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RESEARCH ARTICLE-BIOLOGICAL SCIENCES

Selection and Performance of Antifungal Lactic Acid Bacteria in Corn Mini-Silos

Natalí Ivana Carrizo[1] · José Agustín Carabajal Torrez[1] · Fátima Romina Elizabeth Molina[1] · María José Fornaguera[1] · Gladys Irma Martos[1] · Ana Yanina Bustos[2] · Carla Luciana Gerez[1]

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Abstract
Silage is a method of forage conservation based on lactic fermentation. In order to improve the fermentative performance in corn silages, the aims of the present work were as follows: (I) to screen lactic acid bacteria with acidifying activity, (II) to study their antifungal activity against filamentous fungi and yeasts and (III) to evaluate their performance as antifungal in mini-silos. The acidifying activity was determined by measuring the pH and total titratable acidity. Those most acidifying bacteria were incubated in plates together with fungal markers strains to observe the presence or absence of inhibition halos, in addition to studying the nature of the antifungal metabolites by means of the treatment with proteases. The strain with the widest spectrum of action was selected to formulate an inoculum used in corn mini-silos. During storage, both silos determined humidity, acidification and microbiological composition, and then aerobic stability. The results showed that *Lactoplantibacillus* (*L.*) *plantarum* cultures had a higher production of lactic acid (7.7–17.5 g/L) than *Pediococcus* (8.8–10.9 g/L). *L. plantarum* CRL363 could inhibit the growth of filamentous fungi and some yeasts and consequently it was used in mini-silos. Compared to the control, the inoculated silo presented low pH values (4.02–4.85) and low fungi and yeast count until 20 days of storage, demonstrating the great potential of CRL363 strain for the formulation of inoculants for corn silage.

Keywords Lactic acid bacteria · Silage · Antifungal activity · Fermentation

1 Introduction

Silage is a method of forage conservation based in lactic fermentation under anaerobic conditions. Lactic acid bacteria (LAB), which are typically found in the material to be ensiled, ferment the soluble carbohydrates of the forage producing mainly lactic acid. The rapid decrease of pH inhibits the growth of contaminant microorganisms. The most important polluting genera in silage are *Fusarium*, *Penicillium* (*Pen.*) and *Aspergillus* (*A.*). Also, yeasts such as *Pichia* (*Pi.*) *anomala, Saccharomyces* (*S.*) *cerevisiae*, *Candida* (*C.*) *albicans* are observed as contaminants [1–3]. This aerobic fungal deterioration of silos is a frequent problem that modifies the nutritional and organoleptic characteristics of the forage causing rejection by animals, less production and quality of milk and also a serious risk to human and animal health [4, 5].

The current trend raises the use of lactic inoculants to standardize the final product and guarantees the conservation of the silos [3, 6]. According to the composition, different categories of additives can be mentioned: homofermentative LAB, obligatory heterofermentative LAB, combined inoculants (with obligatory content of heterofermentative and homofermentative LAB) [3]. Also, facultative heterofermentative strains including *Lactoplantibacillus* (*L.*) *plantarum*, *Lacticaseibacillus casei* and various *Pediococcus* (*P.*) species are the oldest and widely used bacteria as silage additives. In this sense, several previous studies have evaluated the effectiveness of homofermentative and heterofermentative LAB in silos made with different plant matrices [7, 8]. Oliveir et al. [9] reported a meta-analysis of 130

✉ Carla Luciana Gerez
clugerez@cerela.org.ar

[1] Reference Centre for Lactobacilli (CERELA-CONICET), Batalla de Chacabuco 145 (4000), San Miguel de Tucumán, Argentina

[2] Research and Transference Centre of Santiago del Estero (CITSE – CONICET – UNSE), RN9, km 1125 (4206), Santiago del Estero, Argentina

articles where they revealed that the effects of the inoculants varied according to forage type, LAB species and silo scale (laboratory or farm-scale). Other meta-analysis performed by Blajman et al. [7] in corn silage assess the effect of LAB as functional additives on fermentation parameters, microbiological composition and aerobic stability. They concluded that the effect of the inoculants may differ depending on the administration of homofermentative or heterofermentative LAB.

In recent years it has been shown that the antifungal properties of LAB are due to a combination of effects of their fermentation products. In the first place, it is due to the action of organic acids such as lactic, acetic, phenylactic (PLA), hydroxyphenylactic, indolactic acids among others, being acetic acid the most powerful inhibitor of fungal growth [10, 11]. Compounds of a peptide nature produced by certain LAB strains, especially *L. plantarum*, have also shown to have an important inhibitory effect on mycelial development and spore germination [12]. Other active metabolites such as 10-octadecenoic acid, palmitic acid, methyl ester, heptadecanoic acid, 16-methyl ester, stearic acid and lauric acid have demonstrated to distort the structures of hyphae and conidia and prevent the germination of spores [13].

Corn silage (*Zea mays* L.) is one of the most important livestock feeds in Argentina and worldwide [14]. In this sense, most of the studies available in this matrix, focus on the isolation and/or characterization of strains isolated from corn silos [15–17]. Oppositely, recent works report the use of LAB strains as additives in corn silos, showing variable results, since organic acid production, spoilage inhibition and in-silo behavior depend on the strain [8, 14, 18]. Besides, only few studies explored the ability of LAB strains to inhibit the growth of fungi frequently isolated from silos. Considering the importance of an adequate selection of LAB strains for silage, with this work we aim (i) to screen different LAB strains in accordance with their acidification capacity, (ii) to study their antifungal activity against filamentous fungi and yeast, and (iii) to evaluate their in situ performance on corn mini-silos.

2 Materials and Methods

2.1 Microorganisms and Culture Conditions

One hundred forty six strains of *P. acidilactici* (16), *P. pentosaceus* (16) and *L. plantarum* (114) (Supplementary Information Table SI-1) isolated from vegetables and belonging and kept in Centro de Referencia para Lactobacilos (CERELA-CONICET) culture collection, were used in this study. Before experimental use, cultures were propagated (1%, v/v) in MRS medium [19] and incubated at 37 °C for 18 h without agitation.

The fungi strains used in this study were *A. niger* CH1, CH2 and CH3, *A. japonicus* CH5, *Pen. sp.* CH6, *Pen. roqueforti* CH4, *Pen. digitatum* INTA2, *C. tropicalis* CH6, *Metschnikowia* (*M.*) *pulcherrima* CH7, *Pi. anomala* CH8, *S. cerevisiae* CH9. All fungi strains were isolated from contaminated cereals and vegetables; while the yeasts were isolated from vegetables. Fungi strains were grown on potato dextrose agar medium (Britania, Argentina) at 25 °C for 7 days. Conidias were collected in sterile Tween 80 at 0.05% (v/v), counted in Buerkner hemocytometer, and adjusted to 10^5 per mL of sterile water. Yeasts were grown in YPD medium (1.0% w/v yeast extract, 2.0% w/v peptone, 2.0% w/v glucose) at 30 °C for 24 h.

2.2 Acidification Capacity and LAB Selection

One hundred forty six LAB were assayed for acidification activity at 37 °C in soybean culture medium (SM) containing (g/L): 10 soybean flour, 20 glucose, 10 yeast extract, 3.6 Na_2HPO_4, 5.6 KH_2PO_4, 0.038 $MnSO_4$ and 0.05 $MgSO_4$. The strains were inoculated at 0.1% v/v (initial viable count: 7 log CFU/mL) in 100 mL of SM broth and samples were taken at 4, 6 and 24 h. The acidification activity was determined by pH and total titratable acidity (TTA) measurements. Dornic solution (0.1 N NaOH) was used to titrate acids measurement and phenolphthalein as pH indicator. The TTA expressed as Dornic Grade (1°D represents 0.01 g lactic acid in 100 ml of culture). The organic acids production by selected LAB was determined after 24 h fermentation in SM medium by high-performance liquid chromatography [20].

