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Control of spoilage fungi by lactic acid bacteria

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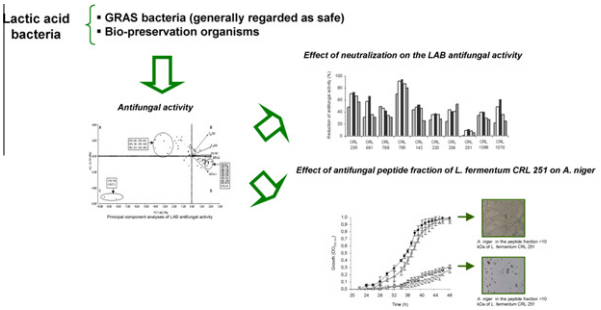
HIGHLIGHTS

- Ten LAB strains were selected from a total of 91 due to their high antifungal effect.
- Lactic, acetic and phenyllactic acids (PLA) were responsible for antifungal activity.
- This is the first report on antifungal peptide/s produced by *Lactobacillus fermentum*.
- This peptide/s was thermostable, <10 kDa, active at pH 4–7 and sensitive to trypsin.

GRAPHICAL ABSTRACT



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ABSTRACT

The evaluation of the potentiality of lactic acid bacteria (LAB) strains isolated from different origins to inhibit mould growth and to identify and characterize the antifungal metabolites were the aims of this study. From a total of ninety-one LAB strains tested, ten were selected due to their high inhibitory effect (>80%). The antifungal activity of the majority of the selected LAB strains was lost after the neutralization treatment determining the acidic nature of the antifungal metabolites. Lactic, acetic and phenyllactic (PLA) acids were identified as being responsible for antifungal effect in the 10 cell-free supernatants (CFS) evaluated. Amongst the strains evaluated, only *Lactobacillus fermentum* CRL 251 produced fungus inhibitory peptide/s, smaller than 10 kDa, thermostable, active in the pH range of 4–7 and sensitive to trypsin. This is the first report on antifungal peptide/s produced by a *L. fermentum* strain.

1. Introduction

Fungi are robust organisms capable of growing on all kinds of foods, including cereals, meats and fruits. They are important spoilage organisms in different foods causing significant economic losses in the industry. Several strategies have been used, to extend the shelf life of vegetables and food such as heat treatments, irradiating the goods with infrared rays or microwaves, using modified atmospheres during packaging, or by adding chemical preservatives (such as sorbic, benzoic, and propionic acids) (Gould, 1996). However, some fungi are able to adapt to the presence of certain preservatives (Brul and Coote, 1999; Hesse et al., 2002; Viljoen, 2001). In addition, consumers are increasingly demanding higher quality vegetable products and foods that are free of chemical pesticides with extended shelf life. This last feature is an important aspect to consider when discussing the need for new preservation methods to inhibit the growth of undesirable contaminating fungi.

The bio-preservation or the use of microorganisms and/or their metabolites to prevent spoilage and to extend the shelf life of foods has gained the interest of producers due to consumers' demands (Stiles, 1996). Lactic acid bacteria (LAB) have been used for centuries as bio-preservation organisms in foods preventing the growth of spoilage microorganisms through the production of lactic acid; moreover, LAB are able to produce different kinds of bioactive molecules, such as organic acids, fatty acids, hydrogen peroxide and bacteriocins. In recent years, considerable effort has been directed

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to harness the antifungal activity of LAB in order to reduce fungal spoilage of foods (Gerez et al., 2010a,b; Mauch et al., 2010; Ryan et al., 2008; Valerio et al., 2009; Voulgari et al., 2010). Various screenings have been undertaken aimed at identifying LAB with antifungal properties isolated from different sources e.g. cereals (Ndagano et al., 2011; Rouse et al., 2008), sourdough (Coda et al., 2011; Corsetti et al., 1998; Gerez et al., 2009; Lavermicocca et al., 2000), vegetables (Gerez et al., 2010b; Magnusson and Schnürer, 2001) and dairy food (Schwenninger et al., 2005; Voulgari et al., 2010). Antifungal compounds produced by LAB that have been characterized include organic acids such as phenyllactic acid (Gerez et al., 2009; Lavermicocca et al., 2000), proteinaceous compounds (Magnusson and Schnürer, 2001; Rouse et al., 2008), reuterin (Chung et al., 1989), cyclic dipeptides (Ström et al., 2002; Yang and Chang, 2010), and fatty acids (Sjogren et al., 2003).

