* ATCC 15713, *X. campestris* 2133.2, *Monilinia laxa* ISCI 334, and *B. cinerea* CECT 2100. [11,15]. Fraction A, consisting of pH-neutralized supernatants, was used for the detection of inhibition by organic acids. Inhibition by means of hydrogen peroxide was tested in fraction B, which consisted of pH-neutralized supernatants treated with 0.1 mg catalase/ml (Sigma, St Louis, MO, USA) at 37°C for 1 h. Fractions C, D, and E were used for the detection of bacteriocin-like compounds and consisted of pH neutralized supernatants treated separately with proteinase K (fraction C), trypsin (fraction D), and α-chymotrypsin (fraction E) (Sigma) at a concentration of 1 mg enzyme/ml for 1 h at 37°C. The reactions with catalase and proteases were stopped by incubating the samples for 10 min at 65°C before antimicrobial activity was assayed. For each treated aliquot (fraction A, B, C, D or E), 100 µl was inoculated in triplicate in 100 µl of LBP or MRS.02 containing 10^3 cells or spores/ml of the test bacteria or fungi, respectively. Plates were incubated at 23°C for 48 h and the positive or negative growth of the tested strain was recorded. Each assay was done in triplicate and controls consisted of cell-free untreated supernatants and untreated cultures.

Survival of LAB strains in apple wounds in postharvest conditions. The survival of representative strains of LAB in postharvest conditions was monitored in apple wounds. The strains used were *Lactobacillus sakei* ATCC 31063, *Leuconostoc fallax* ATCC 700006, *Lb. plantarum* ATCC 14917, *Pediococcus parvulus* ATCC 19371, *P. dextrinicus* ATCC 33087, *Lb. buchnerii* ATCC 4005, and *Lactococcus lactis* ATCC 15577. Spontaneous mutants of these strains resistant to rifampicin were selected and used in the assay. Golden Delicious apples were disinfected and inoculated as described [37]. The apples were left to stand for 1 hour to remove excess water and then stored in plastic boxes on fruit pack trays. The LAB concentration was determined immediately after inoculation. Apples were maintained for 142 days under postharvest conditions (0.5–1°C, 1.2–1.3% O_2, and 1.2–2.0% CO_2). Final concentrations of LAB were measured after the incubation period. Samples for bacterial enumeration were obtained and plated as described [37].

Results

Lactic acid bacteria in fresh fruit and vegetables. LAB were detected in 56% of the samples from group I, 77% of the samples from group II, and 100% of the samples from group III, indicating a wide distribution of LAB in fresh fruits and vegetables of different origins and processing grades. Moreover, the measured densities of LAB differed significantly among product types and sources.