2.3 Antifungal Activity of Selected LAB

Antifungal activity of selected LAB was evaluated by antifungal screening assay [21] as described below. The assay was performed in a 24-well cell-culture plate (Greiner Bio One, Buenos Aires, Argentina) containing 500 µl of 1.5% (w/v) SM agar. Selected LAB was spotted at the center of a well with 10 µl of an outgrown culture and incubated for 48 h. Thereafter, wells were overlaid with 500 µl of 0.5% Sabouraud's glucose soft-agar (Britania, Argentina) and inoculated either with 10^3 fungal spores per mL or 10^4 yeast per mL. The plates were incubated for 24–48 h at optimum conditions for the indicator strain. All experiments were performed as duplicates. The inhibition areas were visually recorded daily. The results were expressed as: negative (absence of zone of inhibition), positive ++ (zone of inhibition defined on LAB colony) and strongly positive +++ (absence of fungal growth in 100% of well).

In addition, selected strain was evaluated in neutral conditions to study the nature of antifungal activity. For this purpose, SM agar and Sabouraud's glucose soft-agar media with 0.1 M de K_2HPO_4 pH = 7 were used.

2.4 Effects of Proteolytic Enzyme on Antifungal Activity

Taking into account that *L. plantarum* CRL 237, CRL 363 and CRL 510 were active at neutral pH against *Pen.* sp. CH6, *Pen. roqueforti* CH4 and *Pen. digitatum* INTA2, the potential peptide nature of the antifungal compounds was evaluated.

LAB cultures were grown in SM medium broth at 37 °C for 24 h. Cell-free supernatants (Sb) obtained by centrifugation at 9000 g for 10 min at 4 °C were filtered (0.2 lm-pore-size, Sartorius AG, Goettingen, Germany). The Sbs were treated with pepsin (Sigma, 2000 U/mL of Sb) during 1 h at 37 °C and these were neutralized (pH 7). The antifungal activity of Sbs was determined using the Microtitre Plate Well Assay [22]. Conidial suspensions (10 µl) containing 10^4 spore per ml were added to 190 µl of: (i) SM broth pH 7 (SMn), (ii) SM acid (SMa), (iii) Sb acid (pH 3.5, Sba) and (iv) Sb treated with pepsin and neutralized (Sb-np). The cultures were incubated at 30 °C after 48 h. Fungal growth was determined by measuring the optical density ($OD_{580\,nm}$) in a spectrophotometer (VERSAmax, Molecular Devices, USA).

2.5 Use of *L. plantarum* CRL 363 as Inoculants in Ensilage

The chopped corn (Burruyacu, Tucumán, Argentina) was transferred with refrigeration to the laboratory. The humidity percentage of chopped corn was determined using an MB45 moisture analyzer (Ohaus). The silage was made using a small-scale system of fermentation. Mini-silos were sprayed with a cell suspension of *L. plantarum* CRL 363 in sterile water (S363) or only with sterile water (untreated control, S-control). All treatments were applied at a rate of 20 mL/kg corn of the corresponding solution. The forage with or without treatment was divided into 50 g that were packed in black bags of 60 × 40 mm, 75 microns. The mini-silos were stored at 25 °C in an oven for 45 days. During storage, a bag silo was processed each time and physical (moisture), chemical (pH, organic acids concentration) and microbiological parameters were determined.

Chemical (pH and organic acids) and microbiological (total mesophilic bacteria, LAB, fungi and yeast) parameters were determined in these samples. For this, forage samples (10 g) and sterile distilled water (90 mL) were homogenized by stirring. The pH was measured using a portable peachimeter (Sartorius, model PT-10), and the organic acids present in the silos were determined by high pressure liquid chromatography (HPLC) as was previously described, [10].

The microbiological analysis was performed according to Burns et al. [8]. Total LAB were enumerated in MRS medium agar (37 °C, 48 h, microaerophilia) and yeasts and filamentous fungi in chloramphenicol glucose agar (Britania, Argentina) (25 °C, 5–7 d, aerophilia). In addition, count of total aerobic mesophilic bacteria (PCA medium, 30 °C, 48 h in aerophilia) was determined.

At the end of the silage process (45 days), aerobic stability was determined according to Burns et al. [8]. Briefly, the silos were exposed to the air and the internal temperature of the silo was recorded daily.

2.6 Statistical Analysis

All assays were performed in three independent experiments and mean values ± standard deviation (SD) are given. Data were compared by analysis of variance (Anova) and Dunnett t-test. The statistical significance ($P < 0.05$) was determined by using InfoStat2006p.3 software.

3 Results

3.1 Acidification Activity and LAB Selection

The rate of acidification and the type of organic acids produced are relevant parameters in the selection of LAB used as silo inoculants. As shown in Fig. 1, all the pediococci evaluated, were able to grow and acidify the culture medium, while 39 strains of *L. plantarum* did not grow after 24 h. In general, *L. plantarum* strains showed the highest acidifying activity ($\Delta pH > 2.5$ and $\Delta TTA > 75°D$) compared to pediococci ($\Delta pH < 2.0$ and $\Delta TTA < 50°D$) at the end of the assay. The 24 best- performing strains were selected according to the results obtained at 6 h incubation (see criteria in Fig. 1). The organic acids production of the strains under study is shown in Table 1. As was expected, the pH decrease was correlated with the production of organic acids by the LAB. In general, a higher production of lactic acid was observed in the cultures of *L. plantarum* (ranged from 7.7 to 17.5 g/L) compared to *Pedicoccus* (ranged from 8.8 to 10.9 g/L).

3.2 Antifungal Activity of Selected LAB

Fungal spoilage of silos is a frequent problem that entails the reduction of their nutritional value and risk to human and animal health due to the possible presence of mycotoxins. Therefore, we studied the antifungal activity of LAB, previously selected for its acidifying capacity. Also, the chemical nature of the bioactive metabolites was characterized. All the studied strains were able to inhibit the growth of at least one