Certain authors have shown that only a limited number of fungal genera and species are able to grow and contaminate a determined type of food (Dijksterhuis and Samson, 2002; Filtenborg et al., 1996), e.g. the most common spoilage fungi of cereal-based products belong to the genera *Penicillium* (*P*), *Aspergillus* (*A.*), *Fusarium* (*F.*) (Keshri et al., 2002); while *Penicillium digitatum* and *Penicillium italicum* are the main postharvest pathogens agents in citrus fruits (Fogliata et al., 2000). In a previous work, we selected antifungal LAB strains able to inhibiting the growth of fungal contaminants of bread or citrus. Four LAB strains isolated from sourdough (*Lactobacillus plantarum* CRL 778, *Lactobacillus reuteri* CRL 1100, *Lactobacillus brevis* CRL 772, and *L. brevis* CRL 796) were able to inhibit the mycelial growth of spoilage molds found in small bakeries (Gerez et al., 2009). More recently, a ready-to-use antifungal starter formulated with *L. plantarum* CRL 778 significantly extended the shelf life of packaged bread (Gerez et al., 2010a). In addition, the effect of LAB on postharvest pathogenic fungi of lemon was determined using the Well Diffusion Agar method and the effectiveness of the metabolites involved was also evaluated (Gerez et al., 2010b).

The aim of this present study was to select efficient antifungal LAB strains isolated from different sources, able to inhibit contaminant fungi of bread and citrus using the Microtitre Plate Well Assay and to identify and characterize the antifungal metabolites.

2. Materials and methods

2.1. Microorganisms and culture conditions

LAB strains (91) isolated from different sources and belonging to the Culture Collection of the Centro de Referencia para Lactobacilos (CRL) (CERELA-CONICET), Tucumán, Argentina, were used (Table 1). The mould strains used in this study were *Aspergillus* (*A.*) *niger* CH 101, *Penicillium* (*P.*) sp. CH 102 and *Fusarium* (*F.*) *graminearum* CH 103, previously isolated from contaminated bread; and *P. digitatum* INTA2 and *Geotrichum* (*G.*) *citri-aurantii* INTA1, isolated from a commercial citrus fruit packing industry of National Agricultural Institute (INTA) Famaillá, Tucumán, Argentina. All fungal strains were used as indicators in the bioassay determinations.

LAB cultures were grown in MRS (De Man et al., 1960) broth (pH 6.5) at 37 °C for 24 h without agitation. Cell-free supernatants (CFS) obtained by centrifugation at 9000*g* for 10 min at 4 °C (IEC model B-22 M, International Equipment Company, USA) were filtered (0.2 μm-pore-size, Sartorius AG, Goettingen, Germany) and stored at −20 °C until used for antifungal assays.

The fungi strains were grown on Czapek-Dox medium (0.3% $NaNO_3$, 0.1% K_2HPO_4, 0.05% KCl, 0.05% $MgSO_4$, 0.001 FeSO4, 3% sacarose, 0.5% yeast extract 1.5% agar) at 25 °C for 7 days. The conidias were collected in sterile Tween 80 at 0.05% (v v^{-1}) and counted at the microscope in a haemacytometer chamber. Dilutions with collection fluid were used to adjust to different concentrations (10^3–10^6 conidia ml^{-1}).

Table 1
LAB strains evaluated for their antifungal activity.

LAB Strains (CRL)	Source
L. acidophilus 1063, 1064, 1065	Dairy products
L. brevis 376	
L. casei 59, 69, 75, 87, 143, 168, 234, 237, 239, 429, 206, 225, 295, 645	
L. paracasei 717	
L. bulgaricus 142, 406	
L. fermentum 220, 236, 251, 345	
L. plantarum 92, 93, 95, 99, 101, 107, 110, 121, 130, 133, 136, 137, 140,	
182, 183, 358	
L. rhamnosus 186, 201	
Ent. faecium 176	
Ent. mundtii 35	
Lactococcus lactis 1109	
St. thermophilus 414	
L. paracasei 1501	Tomato extract
L. plantarum 1073, 1093	Pea
L. plantarum 681	Sausages
L. curvatus 705	
L. paracasei 686	
L. plantarum 759, 768, 769, 775, 778, 783, 785, 788, 794, 795	Sourdough
L. reuteri 1097, 1098, 1099, 1100	
P. acidilactici 770, 767	
P. pentosaceus 761, 791, 792	
L. curvatus 760	
L. brevis 763, 772, 780, 781, 796	
L. plantarum 725	Bagasse
L. plantarum 353	Ensilage
L. acidophilus 1070	
Pediococcus pentosaceus 908	Cabbage
L. collinoides 1013	Apple juice
L. coryniformis 1001	ATCC 25602
L. paracasei 997	ATCC 25598
L. plantarum 948	ATCC 10241
L. mali 1003	ATCC 27053
Weissella ascens 962	ATCC 12706
L. rhamnosus 932	ATCC 7469
L. rhamnosus 981	ATCC 15820
Leuconostoc mesenteroides 742	NCDO 523