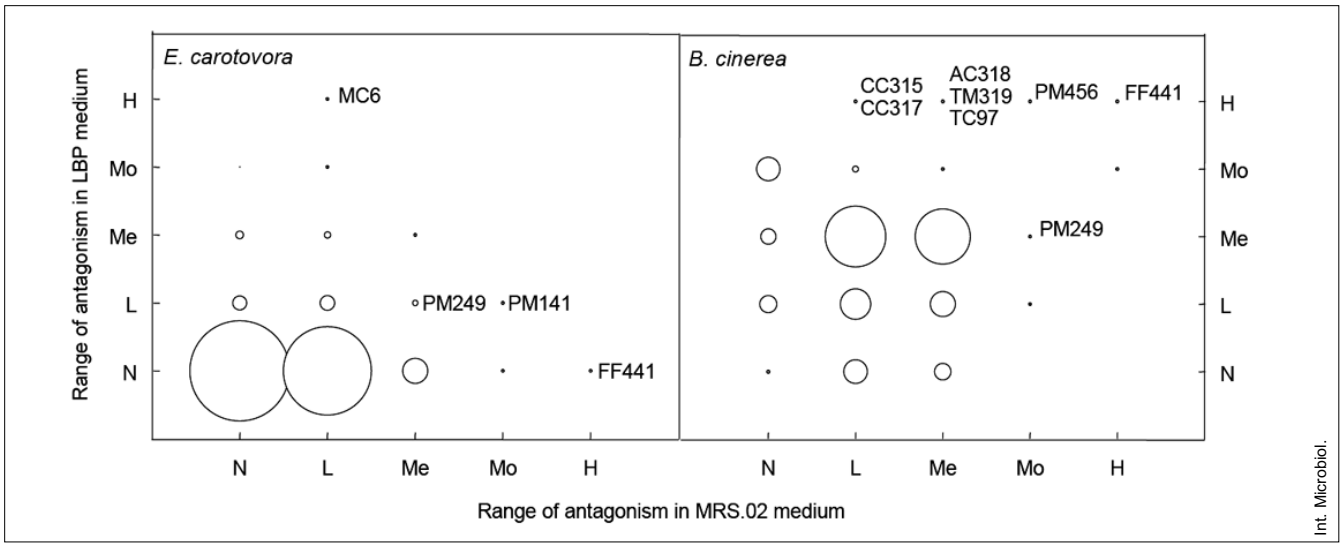

Fig. 1. Scatter plots of normalized antagonistic activity of lactic acid bacteria isolates in MRS.02 and LBP media. Diameters of symbols indicate the strain frequencies. Selected strains with inhibitory activity against each pathogen are indicated. H, high; Mo, moderate; Me, medium; L, low; N, nonsignificant activity.

Mean densities in fresh fruits (group I) accounted for 87 CFU/g (mean of all samples from this group). Bananas, pomegranates, and mandarins were the fruits containing less or no detectable LAB. Pears and apples contained the highest densities of LAB, with values up to 10^4 CFU/g. Samples obtained directly from field stalls always showed higher densities than fruits obtained in markets. The densities of viable LAB found in whole vegetables (1.32×10^2 mean of results from group II products) were not significantly higher than those found in fruits, despite the greater number of samples in which LAB were present. Within group II products, cucumbers, courgettes, and pumpkins were among the vegetables with the lowest surface LAB concentrations. No LAB could be detected on the surface of tubers and bulbs such as potatoes, and onions. In contrast, higher densities were found in tomatoes, lettuces, cabbages, and peppers. LAB were significantly more abundant in group III samples comprising ready-to-eat vegetables from different sources; they were detected in all samples analyzed and accounted for an average value of 2.61×10^6 CFU/g, although densities $>10^7$ CFU/g were also recorded, especially in highly cut vegetables, such as mixed salads, or in samples of soybean sprouts.

Screening of antagonistic activity of lactic acid bacteria. Very few strains showed moderate to high inhibition activity with both media (Fig. 1), which stresses the need to use different media compositions in screening methodologies. Moderate to high inhibition of *X. campestris* in LBP medium was observed in approximately 3% of the tested strains, this pathogen being the most frequently inhibited, and strains TC110 and XM360 the most effective. By contrast, *E. carotovora* was less frequently inhibited, as 1.34% and 0.95% of strains in MRS.02 and LBP showed moderate to high inhibition of this pathogen, respectively. Of the LAB isolates showing moderate to high inhibition patterns (>0.70) towards fungi, 6.30% were effective against *B. cinerea* and 3.80% against M. *laxa*, with strain PM456 being the most efficient. However, in in-vitro assays, none of the strains showed antagonistic activity against *Penicillium expansum*.

Of the tested strains, 3% increased the growth of *P. expansum* by 10–50% compared with control. In addition, 134 strains were able to reduce rot diameters caused by *Penicillium expansum* by 10–50%. Strain TM128 reduced the wound rot diameter by around 50% and was thus identified as the most effective isolate (Fig. 2).

Characterization of selected LAB strains. Based on the intensity of the antagonistic activity against phytopatogenic microorganisms, 19 LAB strains, including TC110, XM360, TM128, PM141, CM135, TC41, BC48, TC54, TC69, TC97, CC317, AC318, TM319, PM249, CC315, PM456, described in a previous work [37], and MC6, SE303, and FF441, were selected as potential biocontrol agents. On the basis of their 16S rRNA gene sequence similarities, the selected strains were identified as *Leuconostoc mesenteroides* (8 isolates), *Ln. citreum* (1 isolate), *Lactobacillus plantarum* (5 isolates), *Lactococcus lactis* (2 isolates), *Weissella cibaria* (2 isolates), and *Enterococcus mudtii* (1 isolate) (Table 1-SI). *Ln. mesenteroides* was selected at high frequencies and had the best antagonistic capacity against fungi, especially *Botrytis cinerea*. *Ln. mesenteroides* strain PM456 showed the highest inhibition capacity when tested