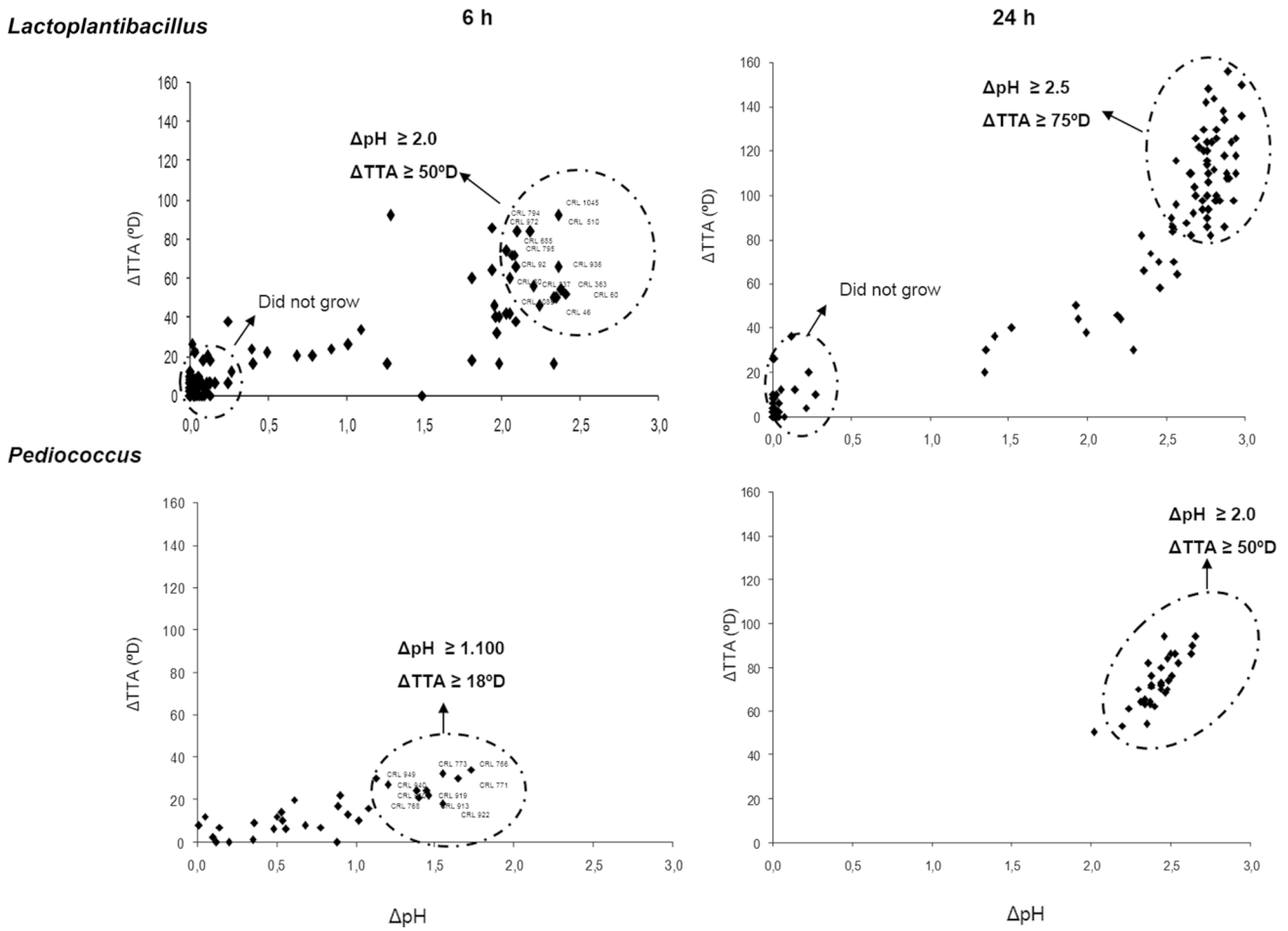

Fig. 1 Acidifying activity (ΔpH and ΔTTA) of 146 LAB strains isolated from vegetables in SM medium at 6 and 24 h. ΔpH and ΔTTA are the difference of the final and initial acidy values

of the fungal strains used as indicators, observing that the lactobacilli showed a greater spectrum of inhibitory activity than the pediococci (Table 2). Regarding the sensitivity of fungi strains, filamentous fungi were more sensitive than yeasts, observing that only *Pi. anomalous* and *M. pulcherrima* were inhibited by *L. plantarum* CRL 363.

Taking into account that the main metabolites produced by LAB are acidic, the inhibitory activity of the strains that showed positive results [(++) or (+++)] was evaluated in neutral conditions. No antifungal activity was detected against *A. niger* CH1, CH2, CH3 and *A. japonicus* CH5 fungi under neutral conditions (absence of halo of growth inhibition). These results demonstrated the acidic nature of most of the bioactive metabolites produced by LAB (Table 3). In this line, *Pi. anomala* and *M. pulcherrima* yeasts, were not inhibited by *L. plantarum* CRL 363 in neutral medium.

It is interesting to note that certain strains of LAB (e.g., *L. plantarum* CRL 46, 60, 80 y 237, 363, 510; *P. acidilacti* CRL 922 y 913) were able to inhibit *Pen.* sp. fungi. CH6, *Pen. digitatum* INTA2 and *Pen. roqueforti* CH4 in neutral conditions (Table 3). These results suggest that the selected LAB have more than one antifungal mechanism of action and their efficacy depends on the fungal strain. Additionally, the Sb of *L. plantarum* CRL 237, CRL 363 and CRL 510 were treated with the protease pepsin. Figure 2 showed the growth of *Pen. digitatum* INTA2 with Sb of *L. plantarum* CRL 363 in neutral and acid conditions and neutral with pepsin treatment. The growth of *Pen. digitatum* INTA2 was affected by the pH in the control SM medium once the higher growth was reached in the SMn medium (pH 7) compared to that observed in SMa (pH 2.4–3). Pepsin treatment did not modify the SbN inhibitory effect of strains CRL 363 on *Pen. digitatum* INTA2 indicating that the antifungal substance is not sensitive to the action of this protease. Similar performance was observed in CRL 237 and CRL 510 strains (data not shown).

Table 1 Production of organic acids by strains of *Lactoplantibacillus* and *Pediococcus* in SM medium at 24 h of fermentation

LAB	Organic acid (g/L)		
	Lactic acid	Acetic acid	Phenyl lactic acid
L. plantarum CRL 46	17.5 ± 0.9	0.22 ± 0.06	0.022 ± 0.001
L. plantarum CRL 60	11.3 ± 0.5	0.19 ± 0.04	0.02 ± 0.001
L. plantarum CRL 80	7.7 ± 0.0	0.24 ± 0.03	0.016 ± 0.001
L. plantarum CRL 92	13.9 ± 0.6	0.28 ± 0.06	0.037 ± 0.002
L. plantarum CRL 237	13.8 ± 0.4	0.23 ± 0.07	0.020 ± 0.001
L. plantarum CRL 363	16.0 ± 0.3	0.23 ± 0.06	0.045 ± 0.001
L. plantarum CRL 510	8.9 ± 0.6	0.19 ± 0.01	0.031 ± 0.001
L. plantarum CRL 685	13.3 ± 0.9	0.32 ± 0.04	0.033 ± 0.001
L. plantarum CRL 794	15.3 ± 0.4	0.29 ± 0.03	0.049 ± 0.001
L. plantarum CRL 795	12.9 ± 0.9	0.29 ± 0.05	0.039 ± 0.001
L. plantarum CRL 936	14.4 ± 0.6	0.30 ± 0.00	0.034 ± 0.004
L. plantarum CRL 972	12.9 ± 0.8	0.31 ± 0.04	0.032 ± 0.001
L. plantarum CRL 1045	12.2 ± 0.9	0.33 ± 0.05	0.032 ± 0.001
L. plantarum CRL 1089	14.3 ± 0.3	0.28 ± 0.07	0.042 ± 0.001
P. pentasaceus CRL 766	9.9 ± 0.6	0.28 ± 0.03	0.012 ± 0.003
P. pentosaceus CRL 768	9.6 ± 0.5	0.25 ± 0.05	0.013 ± 0.004
P. pentosaceus CRL 771	10.3 ± 0.7	0.21 ± 0.03	0.012 ± 0.003
P. pentosaceus CRL 773	9.03 ± 0.4	0.14 ± 0.02	0.011 ± 0.001
P. acidilactici CRL 910	10.26 ± 0.6	0.21 ± 0.01	0.000 ± 0.000
P. acidilactici CRL 922	10.98 ± 0.2	0.19 ± 0.04	0.000 ± 0.000
P. acidilactici CRL 913	9.64 ± 0.6	0.18 ± 0.04	0.184 ± 0.075
P. acidilactici CRL 914	9.3 ± 0.5	0.19 ± 0.04	0.155 ± 0.001
P. acidilactici CRL 919	8.9 ± 0.4	0.13 ± 0.07	0.012 ± 0.003
P. acidilactici CRL 940	8.8 ± 0.3	0.11 ± 0.05	0.009 ± 0.002

From these results, *L. plantarum* CRL 363 was selected for further studies as it has a wide spectrum of activity and even maintains its activity in neutral conditions.