2.2. Antifungal assay

The antifungal activity of the CFS from the different LAB strains was determined by Microtitre Plate Well Assay (Lavermicocca et al., 2003) as described below. Conidial suspensions (10 μl) containing 10^4 conidia per ml were added to 190 μl of the CFS of each LAB strain. The assays were performed in sterile multiwell microdilution plates (96 sterile wells) (Corning Incorporated, USA). Fungal growth was determined by measuring the optical density (OD_{580nm}) at 30 °C after 48 h in a spectrophotometer (VERSAmax, Molecular Devices, USA). Conidia of each strain inoculated in MRS broth were used as control. The antifungal activity of the LAB strains was expressed as the fungal growth inhibition (%) measured as described above. In all trials, the percentages of inhibition [Inhib% = $100 - (\Delta OD_{LAB} \times 100/\Delta OD_{Control})$] was determined.

2.3. Characterization of the antifungal compounds

The LAB CFS (pH 3.5) were subjected to different treatments to determine the nature of the antifungal compounds: exposure to high temperature (100 °C for 10 min), neutralization to pH 7.0 (with 0.1 M NaOH), or subjected to the action of the following enzymes: catalase (Sigma Chemical Company, St. Louis, MO, USA, pH

7.0), trypsin (Sigma, pH 7.6), pepsin A (Sigma, pH 2.0) or proteinase K (Invitrogen, CA USA, pH 7.6). The pH values of the CFS were adjusted to the optimum pH of each enzyme. The mixture was prepared as follows: 100 μg of each enzyme/ml of CFS incubated 1 h at 37 °C (trypsin and pepsin A), or 30 min at 45 °C (proteinase K) or 30 min. at 37 °C (catalase). The antifungal activity of treated CFS was determined by measuring the OD_{580nm} using the microtiter plate assay as described above.

2.4. Organic acids determination

The organic acids present in the 24 h CFS were determined by High Performance Liquid Chromatography (HPLC) and enzymatic methods. Lactic and phenyllactic acids were determined by HPLC (ISCO 2350 model, Nebraska, USA) using an ion-exclusion Aminex 87 H column (300 × 78 mm, Bio-Rad Laboratories, CA, USA) under the following conditions: mobile phase (2.5 mM) H_2SO_4; flow rate 0.6 ml/min; temperature of column 45 °C. An UV (210 nm) detector (ISCO V_4 model) connected to the software (Peak Simple II) for data analyses was used. Acetic acid was determined by a commercial kit (Acetic acid assay, Boehringer Mannheim, Germany).

2.5. Determination of the antifungal peptide/s molecular weight

The separation of the metabolites present in the CFS (48 h) of *Lactobacillus fermentum* CRL 251 and non-fermented MRS medium (control) according to their molecular weight were performed by ultrafiltration using Centricon (10 kDa Ultracel® YM membranes, Millipore) non-sterile filters. The CFS (1.5 ml) was placed in the sample reservoir and centrifuged (IEC Multi RF) at 3500*g* at 10 °C for 2 h to avoid possible inactivation of the biofungicides. The metabolites higher than 10 kDa were retained in the membrane and re-suspended in MRS without acetate. Both fractions, peptides >10 kDa and <10 kDa were sterilized by filtration (0.22 μm) and neutralized to pH 7.0 to eliminate the effect of organic acids. The antifungal activity against the most resistant assayed fungus strain, *A. niger* CH 101, was determined by measuring OD_{580nm} as described previously. The peptides fractions (peptides >10 and <10 kDa) obtained from non-fermented MRS medium were used as control.

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 InfoStat 2006p.3 software. The principal component (PCA) and cluster analysis were applied to the LAB antifungal activity data.