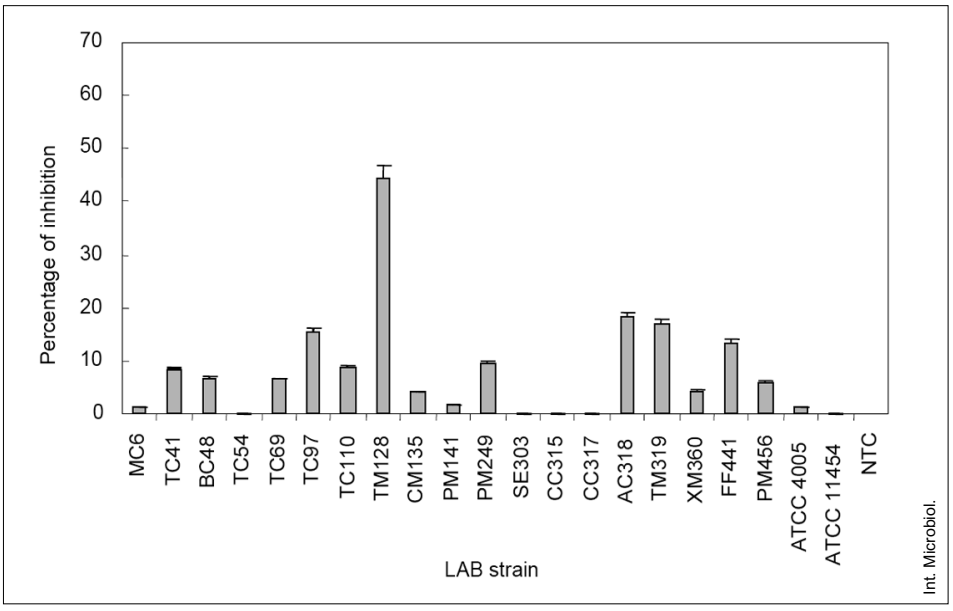

Fig. 2. Inhibition of blue mold rot infections in apple wounds treated with selected lactic acid bacteria. Values correspond to the severity of rot diameter compared to non-treated controls and are means of three replicates. Mean standard errors are represented by bars.

against all pathogenic fungi, including *Monilinia laxa*. By contrast, *Lc. plantarum* strains showed little fungal inhibition. Other phytopathogenic bacteria, such as *Erwinia carotovora* and *Xanthomonas campestris*, were efficiently inhibited by *Enterococcus mudtii* strain MC6 and *Lc. plantarum* strain TC110, respectively.

All selected strains had optimal growth temperatures on MRS plates at 25 or 37°C and were able to grow at temperatures ranging from 8°C to 45°C, except *Ln. mesenteroides* strain PM456 and *Lc. lactis* strain FF441, which did not grow at temperatures <15°C (Table 1-SI). Dextran production was confirmed in all *Leuconostoc* spp. and *Weissella* strains, except *Ln. mesenteroides* strain PM456.

Table 1. Viable cell counts of lactic acid bacteria upon inoculation of apple wounds and after 142 days under postharvest conditions[a]

	Viable cell counts (CFU/wound)[b]	
Strain	Upon inoculation	After postharvest
Lb. sakei ATCC 31063	2.97×10^7	3.71×10^5
Ln. fallax ATCC 700006	4.50×10^7	7.09×10^4
Lb. plantarum ATCC 14917	2.03×10^7	6.46×10^4
P. parvulus ATCC 19371	3.76×10^7	2.78×10^5
P. dextrinicus ATCC 33087	1.84×10^7	9.60×10^4
Lb. buchnerii ATCC 4005	3.89×10^7	4.35×10^4
Lc. lactis ATCC 15577	2.59×10^7	2.52×10^5

[a]0.5–1°C, 1.2–1.3% O_2, and 1.2–2.0% CO_2.
[b]Values are means of three replications.

The major antimicrobial effect produced by LAB strains was acidification of the medium. In fact, many pH-neutralized supernatants (fraction A) lost their inhibition ability. In in-vitro tests, organic acids were the only substances produced by the selected LAB that were capable of inhibiting fungal growth. When strains BC48, TM128, PM141, and FF441 were tested against *E. carotovora*, a significant effect mediated by the production of hydrogen peroxide as the inhibition compound was detected.