3.3 Use of *L. plantarum* CRL 363 as Inoculants in Corn Ensilage

Silage was made with maize using a small-scale system of fermentation. Figure SI-1 shows the mini-silos produced with and without (S- control) the addition of the CRL 363 strain. At the beginning of storage, plant material with vital color and texture and without the presence of strong or unpleasant odors was observed in both mini-silos. However, after 20 days, in the control silo, the forage mass gradually darkened and the leaves and stems began to wilt, while a strong and persistent acidic odor developed. The addition of the lactic strain as an additive (S363) allowed to maintain a better color, texture and odor than the control silo. From day 45, both silos showed signs of pronounced wilt with characteristics of global deterioration, changes that were most evident in the control silo (Supplementary Information Fig. SI-1).

The humidity of the silos was determined at the beginning and after 45 days of storage. The initial values were within the expected parameters (60–67%) while at 45 days an increase of 10% in the percentage of humidity (71–77%) was observed in both silos.

Figure 3 shows the results of the microbial counts of the mini-silos at different times. At day 2, the total mesophyll count was slightly higher in the control silo than in the S363. Then, the count increased to 1.32 and 2.42 Log UFC/g at 20 and 45 days, respectively, in S363 while in the S-control decreased (1.98 logarithmic units) until 20 days, and then increased after 45 days.

As expected, the initial LAB count was higher in the silo S363. However, on day 2, both silos reach their maximum value of 9 log CFU/g and then drop (approximately 1 log CFU, at the end of storage). The yeast and fungi count increased from 6.5 to 7.5 log CFU in both silos at 2 days of storage. From this day, in the S-control the count gradually increased until reaching a value close to 9 log CFU at the end of storage, while in the S363 the count did not change significantly. Fungal contamination of the S-control was also visually verified when the mini-silos were opened.

Rapid acidification of silos is vital in the silage process to prevent the growth of contaminating microbiota. The pH values and the content of organic acids are shown in Fig. 3. The S-control had a drop in pH of almost 1.4 units on the second day of storage, which is coincident with an increase in lactic acid (0.45 g/L) and acetic (0.15 g/L) in the silo. Additionally, ethanol was detected on day 2. Subsequently, a gradual increase in the pH value was recorded from day 20 together with a decrease in the content of the acids. A similar trend was observed in the S363 silo, except that a greater decrease in pH was observed after 2 days of storage (2 units) associated with lactic acid production (0.42 g/L), which decreased at 20 days to fully descend at day 45. Furthermore, ethanol was only detected on day 2 and acetic acid reached a high production at 20 days of storage.

The aerobic stability was also evaluated, showing that all mini-silos were stable for 5 days. Then, in all the silos a decrease in temperature was observed coincident with the room temperature. At the end of the aerobic exposure, the plant material was deteriorated and withered, mainly in the S-control as shown in supplementary Information Fig. SI-2.

Table 2 Spectrum of antifungal action of *Lactoplantibacillus* and *Pediococcus* at free pH

LAB	*Fungus*										
	A. niger CH1	*A. niger* CH2	*A. niger* CH3	*A. japonicus* CH5	*Penicillium sp.* CH6	*Pen. roqueforti* CH4	*Pen. digitatum* INTA2	*C. tropicalis*	*S. cerevisiae*	*Pi. anómala*	*M. pulcherrima*
L. plantarum CRL 46	+++	−	++	++	+++	+++	+++	−	−	−	−
L.plantarum CRL 60	+++	−	++	++	+++	+++	+++	−	−	−	−
L.plantarum CRL 80	++	−	++	++	+++	+++	+++	−	−	−	−
L.plantarum CRL 92	−	−	−	++	−	−	+++	−	−	−	−
L.plantarum CRL 237	++	++	++	++	+++	+++	+++	−	−	−	−
L.plantarum CRL 363	+++	++	+++	+++	+++	+++	+++	−	−	++	+++
L. plantarum CRL 510	+++	+++	++	++	+++	+++	+++	−	−	−	−
L.plantarum CRL 685	−	−	−	−	+++	−	+++	−	−	−	−
L.plantarum CRL 794	++	−	−	++	+++	++	+++	−	−	−	−
L.plantarum CRL 795	++	−	−	++	+++	++	+++	−	−	−	−
L.plantarum CRL 936	++	−	−	++	+++	−	+++	−	−	−	−
L.plantarum CRL 972	+++	+++	−	++	+++	−	+++	−	−	−	−
L.plantarum CRL 1045	++	+++	++	++	+++	−	+++	−	−	−	−
L.plantarum CRL 1089	−	−	−	++	+++	−	+++	−	−	−	−
P.pentasaceus CRL 766	−	−	−	−	+++	+++	+++	−	−	−	−
P.pentosaceus CRL 768	−	−	−	−	+++	+++	+++	−	−	−	−
P.pentosaceus CRL 771	−	−	−	−	+++	++	+++	−	−	−	−
P.pentosaceus CRL 773	++	−	−	++	+++	++	+++	−	−	−	−
P. acidilactici CRL 910	−	−	−	+++	+++	−	+++	−	−	−	−
P. acidilactici CRL 922	++	−	−	++	+++	−	+++	−	−	−	−
P. acidilactici CRL 913	−	−	−	−	+++	−	+++	−	−	−	−
P. acidilactici CRL 914	−	−	−	−	+++	−	+++	−	−	−	−
P. acidilactici CRL 919	−	−	−	−	+++	−	+++	−	−	−	−
P. acidilactici CRL 940	−	−	−	−	+++	−	+++	−	−	−	−

Negative − (absence of zone of inhibition), positive ++ (zone of inhibition defined on LAB colony), strongly positive +++ (absence of fungal growth in 100% of well)

Table 3 Spectrum of antifungal action of *Lactoplantibacillus* and *Pediococcus* at neutral pH