3. Results

3.1. Selection of LAB with antifungal activity

The antifungal activity of the CFS LAB strains was evaluated. To facilitate the analysis and interpretation of the results, the LAB strains were arbitrarily classified, according to their antifungal activity (percentages of inhibition), into 5 levels: 80–100% (Level 1), 60–79% (Level 2), 40–59% (Level 3), 20–39% (Level 4) and 0–19% (Level 5) (Fig. 1). *A. niger* CH 101 was inhibited by 52% of the assayed LAB strains in level 2 and only 18% with activity of level 1. Respect to *Penicillium* genus, 83% of LAB strains inhibited

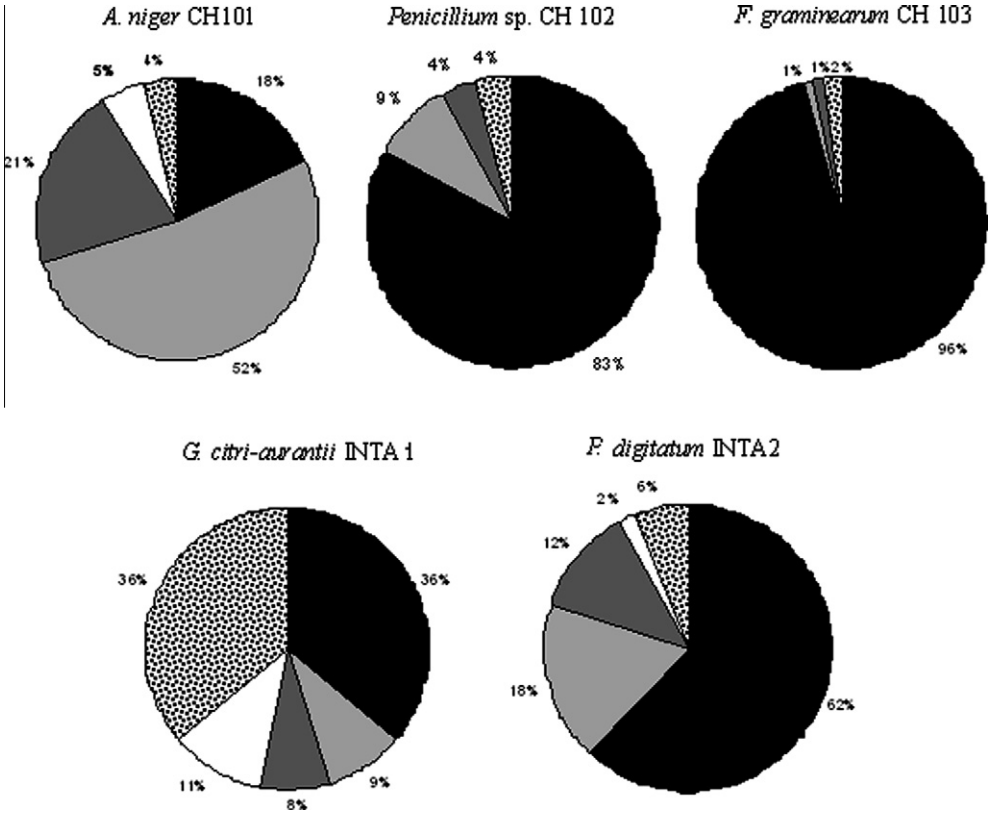

Fig. 1. Classification of LAB strains according to their degree of inhibition on *A. niger* CH101, *Penicillium* sp. CH 102, *F. graminearum* CH 103, *G. citri-aurantii* INTA1 and *P. digitatum* INTA2. The LAB strains were classified according to their antifungal activity (percentages of inhibition), into 5 levels: 80–100% (Level 1,■), 60–79% (Level 2, ▒), 40–59% (Level 3, ▓), 20–39% (Level 4, □) and 0–19% (Level 5, ⁙).

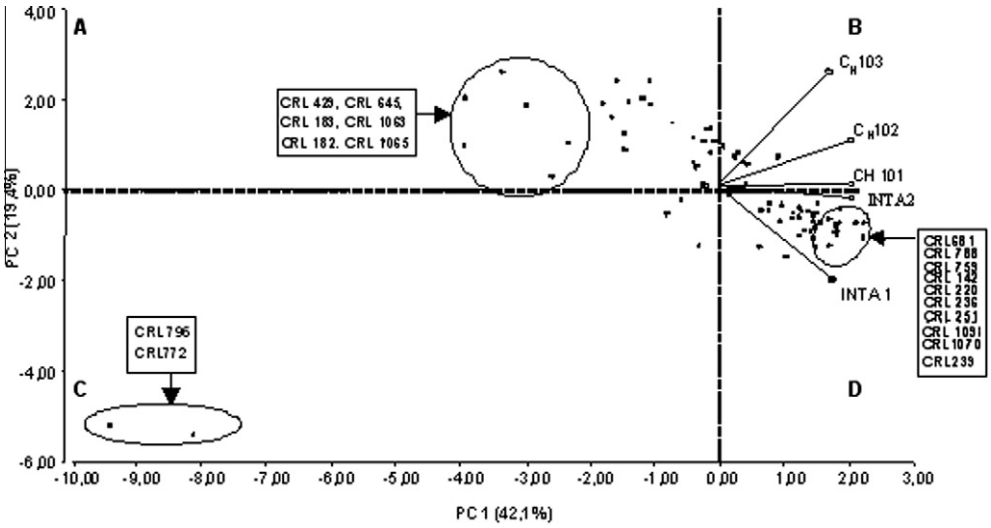

Fig. 2. Principal component analyses of LAB antifungal activity: PC1 vs. PC2.