Survival of LAB in apple wounds in postharvest conditions. All representative LAB strains inoculated in apple wounds and incubated under postharvest conditions of 0.5–1.0°C, 1.2–1.3% O_2, and 1.2–2.0% CO_2 for 142 days recovered well. At the end of the experiment, LAB population concentrations remained at 4.0×10^4 CFU/wound, and a reduction in the population by 2–3 log CFU/wound compared to the starting levels was determined (Table 1). No visible effects on wounds, such as browning and off odors, were observed.

Discussion

The densities of LAB in fruit and vegetable products usually range from 10^2 to 10^6 CFU/wound. In the present study, the highest concentrations were in ready-to-eat vegetables. This was due to the presence of cut surfaces, which allow higher nutrient availability [25] and affects not only LAB but all the microbiota related to the fresh product [4]. The LAB population levels found here are in agreement with data reported for ready-to-eat salads in other studies [10]. However, despite

the relatively high abundances of LAB, only a low percentage of these bacteria had inhibitory abilities. In vitro antagonistic assays revealed that LAB strains isolated from fresh fruit and vegetable products had antifungal activity against *B. cinerea* and *M. laxa*. A previous work using the same isolates showed that most of the selected strains had good antagonistic activity against foodborne pathogens, including *Listeria monocytogenes*, *Salmonella thyphimurium*, and *Escherichia coli* [37]. Although no activity was found for any of the 496 strains tested in an in vitro assay against *P. expansum*, relatively good inhibitors were detected in the ex vivo analysis, which suggests that in vitro assays are not fully predictive of the inhibitory action of fungal infection confirmed in ex vivo assays. A complete set of ex vivo tests including all isolates were needed to completely discard the lack of inhibition towards *P. expansum*. The efficacy of the best strain identified in the present study, *W. cibaria* TM128, is of the same order as that reported for the yeast *Cryptococcus laurentii*, which reduces *P. expansum* rot by 40% [38]. The inhibition capacity of this yeast strain increases to 80–90% in the presence of certain supplements, such as salicylic acid and cytokinins, which induce resistance or retard senescence in plant tissues [38,39]. Bacterial biocontrol agents also have proven to effectively control blue mold rot. These antagonistic bacteria include *P. agglomerans* EPS125 [8] and *Aureobasidium pullulans* Ach1-1 [6], and they inhibit growth by competing for space and nutrients. A major advantage of using LAB as biocontrol agents is that they are considered GRAS (generally recognized as safe) and usually comply with all recommendations for food products [35]. Moreover, LAB are natural colonizers of fresh fruit and have been previously described as good antagonists of several bacteria and fungi in different food products [5,30]. The application of LAB strains in meat products has been successfully tested [32], and their potential use as antifungal biocontrol agents in food products has been studied as well [28]. An additional advantage of the strains reported here is the absence of adverse effects of spoilage, such as browning or off odors.

The survival of LAB in postharvest conditions makes these bacteria most adequate to prevent postharvest spoilage. Moreover, the heterofermentative metabolism of *Leuconostoc* and *Weissella* strains may produce significant amounts of CO_2, a reported inhibitor of *P. expansum* in environments with low oxygen concentrations [33].

Studies on the antifungal properties of LAB are relatively scarce; when this effect was reported, it was attributed to the production of proteinaceous compounds [17] or organic acids [5]. The preferred antimicrobial substances produced by the strains described in this study are organic acids; this was the case for all the bacteria and fungi tested. The combination of different organic acids, such as lactic and propionic, has been reported to have a synergistic fungistatic effect [1]. Moreover, the acidification of fruit tissue can reduce the postharvest decay caused by pathogens, such as *P. expansum* and *Alternaria alternata* [27]. The antifungal activity of hydrogen peroxide, which has been proven in vitro, seems to have no effect in plant tissues [12]. Nevertheless, hydrogen peroxide production has only been detected in vitro for strains BC48, TM128, PM141, and FF441, and is effective against *E. carotovora*. Other mechanisms, such as competition for substrate and space and competitive exclusion of the pathogen from entry sites in the fruit [26], might explain the inhibitory effect of LAB on fungal infection [2]. This proposed mechanism is not likely to occur with strain TM128, since, when inoculated at low doses, this strain hardly grows on apple tissue [37].