LAB	*Fungus*										
	A. niger CH1	*A. niger*CH2	*A. niger*CH3	*A. japonicus* CH5	*Penicillium sp.* CH6	*Pen. roqueforti* CH4	*Pen. digitatum* INTA2	*C. tropicalis*	*S. cerevisiae*	*Pi. anómala*	*M. pulcherrima*
L. plantarum CRL 46	–	ND	–	–	++	+++	+++	ND	ND	ND	ND
L. plantarum CRL 60	–	ND	–	–	++	–	+++	ND	ND	ND	ND
L. plantarum CRL 80	–	ND	–	–	++	+++	+++	ND	ND	ND	ND
L. plantarum CRL 92	ND	ND	ND	–	ND	ND	++	ND	ND	ND	ND
L. plantarum CRL 237	–	–	–	–	++	–	++	ND	ND	ND	ND
L. plantarum CRL 363	–	–	–	–	+++	++	++	ND	ND	–	–
L. plantarum CRL 510	–	–	–	–	+++	+++	++	ND	ND	ND	ND
L. plantarum CRL 685	ND	ND	ND	ND	–	ND	–	ND	ND	ND	ND
L. plantarum CRL 794	–	ND	ND	–	+++	++	+++	ND	ND	ND	ND
L. plantarum CRL 795	–	ND	ND	–	++	+++	++	ND	ND	ND	ND
L. plantarum CRL 936	–	ND	ND	–	–	ND	–	ND	ND	ND	ND
L. plantarum CRL 972	–	–	ND	–	–	ND	–	ND	ND	ND	ND
L. plantarum CRL 1045	–	–	–	–	–	ND	–	ND	ND	ND	ND
L. plantarum CRL 1089	ND	ND	ND	–	–	ND	–	ND	ND	ND	ND
P. pentasaceus CRL 766	ND	ND	ND	ND	–	–	–	ND	ND	ND	ND
P. pentosaceus CRL 768	ND	ND	ND	ND	–	–	–	ND	ND	ND	ND
P. pentosaceus CRL 771	ND	ND	ND	ND	–	–	–	ND	ND	ND	ND
P. pentosaceus CRL 773	–	ND	ND	–	–	–	–	ND	ND	ND	ND
P. acidilactici CRL 910	ND	ND	ND	–	–	ND	–	ND	ND	ND	ND
P. acidilactici CRL 922	–	ND	ND	–	++	ND	–	ND	ND	ND	ND
P. acidilactici CRL 913	ND	ND	ND	ND	++	ND	–	ND	ND	ND	ND
P. acidilactici CRL 914	ND	ND	ND	ND	–	ND	–	ND	ND	ND	ND

Negative – (absence of zone of inhibition), positive + + (zone of inhibition defined on LAB colony), strongly positive +++ (absence of fungal growth in 100% of well). ND: not determined

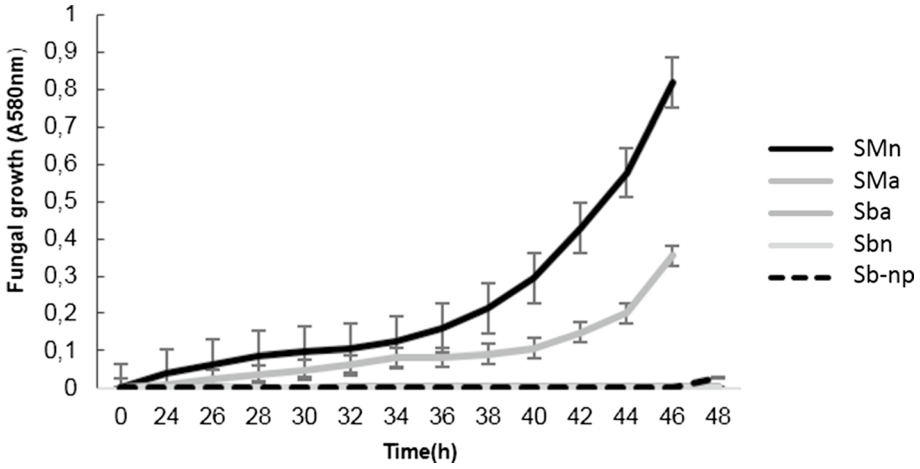

Fig. 2 Growth curves of *Pen. digitatum* INTA2 in culture supernatants of *L. plantarum* CRL 363 in acidic (SbA), neutral (SbN), and neutral conditions treated with pepsin (Sb-NP)

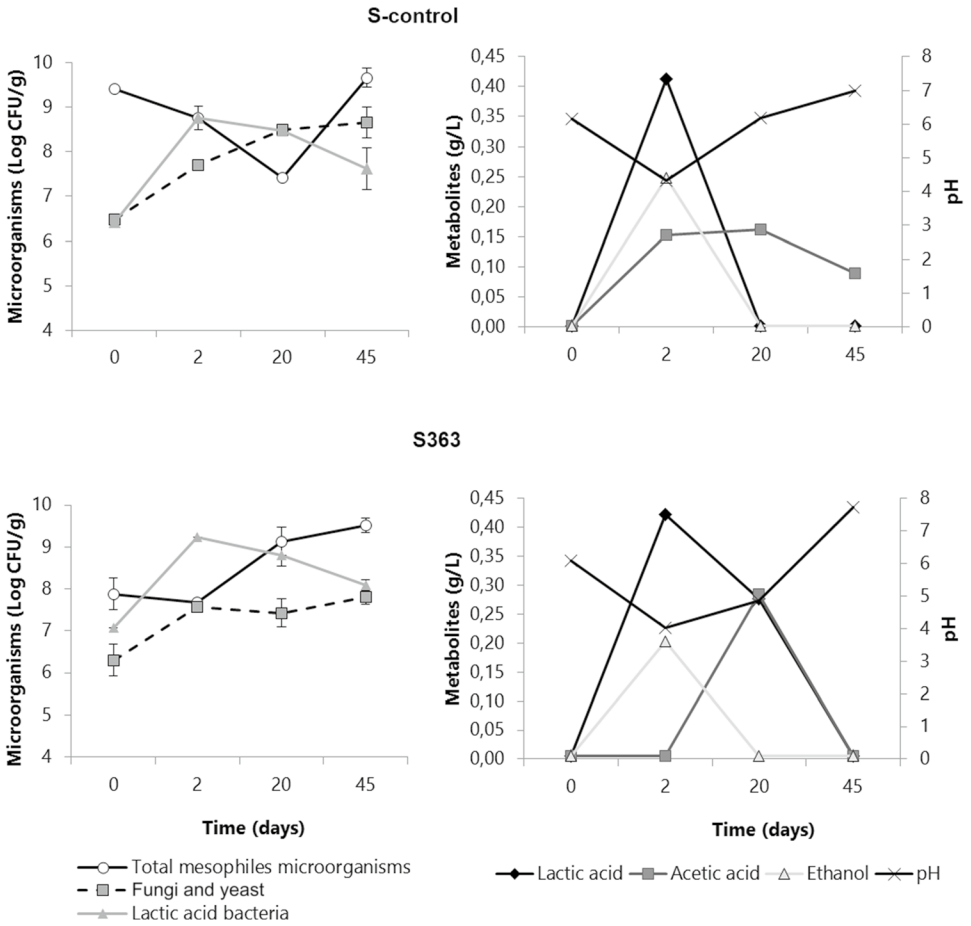

Fig. 3 Chemical and microbiological analysis of inoculated and uninoculated silages

4 Discussion

The application of LAB as bio-inoculants is the most widely used form of silos preservation today. Different LAB were tested to achieve this goal, showing a strong variability in the results, probably due by the great microbiological diversity of the forage and the different experimental conditions of the silos [4, 7, 18]. Inoculant source, acidifying activity, tolerance to technological conditions (temperature, spray drying, lyophilization, among others), are the main criteria reported for the selection of LAB inoculants. However, only a few studies explored the ability of LAB strains to inhibit the growth of fungi frequently isolated from silos [23].

Therefore, the present work was designed to screen LAB based on their acidifying capacity and antifungal activity to be proposed as potential corn silage inoculants.