Penicillium sp. CH 102 while 62% inhibited *P. digitatum* INTA 2 with activity of level 1. The majority (96%) of the assayed LAB strains produced a high inhibition percentage (level 1) of the germination of *F. graminearum* CH 103 spores. The most resistant fungus was *A. niger* CH 101, and the most sensitive was *F. graminearum* CH 103; while *P. digitatum* INTA 2 and *Penicillium* sp. CH 102 showed intermediate sensitivity to the inhibitory effect by LAB.

To compare the antifungal profile of LAB strains, data were analyzed by the PCA method (Fig. 2). The LAB strains, represented as dots in the figure, are disposed in different quadrants according to their inhibitory activity on the fungi strains. Distances between dots indicate differences in their antifungal profiles. Principal component 1 and 2 explained 61% of the observed variability of the results. The strains *L. casei* CRL 645 and CRL 429, *L. plantarum* CRL 182 and CRL 183, *L. acidophillus* CRL 1063 and CRL 1065, which showed less inhibitory effect against *A. niger* CH 101, *G. citri-aurantii* INTA 1, *Penicillium* sp. CH 102 and *P. digitatum* INTA 2 were situated in quadrant A. Only two strains (*L. brevis* CRL 772 and CRL 796) that did not show inhibitory effect on the fungal strains tested were placed in the quadrant C. The seventy-two LAB strains with high percentages of inhibition (>60% of inhibition) were located in quadrant D. In this group, 10 strains (*L. casei* CRL 239, *L. plantarum* CRL 681, CRL 788, CRL 759 and CRL 142, *L. fermentum* CRL 220, CRL 236 and CRL 251, *L. reuteri* CRL 1098 and *L. acidophilus* CRL 1070)

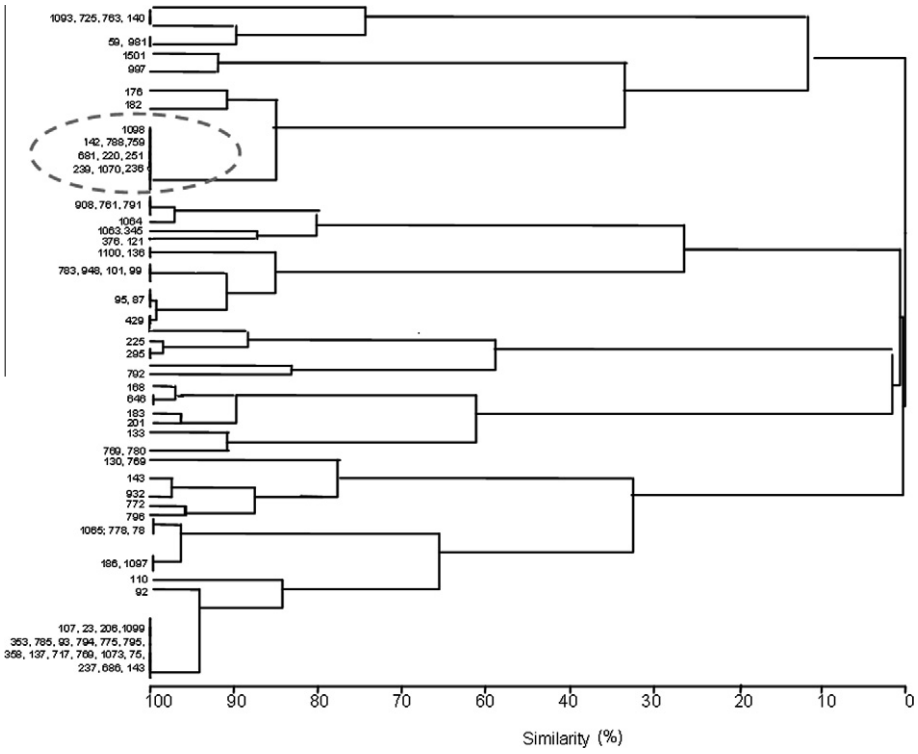

Fig. 3. Cluster analysis of the LAB antifungal activity. The numbers corresponds to strains belonging to the Culture Collection of the Centro de Referencia para Lactobacilos (CRL). Selected strains with similarity (100%) of antifungal activity (gray circle).

had inhibitory effect >80% on all fungi tested (Fig. 2) and were selected for further studies. The PCA analysis revealed low variability between the antifungal activities of these compared to the remaining LAB strains (82).