Dextran production and accumulation are related in certain cases to biofilm formation [36], which contributes to colonization and can prevent fungal attack. However, the specific role of biofilm formation in biocontrol has not yet been established [7]. The production of dextran was observed in all LAB strains that inhibited *P. expansum* ex vivo; however, the expected effect of the accumulation of this exopolysaccharide must occur in combination with other mechanisms since not all dextran-producing bacteria inhibited the development of blue mold rot.

Compared with such studies in fungi, little work has been done on the biocontrol of spoilage bacteria, such as *Erwinia carotovora* or *Xanthomonas*. However, biocontrol agents of these pathogens have been described, including a *Pseudomonas fluorescens* strain producing 2,4-diacetylphloroglucinol, which inhibits the soft rot of potatoes caused by *Erwinia carotovora* [13]. More recently, a strain of *Lysobacter antibioticus* has been described as biocontrol agent of rice blight, caused by *Xanthomonas oryzae* [19]. In vitro assays showed that LAB could inhibit these pathogens and encourage the development of biocontrol agent from this bacterial group. Moreover, some LAB strains are able to inhibit more than one phytopathogen, which must be taken into account in considering a wide range of plant protection.

In conclusion, strains such as PM456, FF441 or TM128 have the potential to prevent fresh fruits and vegetables spoilage. Strain TM128 may be used to prevent the development of blue mold, a finding reinforced not only by the observed inhibition capacity but also by other characteristics. First, because LAB strains are considered as GRAS microorganisms they can be used in food products [35]. In addition, although the optimum growth temperature for most strains is 25°C, they have a wide growth range, which allows their application under different conditions, including at refrigeration temperatures. Further research should confirm the mechanisms of inhibition and determine the optimal conditions of LAB application.