The rapid decrease in pH is a desirable behavior to avoid the increase in undesirable microorganisms in the silage [24]. The decline in pH reflects the concentration of lactic acid, main product of the fermentation. Our trials showed that the 32 *Pediococcus* strains tested had acidification activity, while only 39 strains of the 114 *L. plantarum* strains tested did not grow in the culture after 24 h. It was observed that *L. plantarum* strains showed the highest acidifying activity. In this sense, it is important to highlight that *L. plantarum* strains have a high biosynthetic capacity and a great adaptation to plant substrates as has been reported by different authors [25]. This great metabolic capacity may be related to the size of its genome, 50% larger than the majority of LAB, and also to the higher acidification rate observed in some *L. plantarum* strains [26]. Nonetheless, some evidence suggest that pediococci initiate the fermentation process in silos, since the high initial pH is not optimal for lactobacilli growth [27].

The bio-control activity of LAB strains was evaluated against the most prevalent fungi present in silage in our country [28]. In this study, 146 LAB strains were screened for antifungal activity against yeasts and filamentous fungi, the most common contaminants in silos. Results obtained evidenced that the antifungal ability was dependent on the LAB strain and the fungus species. All LAB strains (in vitro) were able to reduce or inhibit at least one of the fungal strains used as indicators, being lactobacilli the most effective. Regarding the sensitivity, filamentous fungi were more sensitive than yeasts. In general, *Aspergillus* were the more resistant strains, however, many tested lactobacilli, showed significant antifungal activity against them. Previous works showed that some LAB and also *Bifidobacterium* strains were less effective as antagonistic agents of *Aspergillus* strains. In fact, Dogi et al. [23] reported that *Lacticaseibacillus rhamnosus* and *L. plantarum* reduced fungal growth rate of *A. parasiticus* only at certain water activity conditions. In a similar way, three *Lactobacillus acidophilus* strains and two *Bifidobacterium* strains were not effective in preventing the growth of this *Aspergillus* strain [29]. In a same line, a recent work performed by Ben Taheur et al. [30], showed that none of the four LAB tested were able to completely inhibit the fungal growth of *A. flavus* and *A. carbonarius*.

Nevertheless, only *L. plantarum* CRL 363 was able to inhibit *Pi. anomalous* and *M. pulcherrima* growth. The resistance of yeasts to the inhibitory action of LAB was previously reported by Lipinska et al. [31] since none of the 60 *Lactobacillus* sp. (60) strains tested could significantly inhibited the growth of *C. vini*.

The inhibitory activity of the LAB on spores germination was removed after neutralization indicating the acidic nature of the metabolites involved. In fact, organic acids were identified and quantified in the LAB-positive supernatant (Table 1). Therefore, the antifungal effect of LAB could be related to both natures of the organic acid produced—lactic and acetic- and the low pH (3.5–4.0) reached after fermentation. These organic acids have been described as an antimicrobial agent with broad-spectrum activity against bacterial and fungal pathogens [32]. The acetic and lactic acid can inhibit the growth of yeasts and filamentous fungi, improving aerobic stability [3, 33]. The production of PLA would also contribute to the antifungal activity of some strains. Antifungal activity of PLA was effective against several species isolated from bakery products, including some mycotoxigenic strains [20, 34].

Organic acid production may be the main cause of growth inhibition of filamentous fungi and yeasts, however, other active antifungal metabolites have been reported (palmitic acid, heptadecanoic acid, esters, proteinaceous compounds or low-molecular weight peptides) [10, 13, 35]. Antifungal peptides have been widely studied previously [20, 35–41], showing that most of the peptides reported are derived from hydrolysis of the media components in the presence of LAB and, also, LAB can produce peptides as their secondary metabolites under certain conditions. In this study, only three LAB strains showed inhibitory effect in neutral conditions which could match the presence of an antifungal peptide. This metabolite was not sensitive to pepsin, therefore more studies will be necessary to unravel its nature.

For a successful application of inoculants, the silo matrix needs to be compatible with the strains used. This compatibility is reflected by the ability of the microorganisms to use carbohydrates present in the forage and to produce metabolites of interest such as organic acids. Based on the previous results, silage was made with fresh-cut maize using a small-scale system of fermentation with and without (S- control) the addition of the CRL 363 strain, selected for its greater spectrum of antifungal activity. In this sense, a meta-analysis performed by Blajman et al. [7], showed that from a total of 276 studies assessing the effects of using LAB as inoculants on corn silage, 140 used *L. plantarum* (alone or combined with other LAB), being the second most used strain after *Lactobacillus buchneri*.

In relation to the appearance of the silos, it was observed that after 20 days, the addition of the lactic strain as an additive (S363) allowed them to maintain a better color, texture and odor than the control silo. These results may be related to the lower yeast and fungi count in the inoculated silo (1 logarithmic unit lower than in the control silo), which in turn could be due to the higher concentration of organic acids detected. Regarding LAB, the initial count was higher in the inoculated silo, reaching the maximum value on day 2 for both assays, and decreasing gradually until the end of the storage. This trend was also reported by other authors who

observed a peak of LAB at day 7 followed by a decrease in cell count in maize stover silos with and without molasses [42]. Similar behaviors were reported by Burns et al. [8] for a maize silage inoculated with three LAB strains. Besides, after 2 days of fermentation the pH of both silos significantly decreased from 6 to 4, related to the peak of lactic acid concentration in both cases, indicating that natural fermentation had occurred. This pH values are in the range of previously reported values for maize silages (3.7–4.0) since it is a crop that has low buffering capacity [8].

At day 45, both silos showed signs of pronounced wilt with characteristics of global deterioration, changes that were most evident in the control silo. Coincidentally, in both silos high levels of total mesophilic bacteria, yeasts and fungi counts were detected, as well as a decrease in the concentration of LAB. Unusually, no organic acids were detected in both silos, with the exception of a little amount of acetic acid in the control silo, probably produced by the coliform bacteria present in forage. Besides, an increase in pH was observed in this storage time in both silos which could be related to high ammonia nitrogen values. On the contrary, most of the available literature show low pH values for inoculated silos [8, 14]. The addition of molasses or other carbon source is a reported strategy to increase carbohydrate content and lactic acid production [43, 44].

Finally, control and inoculated mini-silos showed stability for 5 days and then a decrease in temperature was observed together with the room temperature, which could indicate poor thermal insulation of the system. Additionally, a high concentration of yeasts was registered in the silos, which can be acid tolerant and lactate assimilators and therefore consume carbohydrates and acids produced during fermentation [5, 6]. This generates an increase in the temperature and pH of the ensiled material and also causes spoilage of the silo as observed in our study. On the contrary, there were other authors that reported high stability of silos inoculated with LAB [8], suggesting that the behavior mentioned before is strain dependent.

5 Conclusions

Bacterial inoculants promote fast and efficient fermentation of ensiled materials, which increases the quality and quantity of the final product. These additives have some advantages over other types of additives such as their low cost, its safe handling and its low application rate, as well as the fact that it does not pollute the environment. According to our in vitro and in vivo assays, *L. plantarum* CRL 363 could be used as a bioinoculant for silos with the addition of an adequate concentration of soluble carbohydrates to improve the lactic acid production. In this way it would be possible to maintain a low pH and the silage would be better preserved throughout the storage period.

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Data Availability Experimental data are available from the authors upon request.

Declaration

Conflicts of interest The authors declare that they have no competing interests.