The ten selected strains were previously isolated from different sources (Table 1). No relationship between the source of isolation and antifungal activity was found. The similarity between the LAB strains according to their ability to inhibit fungal growth was also determined by cluster analysis (Fig. 3). The antifungal activity of selected strains showed 100% similarity between each other (gray circle) and 85% similarity with *L. plantarum* CRL176 and CRL182 because they inhibit more than 80% fungal growth of four of the fungi tested. By contrast, no similarity between these ten strains with *L. brevis* CRL 796 and 772, used as negative control in this study, was observed.

3.2. Characterization of the bioactive metabolites

The antifungal activity of the CFS from the selected LAB strains remained unchanged ($p > 0.05$) after heating or treatment with catalase (data not shown). In the majority (>90%) of the assayed CFS, neutralization caused a significant decrease of the inhibitory activity (40–80%), which was dependant on the fungus and LAB strains evaluated (Fig. 4), indicating the acidic nature of the metabolites involved. The concentrations of organic acids present in the CFS of the 10 strains were determined. The HPLC analysis revealed the presence of lactic, acetic, and phenyllactic acids (PLA) (Table 2). The highest and smallest lactic acid concentrations, 267.9 and 118.9 mM were found in the CFS of *L. plantarum* CRL 759 and *L. fermentum* CRL 220, respectively. The production of acetate varied among the strains (10–27.5 mM); while PLA was detected in low concentrations (0.2–3.5 mM).

Only *L. fermentum* CRL 251 of the selected 10 strains produced antifungal compound/s which was not of acidic nature as the neutralization did not produce significant reduction ($p > 0.05$) of the antifungal activity of the CFS of this strain; moreover, similar effect was observed after expose the CFS to the heat treatment. To determine the peptide nature of the active compounds produced by this strain, its CFS was treated with different protease enzymes. The antifungal activity of *L. fermentum* CRL 251 was decreased by 50, 4 and 3% after treatment with trypsin, proteinase K and pepsin, respectively.

The molecular weight of the peptide/s was determined by ultrafiltration of the CFS; two fractions were obtained, one with peptides <10 kDa and another one with peptides >10 kDa. The growth of *A. niger* CH 101 in both peptides fractions of MRS (control) was similar to that in the MRS medium. The antifungal activity of the *L. fermentum* CRL 251 CFS and the peptide fraction <10 kDa was similar producing 85% inhibition respect to control (MRS), while the fraction with peptides >10 kDa did not show inhibitory activity (Fig. 5).

Table 2
Organic acids produced by antifungal LAB.

LAB	Organic acids [a] (mmol l^{-1})		
	Lactic	Acetic	Phenyllactic
L. casei CRL 239	202.9 ± 0.1	16.3 ± 1.2	1.3 ± 0.4
L. plantarum CRL 681	216.7 ± 0.2	13.2 ± 0.5	2.7 ± 0.7
L. plantarum CRL 788	249.7 ± 0.5	13.5 ± 0.9	3.5 ± 0.2
L. plantarum CRL 759	267.9 ± 0.8	20.0 ± 0.1	1.9 ± 0.1
L. plantarum CRL 142	236.0 ± 0.2	12.8 ± 0.7	0.2 ± 0.1
L. fermentum CRL 220	118.9 ± 0.9	27.5 ± 0.4	2.2 ± 0.2
L. fermentum CRL 236	160.8 ± 0.7	27.4 ± 0.5	1.2 ± 0.1
L. fermentum CRL 251	161.3 ± 7.1	26.3 ± 0.6	1.3 ± 0.3
L. reuteri CRL 1098	153.3 ± 0.4	10.6 ± 0.5	1.9 ± 0.4
L. acidophillus CRL 1070	195.1 ± 1.2	11.7 ± 1.6	1.1 ± 0.1

[a] Concentration of organic acids present in CFS (24-h).