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References

1. Adams MR, Hall CJ (1988) Growth inhibition of foodborne pathogens by lactic and acetic acids and their mixtures. Int J Food Sci Technol 23:287-291
2. Andrews JH (1992) Biological control in the phyllosphere. Annu Rev Phytopatol 30:603-635
3. Andrews JH, Harris RF (2000) The ecology and biogeography of microorganisms on plant surfaces. Annu Rev Phytopathol 38:145-180
4. Badosa E, Trias R, Parés D, Pla M, Montesinos E (2008) Microbiological quality of fresh fruit and vegetable products in Catalonia (Spain) using normalised plate-counting methods and real time polymerase chain reaction (QPCR). J Sci Food Agr 88:605-611
5. Batish VK, Roy U, Lal R, Grover S (1997) Antifungal attributes of lactic acid bacteria—a review. Crit Rev Biotechnol 17:209-225
6. Bencheqroun SK, Bajji M, Massart S, Labhilili M, El Jaafari S, Jijakli MH (2007) *In vitro* and *in situ* study of postharvest apple blue mold biocontrol by *Aureobasidium pullulans*: evidence for the involvement of competition for nutrients. Postharvest Biol Technol 46:128-135
7. Bianciotto V, Andreotti S, Balestrini R, Bonfante P, Perotto S (2001) Mucoid mutants of the biocontrol strain *Pseudomonas fluorescens* CHA0 show increased ability in biofilm formation on mycorrhizal and nonmycorrhizal carrot roots. Mol Plant Microbe Interact 14:255-260
8. Bonaterra A, Mari M, Casalini C, Montesinos E (2003) Biological control of *Monilinia laxa* and *Rhizopus stolonifer* in postharvest of stone fruit by *Pantoea agglomerans* EPS125 and putative mechanisms of antagonism. Int J Food Microbiol 84:93-104
9. Cabrefiga J, Bonaterra A, Montesinos E (2007) Mechanisms of antagonism of *Pseudomonas fluorescens* EPS62e against *Erwinia amylovora*, the causal agent of fire blight. Int Microbiol 10:123-132
10. Carlin F, Nguyen-The C, Cudennec P, Reich M (1989) Microbiological spoilage of fresh, ready-to-use grated carrots. Sci Aliment 9:371-386
11. Chang YH, Kim JK, Kim WY, Kim YB, Park YH (2001) Selection of a potential probiotic *Lactobacillus* strain and subsequent in vivo studies. Anton Leeuw Int J G 80:193-199
12. Chen L, Ingham BH, Ingham SC (2004) Survival of *Penicillium expansum* and patulin production on stored apples after wash treatments. J Food Sci 69:669-675
13. Cronin D, Moeënne-Loccoz Y, Fenton A, Dunne C, Dowling DN, O'Gara F (1997) Ecological interaction of a biocontrol *Pseudomonas fluorescens* strain producing 2,4-diacetylphloroglucinol with the soft rot potato pathogen *Erwinia carotovora* subsp. *atroseptica*. FEMS Microbiol Ecol 23:95-106
14. Francés J, Bonaterra A, Moreno MC, Cabrefiga J, Badosa E, Montesinos E (2006) Pathogen agressiveness and postharvest biocontrol efficiency in *Pantoea agglomerans*. Postharvest Biol Technol 39:299-307
15. Glass MD, Courtney PD, LeJeune JT, Ward LA (2004) Effects of *Lactobacillus acidophilus* and *Lactobacillus reuteri* cell-free supernatants on *Cryptosporidium* viability and infectivity in vitro. Food Microbiol 21:423-429
16. Gómez R, Muñoz M, Ancos B, Cano P (2002) New procedure for the detection of lactic acid bacteria in vegetables producing antibacterial substances. Lebensm-Wiss u-Technol 35:284-288
17. Gourama H (1997) Inhibition of growth and mycotoxin production of *Penicillium* by *Lactobacillus* species. Lebensm-Wiss u-Technol 30:279-283
18. Janisiewicz W, Jeffers S (1997) Efficacy of commercial formulation of two biofungicides for control of blue mould and gray mould of apples in cold storage. Crop Prot 16:629-633
19. Ji GH, Wei LF, He YQ, Wu YP, Bai XH (2008) Biological control of rice bacterial blight by *Lysobacter antibioticus* strain 13-1. Biol Control 45:288-296
20. Klement Z (1963) Rapid detection of pathogenicity of phytopathogenic pseudomonads. Nature 129:299-300
21. Landa BB, Hervás A, Bettiol W, Jiménez-Díaz RM (1997) Antagonistic activity of bacteria from chickpea rhizosphere against *Fusarium oxysporum* f. sp. *ciceris*. Phytoparasitica 25:305-318
22. Lisboa MP, Bonatto D, Bizani D, Henriques JAP, Brandelli A (2006) Characterization of a bacteriocin-like substance produced by *Bacillus amyloliquefaciens* isolated from the Brazilian Atlantic Forest. Int Microbiol 9:111-118
23. Montesinos E (2003) Development, registration and commercialization of microbial pesticides for plant protection. Int Microbiol 6:245-252
24. Obagwu J, Korsten L (2003) Integrated control of citrus green and blue moulds using *Bacillus subtilis* in combination with sodium bicarbonate or hot water. Postharvest Biol Technol 28:187-194
25. Ongeng D, Devlieghere F, Debevere J, Coosemans J, Ryckeboer J (2006) The efficacy of electrolysed oxidizing water for inactivating spoilage microorganisms in process water and on minimally processed vegetables. Int J Food Microbiol 109:187-197
26. Parente E, Ricciardi A (1999) Production, recovery and purification of bacteriocins from lactic acid bacteria. Appl Microbiol Biotechnol 52:628-638
27. Prusky D, Kobiler I, Akerman M, Miyara I (2006) Effect of acidic solutions and acidic prochloraz on the control of postharvest decay caused by *Alternaria alternata* in mango and persimmon fruit. Postharvest Biol Technol 42:134-141
28. Rouse S, Harnett D, Vaughan A, van Sinderen D (2008) Lactic acid bacteria with potential to eliminate fungal spoilage in foods. J Appl Microbiol 104:915-923
29. Rundberget T, Skaar, I, Flåøyen A (2004) The presence of *Penicillium* and *Penicillium* mycotoxins in food wastes. Int J Food Microbiol 90:181-188
30. Sathe SJ, Nawani NN, Dhakephalkar PK, Kapadnis BP (2007) Antifungal lactic acid bacteria with potential to prolong shelf-life of fresh vegetables. J Appl Microbiol 103:2622-2628
31. Schillinger U, Lücke FK (1987) Identification of lactobacilli from meat and meat products. Food Microbiol 4:199-208
32. Schillinger U, Lücke FK (1989) Antibacterial activity of *Lactobacillus sake* isolated from meat. Appl Environ Microbiol 55:1901-1906
33. Sitton JW, Patterson ME (1992) Effect of high carbon dioxide and low oxygen controlled atmospheres on postharvest decay of apples. Plant Dis 76:992-995
34. Spadaro D, Gullino ML (2004) State of the art and future prospects of the biological control of postharvest fruit diseases. Int J Food Microbiol 91:185-194
35. Stiles M, Holzapfel W (1997) Lactic acid bacteria of foods and their current taxonomy. Int J Food Microbiol 36:1-29
36. Sutherland IW (2001) Biofilm exopolysaccharides: a strong and sticky framework. Microbiology 147:3-9
37. Trias R, Bañeras L, Badosa E, Montesinos E (2008) Bioprotection of Golden Delicious apples and Iceberg lettuce against foodborne bacterial pathogens by lactic acid bacteria. Int J Food Microbiol 123:50-60
38. Yu T, Chen J, Chen R, Huang B, Liu D, Zheng X (2007) Biocontrol of blue and gray mould diseases of pear fruit by integration of antagonistic yeast with salicylic acid. Int J Food Microbiol 116:339-345
39. Zheng X, Yu T, Chen R, Huang B, Wu VCH (2007) Inhibiting *Penicillium expansum* infection on pear fruit by *Cryptococcus laurentii* and cytokinin. Post Biol Technol 45:221-227