References

1. Ogunade, I.M.; Martinez-Tuppia, C.; Queiroz, O.C.M.; Jiang, Y.; Drouin, P.; Wu, F.; Vyas, D.; Adesogan, A.T.: Silage review: Mycotoxins in silage: occurrence, effects, prevention, and mitigation. J. Dairy Sci. **101**, 4034–4059 (2018). https://doi.org/10.3168/jds.2017-13788
2. Casemiro, L.A.; Martins, C.H.; De Souza, F.D.C.P.; Panzeri, H.; Ito, I.Y.: Bacterial, fungal and yeast contamination in six brands of irreversible hydrocolloid impression materials. Braz. Oral Res. **21**(2), 106–111 (2007). https://doi.org/10.1590/S1806-83242007000200003
3. Muck, R.E.; Nadeau, E.M.G.; McAllister, T.A.; Contreras-Govea, F.E.; Santos, M.C.; Kung, L., Jr.: Silage review: recent advances and future uses of silage additives. J. Dairy Sci. **101**, 3980–4000 (2018). https://doi.org/10.3168/jds.2017-13839
4. Ferrero, F.; Prencipe, S.; Spadaro, D.; Lodovica Gullino, M.; Cavallarin, L.; Piano, S.; Tabacco, E.; Borreani, G.: Increase in aflatoxins due to *Aspergillus* section *Flavi* multiplication during the aerobic deterioration of corn silage treated with different bacteria inoculate. J. Dairy Sci. **102**, 1176–1193 (2019). https://doi.org/10.3168/jds.2018-15468
5. Kung, L., Jr.; Shaver, R.D.; Grant, R.J.; Schmidt, R.J.: Silage review: interpretation of chemical, microbial, and organoleptic components of silages. J. Dairy Sci. **101**, 4020–4033 (2018). https://doi.org/10.3168/jds.2017-13909
6. Borreani, G.; Tabacco, E.; Schmidt, R.J.; Holmes, B.J.; Muck, R.E.: Silage review: factors affecting dry matter and quality losses in silages. J. Dairy Sci. **101**, 3952–3979 (2018). https://doi.org/10.3168/jds.2017-13837
7. Blajman, J.E.; Paez, R.B.; Vinderola, C.G.; Lingua, M.S.; Signorini, M.L.: A meta-analysis on the effectiveness of homofermentative and heterofermentative lactic acid bacteria for corn silage. J. Appl. Microbiol. **125**(6), 1655–1669 (2018). https://doi.org/10.1111/jam.14084

8. Burns, P.; Borgo, M.F.; Binetti, A.; Puntillo, M.; Bergamini, C.; Páez, R.; Vinderola, G.: Isolation, characterization and performance of autochthonous spray dried lactic acid bacteria in maize micro and bucket-silos. Front. Microbiol. **9**, 2861 (2018). https://doi.org/10.3389/fmicb.2018.02861
9. Oliveir, A.S.; Weinberg, Z.G.; Ogunade, I.M.; Cervantes, A.A.P.; Arriola, K.G.; Jiang, Y.; Kim, D.; Li, X.; Gonçalves, M.C.M.; Vyas, D.; Adesogan, A.T.: Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. J. Dairy Sci. **100**, 4587–4603 (2017). https://doi.org/10.3168/jds.2016-11815
10. Gerez, C.L.; Torino, M.I.; Rollán, G.; Font de Valdez, G.: Prevention of bread fungi spoilage by using lactic acid bacteria with antifungal properties. Food Control **20**, 144–148 (2009). https://doi.org/10.1016/j.foodcont.2008.03.005
11. Guimarães, A.; Venancio, A.; Abrunhosa, L.: Antifungal effect of organic acids from lactic acid bacteria on *Penicillium nordicum*. Food addit. Contam. **35**(9), 1803–1818 (2018)
12. Gupta, R.; Srivastava, S.: Antifungal effect of antimicrobial peptides (AMPs LR14) derived from *Lactobacillus plantarum* strain LR/14 and their applications in prevention of grain spoilage. Food Microbiol. **42**, 1–7 (2014). https://doi.org/10.1016/j.fm.2014.02.005
13. Deepthi, B.V.; Poornachandra Rao, K.; Chennapa, G.; Naik, M.K.; Chandrashekara, K.T.; Sreenivasa, M.Y.: Antifungal attributes of *Lactobacillus plantarum* MYS6 against fumonisin producing *Fusarium proliferatum* associated with poultry feeds. PLoS ONE **11**(6), e0155122 (2016). https://doi.org/10.1371/journal.pone.0155122
14. Dogi, C.A.; Pellegrino, M.; Poloni, V.; Poloni, L.; Pereyra, C.M.; Sanabria, A.; Pianzzola, M.J.; Dalcero, A.; Cavaglieri, L.: Efficacy of corn silage inoculants on the fermentation quality under farm conditions and their influence on Aspergillus parasitucus, A. flavus and A. fumigatus determined by q-PCR. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. **32**(2), 229–235 (2015). https://doi.org/10.1080/19440049.2014.986223
15. Li, K.; Fu, S.; Zhan, H.; Zhan, Y.; Lucia, L.: Analysis of the chemical composition and morphological structure of banana pseudostem. BioResources **5**, 576–585 (2010)
16. Carvalho, B.F.; Ávila, C.L.S.; Bernardes, T.F.; Pereira, M.N.; Santos, C.; Schwan, R.F.: Fermentation profile and identification of lactic acid bacteria and yeasts of rehydrated corn kernel silage. J. Appl. Microbiol. **122**(3), 589–600 (2017). https://doi.org/10.1111/jam.13371
17. Xu, Z.; Zhang, S.; Zhang, R.; Li, S.; Kong, J.: The changes in dominant lactic acid bacteria and their metabolites during corn stover ensiling. J. Appl. Microbiol. **125**(3), 675–685 (2018). https://doi.org/10.1111/jam.139144
18. Da Silva, N.C.; Nascimento, C.F.; Nascimento, F.A.; De Resende, F.D.; Daniel, J.L.P.; Siqueira, G.R.: Fermentation and aerobic stability of rehydrated corn grain silage treated with different doses of *Lactobacillus buchneri* or a combination of *Lactobacillus plantarum* and *Pediococcus acidilactici*. J. Dairy Sci. **101**(5), 4158–4167 (2018). https://doi.org/10.3168/jds.2017-13797
19. De Man, J.C.; Rogosa, M.; Sharpe, E.M.: A medium for the cultivation of lactobacilli. J. Appl. Microbiol. **23**, 130–135 (1960). https://doi.org/10.1111/j.1365-2672.1960.tb00188.x
20. Gerez, C.L.; Torres, M.J.; Valdez, G.; Rollán, G.: Control of spoilage fungi by lactic acid bacteria. Biol. Control **64**, 231–237 (2013). https://doi.org/10.1016/j.biocontrol.2012.10.009
21. Inglin, R.C.; Stevens, M.J.; Meile, L.; Lacroix, C.; Meile, L.: High-throughput screening assays for antibacterial and antifungal activities of *Lactobacillus* species. J. Microbiol. Methods **114**, 26–29 (2015). https://doi.org/10.1016/j.mimet.2015.04.011
22. Lavermicocca, P.; Valerio, F.; Visconti, A.: Antifungal activity of phenyllactic acid against molds isolated from bakery products. Appl. Environ. Microbiol. **69**(1), 634–640 (2003). https://doi.org/10.1128/AEM.69.1.634-640.2003
23. Dogi, C.A.; Fochesato, A.; Armando, R.; Pribull, B.; De Souza, M.M.; Coelho, I.D.S.; Araújo, D.M.; Dalcero, A.; Cavaglieri, L.: Selection of lactic acid bacteria to promote an efficient silage fermentation capable of inhibiting the activity of *Aspergillus parasiticus* and *Fusarium graminearum* and mycotoxin production. J. Appl. Microbiol. **114**(6), 1650–1660 (2013). https://doi.org/10.1111/jam.12173
24. Muck, R.E.; Filya, I.; Contreras-Govea, F.E.: Inoculant effects on alfalfa silage: in vitro gas and volatile fatty acid production. J. Dairy Sci. **90**(11), 5115–5125 (2007). https://doi.org/10.3168/jds.2006-878
25. Georgieva, R.N.; Iliev, I.N.; Chipeva, V.A.; Dimitonova, S.P.; Samelis, J.; Danova, S.T.: Identification and in vitro characterization of *Lactobacillus plantarum* strains from artisanal Bulgarian white brined cheeses. J. Basic Microbiol. **48**(4), 234–244 (2008). https://doi.org/10.1002/jobm.2007003555
26. Vázquez Aguilar M.M.: Viabilidad y propiedades fisicoquímicas de leche fermentada probiótica. Maestría en Ciencia de Alimentos. Departamento de Ingeniería Química y Alimentos. Escuela de Ingeniería y Ciencias, Universidad de las Américas Puebla, Mexico. Thesis (2008). http://catarina.udlap.mx/u_dl_a/tales/documentos/mca/vazquez_a_mm/
27. Muck, R.E.: Recent advances in silage microbiology. Agric. Food Sci. **22**, 3–15 (2013). https://doi.org/10.23986/afsci.6718
28. Pereyra, M.G.; Alonso, V.A.; Sager, R.; Morlaco, M.B.; Magnoli, C.E.; Astoreca, A.L.; Cavaglieri, L.R.: Fungi and selected mycotoxins from pre-and post-fermented corn silage. J. Appl. Microbiol. **104**(4), 1034–1041 (2008). https://doi.org/10.1111/j.1365-2672.2007.03634.x
29. Kabak, B.; Var, I.: The effect of Lactobacillus and *Bifidobacterium* strains on the growth and AFB 1 production of *Aspergillus flavus*. Acta Aliment. **33**, 371–376 (2004). https://doi.org/10.1556/AAlim.33.2004.4.7
30. Ben Taheur, F.; Mansour, C.; Kouidhi, B.; Chaieb, K.: Use of lactic acid bacteria for the inhibition of *Aspergillus flavus* and *Aspergillus carbonarius* growth and mycotoxin production. Toxicon **166**, 15–23 (2019). https://doi.org/10.1016/j.toxicon.2019.05.004
31. Lipińska, L.; Klewicki, R.; Klewicka, E.; Kołodziejczyk, K.; Sójka, M.; Nowak, A.: Antifungal activity of lactobacillus sp. bacteria in the presence of xylitol and galactosyl-xylitol. BioMed Res. Int. (2016). https://doi.org/10.1155/2016/5897486
32. Zhang, Q.; Li, X.J.; Zhao, M.M.; Yu, Z.: Isolating and evaluating lactic acid bacteria strains for effectiveness of *Leymus chinensis* silage fermentation. Lett. Appl. Microbiol. **59**(4), 391–397 (2014). https://doi.org/10.1111/lam.12291
33. Ávila, C.L.S.; Carvalho, B.F.; Pinto, J.C.; Duarte, W.F.; Schwan, R.F.: The use of Lactobacillus species as starter cultures for enhancing the quality of sugar cane silage. Int. J. Dairy Sci **97**(2), 940–951 (2014)
34. Valerio, F.; Di Biase, M.; Lattanzio, V.M.; Lavermicocca, P.: Improvement of the antifungal activity of lactic acid bacteria by addition to the growth medium of phenylpyruvic acid, a precursor of phenyllactic acid. Int. J. Food Microbiol. **222**, 1–7 (2016). https://doi.org/10.1016/j.ijfoodmicro.2016.01.011
35. Ström, K.; Sjögren, J.; Broberg, A.; Schnürer, J.: *Lactobacillus plantarum* MiLAB 393 produces the antifungal cyclic dipeptides cyclo (L-Phe-L-Pro) and cyclo (L-Phe-trans-4-OH-L-Pro) and 3-phenyllactic acid. Appl. Environ. Microbiol. **68**(9), 4322–4327 (2002). https://doi.org/10.1128/AEM.68.9.4322-4327.2002
36. Magnusson, J.; Ström, K.; Roos, S.; Sjögren, J.; Schnürer, J.: Broad and complex antifungal activity among environmental