4. Discussion

Fungal contamination is one of the main causes of economic losses worldwide in the food industry and agriculture (Legan and Voysey, 1991). Consumerś demand for natural products is increasing, which involves the elimination or decrease of use of chemicals such as food preservatives used in agricultural industry (Brul and Coote, 1999). In this context, LAB may be considered an interesting alternative for bio-conservation. In this study, ninety-one LAB strains isolated from different sources were screened for antifungal activity against fungi strains responsible for food contamination. The inhibition of fungal growth through spectrophotometrical measurements (OD_{580nm}) showed high sensitivity to evaluate inhibition on all fungal strains tested. This method was also reported by other authors (Laine et al., 1996; Lavermicocca et al., 2003; Raaska and Mattila-Sandholm, 1995) as a good tool to examine the antifungal potential of LAB. In this study we observed that the antifungal activity was strain-dependent as well as the evaluated fungal species. From the total of LAB strains evaluated, 10 were selected due to their high inhibitory effect (>80%) on all fungal strains tested.

Many LAB have flavoproteins, NADH oxidases and peroxidases, which are capable of producing hydrogen peroxide in the presence of oxygen. Some authors attributed the inhibitory action of hydrogen peroxide to a strong oxidizing effect of the cells and the destruction of the basic molecular structures of cellular proteins (Condon, 1987; Davidson et al., 1983). In this study, the treatment with catalase of the CFS from the evaluated LAB strains did not

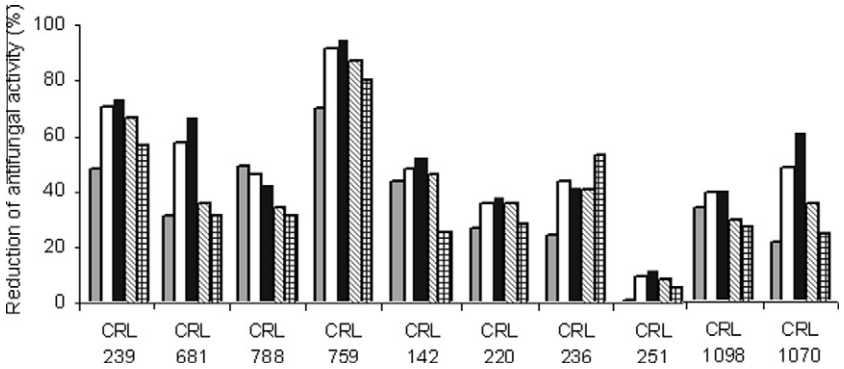

Fig. 4. Effect of neutralization on the LAB antifungal activity on growth of *A. niger* CH 101 (▒), *Penicillium* sp. CH 102 (■), *F. graminearum* CH 103 (□), *G. citri-aurantii* INTA1 (▨) and *P. digitatum* INTA2 (⊞).

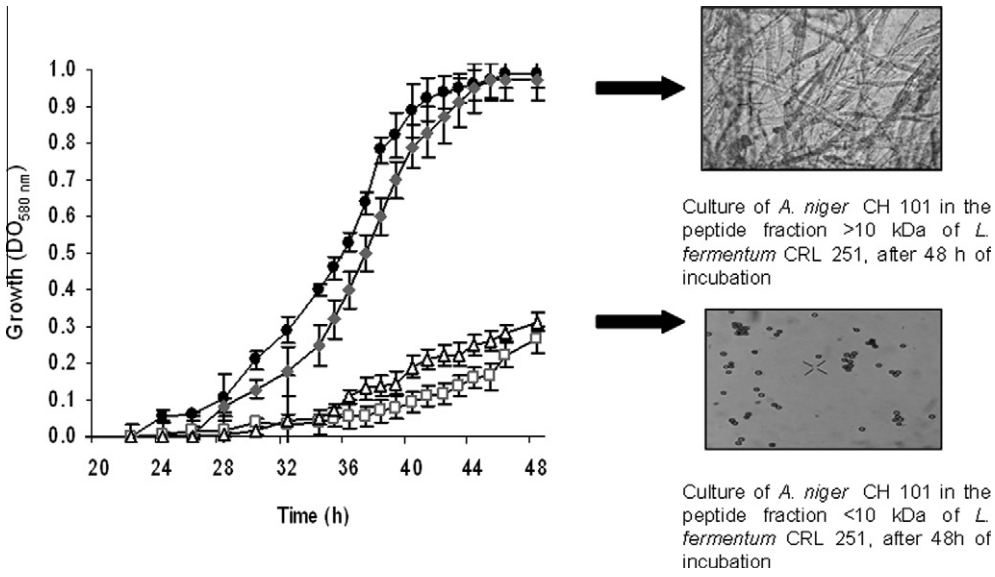

Fig. 5. Effect of antifungal peptide fractions from *L. fermentum* CRL 251 on *A. niger* CH 101. MRS medium control (●), peptides <10 kDa (□), peptides >10 kDa (◈), CFS of *L. fermentum* CRL 251 (△).