Table 1-SI. Major characteristics of selected lactc acid bacteria strains isolated from fresh fruits and vegetables

			Test microorganisms inhibited in vitro[a]				
16S rDNA identification	Strain	Isolation source	High	Moderate	Medium	Dextran production	Optimal temp. (°C)
E. mundtii	MC6	strawberries	Erw	Bot	Mon	–	37 (8–45)
Lb. plantarum	TC41	tomatoes	–	–	Bot	–	25 (8–45)
Lb. plantarum	TC54	tomatoes	–	–	Xan, Bot	–	37 (8–45)
Lb. plantarum	TC69	tomatoes	–	Mon	Bot	–	37 (8–45)
Lb. plantarum	TC97	tomatoes	–	–	Xan, Bot	–	25 (8–45)
Lb. plantarum	TC110	tomatoes	Xan	–	Bot	–	37 (8–45)
Lc. lactis	SE303	soybean sprouts	–	–	Bot	–	25 (8–45)
Lc. lactis	FF441	cheese	Erw, Bot	–	Mon	–	25 (15–45)
Ln. citreum	TM319	tomatoes	Bot	–	–	+	25 (8–45)
Ln. mesenteroides	CM135	cherries	–	–	Mon	+	25 (8–45)
Ln mesenteroides	PM141	peach	–	–	Erw	+	25 (8–45)
Ln. mesenteroides	PM249	peach	–	Bot	Erw	+	25 (8–45)
Ln. mesenteroides	CC315	persimmon	Bot	–	–	+	25 (8–45)
Ln. mesenteroides	CC317	persimmon	Bot	–	–	+	25 (8–45)
Ln. mesenteroides	AC318	eggplant	Bot	Xan	–	+	25 (8–45)
Ln. mesenteroides	XM360	custard apple	Xan	–	–	+	25 (8–45)
Ln. mesenteroides	PM456	green pepper	Bot, Mon	–	–	–	37 (15–45)
W. cibaria	BC48	chards	–	Bot	Mon	+	37 (8–45)
W. cibaria[b]	TM128	tomatoes	–	–	–	+	25 (8–45)

[a]Erw, *Erwinia carotovora* ATCC 15713; Xan, *Xanthomonas campestris* pv *vesicatoria* 2133.2; Mon, *Monilinia laxa* ISCI 334; Bot, *Botrytis cinerea* CECT 2100. Intensity of inhibition is indicated in the same order of the test inhibited microorganisms: high (inhibition range 1.0–0.90); moderate (0.89–0.70) and medium (0.69–0.40).

[b]Included for ex vivo *P. expansum* inhibition.