isolates of lactic acid bacteria. FEMS Microbiol. Lett. **219**, 129–135 (2003). https://doi.org/10.1016/S0378-1097(02)01207-7

37. Coda, R.; Cassone, A.; Rizzello, C.G.; Nionelli, L.; Cardinali, G.; Gobbetti, M.: Antifungal activity of *Wickerhamomyces anomalus* and *Lactobacillus plantarum* during sourdough fermentation: identification of novel compounds and long-term effect during storage of wheat bread. Appl. Environ. Microbiol. **77**, 3484–3492 (2011). https://doi.org/10.1128/AEM.02669-10
38. Rizzello, C.; Cassone, A.; Coda, R.; Gobbetti, M.: Antifungal activity of sourdough fermented wheat germ used as an ingredient for bread making. Food Chem. **127**, 952–959 (2011). https://doi.org/10.1016/j.foodchem.2011.01.063
39. Li, H.; Liu, L.; Zhang, S.; Cui, W.; Lv, J.: Identification of antifungal compounds produced by *Lactobacillus casei* AST18. Curr. Microbiol. **65**, 156–161 (2012). https://doi.org/10.1007/s00284-012-0135-2
40. Garofalo, C.; Zannini, E.; Aquilanti, L.; Silvestri, G.; Fierro, O.; Picariello, G.; Clementi, F.: Selection of sourdough lactobacilli with antifungal activity for use as biopreservatives in bakery products. J. Agric. Food Chem. **60**(31), 7719–7728 (2012). https://doi.org/10.1021/jf301173u
41. Muhialdin, B.J.; Hassan, Z.; Abu Bakar, F.; Algboory, H.L.; Saari, N.: Novel antifungal peptides produced by *Leuconostoc mesenteroides* DU15 effectively inhibit growth of *Aspergillus niger*. J. Food Sci. **80**(5), M1026–M1030 (2015). https://doi.org/10.1111/1750-3841.12844
42. Mitiku, A.A.; Andeta, A.F.; Borremans, A.; Lievens, B.; Bossaert, S.; Crauwels, S.; Van Campenhout, L.: Silage making of maize stover and banana pseudostem under South Ethiopian conditions: evolution of pH, dry matter and microbiological profile. Microb. Biotechnol. **13**(5), 1477–1488 (2020). https://doi.org/10.1111/1751-7915.13626
43. Liu, B.; Yang, Z.; Huan, H.; Gu, H.; Xu, N.; Ding, C.: Impact of molasses and microbial inoculants on fermentation quality, aerobic stability, and bacterial and fungal microbiomes of barley silage. Sci. rep. **10**(1), 1–10 (2020)
44. Chen, S.W.; Chang, Y.Y.; Huang, H.Y.; Kuo, S.M.; Wang, H.T.: Application of condensed molasses fermentation solubles and lactic acid bacteria in corn silage production. Agric. Food Sci. **100**(6), 2722–2731 (2020)