reduce the antifungal activity, indicating that metabolites other than hydrogen peroxide would be responsible for the inhibition of fungal growth. The antifungal effect of the majority of the LAB strains was lost after neutralization confirming the acidic nature of the antifungal metabolites. Lactic acid, acetic acid and PLA were determinated in the 10 CFS strains. The inhibitory activity of the acids could have a specific effect on the metabolic activity or acidification of the cytoplasm, which affects the proton motive force of the membrane directly inhibiting fungal growth (Piper et al., 2001; Young and Foegeding, 1993).

The short-chain organic acids produced by LAB such as acetic acid are commonly used by food manufacturers as antimicrobial preservatives or acidulants in a variety of food products (Davidson and Juneja, 1990). Moreover, a reagent grade (99.99%) of glacial acetic acid was used to fumigate different fruits (cherries, apples, pears, oranges, grapefruits, and lemons) previously infected with *Penicillium* (Sholberg, 1998).

In recent years, the role of organic acids as antifungal metabolites has become more important with the identification of PLA, as antifungal compound produced by different LAB (Lavermicocca et al., 2000; Prema et al., 2010). PLA is regarded as active compound against several fungal species (including some mycotoxigenic isolates such as *A. ochraceus*, *P. verrucosum* and *P. citrinum*) and certain contaminating bacteria, namely *Listeria* sp., *Staphylococcus aureus* and *Enterococcus faecalis* (Gould, 1996). Dallagnol et al. (2011) reported the influence of biosynthetic precursors, intermediates and electron acceptors on the production of PLA by a *L. plantarum* strain. Phenylalanine was the best stimulant compound for PLA production; however, citrate could also increase its synthesis.

Only one out of the ten selected LAB strains, *L. fermentum* CRL 251 produces fungus-inhibitory peptide/s smaller than 10 kDa, thermoresistant and active at a pH range values of 4.0–7.0, corresponding to the pH of 24 h culture and after neutralization, respectively. In addition, the inhibitory peptide/s was sensitive to trypsin, which catalyzes the hydrolysis of peptide bonds preferably formed by arginine and lysine (Glick and Pasternak, 1998). This is the first report about a proteinaceous antifungal compound/s produced by a *L. fermentum* strain. There is no previous evidence at this time, of the role of the protein compounds in the inhibition of mould growth by LAB. However, other authors have reported that some lactic strains such as *Lactococcus lactis* (Roy et al., 1996), *L. casei* (Gourama and Bullerman, 1997) and *P. pentosaceus* (Rouse et al., 2008) produced antifungal metabolites that were sensitive to proteolytic enzymes. The antifungal activity of a *L. casei* strain showed to be suppressed by trypsin (Gourama and Bullerman, 1997). After a partial characterisation, the authors suggested that the main molecule involved in this antifungal activity was a peptide with a molecular weight lower than 1 kDa. Also, Magnusson and Schnürer (2001) showed that the antifungal metabolite produced by the strain *L. coryniformis* Si3 was a small peptide (approximately 3 kDa), highly resistant to heat, whose activity was completely inhibited by proteolytic enzymes. Other proteinaceous compound characterized by Atanossova et al. (2003) from *L. paracasei* M3 with broad fungistatic effects was a hydrophobic protein of approximately 43 kDa.

Various reasons have led to the search for new alternatives to minimize the risks associated to the presence of fungal spoilage in foods and animal feed that include consumerś demands regarding quality and food safety and increasing government concern about environmental and safety issues. Thus, the concepts of biocontrol returned to occupy the place they deserve, exploiting the use of microorganisms and/or its metabolites in different food processes. LAB are food-grade microorganisms whose metabolic diversity and ability to produce natural biocides makes them a versatile biological alternative, technically viable and with high benefit/cost ratio for fungal control (Messens and De Vuyst, 2002; Schwenninger et al., 2005). The current study shows that ten LAB isolated from several environments belonging to different genera and species exhibit antifungal activity against all spoilage fungi evaluated. The inhibitory activity is caused by organic acids and antifungal peptide/s. Further investigations in our laboratory are in progress to elucidate the identification and action mechanism of the antifungal peptide/s produced by *L. fermentum* CRL 251 which is of great interest as biocontrol agent to inhibit spoilage fungi and to extend the shelf life of foods.

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