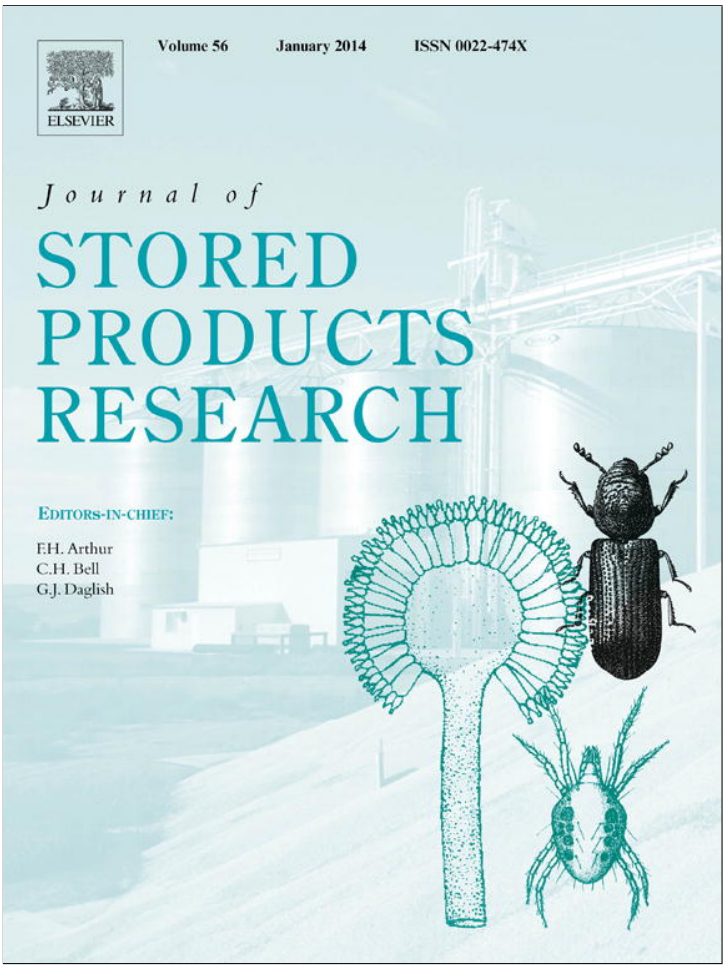

Journal of Stored Products Research 56 (2014) 33–37

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Incidence of lactic acid bacteria and *Aspergillus flavus* in brewer's grains and evaluation of potential antifungal activity of these bacteria

P. Asurmendi [a,b,*], L. Pascual [a], A. Dalcero [a,b], L. Barberis [a]

[a] Departamento de Microbiología e Inmunología, Universidad Nacional de Río Cuarto, Ruta 36 Km. 601, 5800 Río Cuarto, Córdoba, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

ARTICLE INFO

Article history:
Accepted 12 November 2013

Keywords:
Aspergillus section *Flavi*
Lactic acid bacteria
Antimicrobial activity
Brewer's grains

ABSTRACT

Feed destined for animal production as brewer's grains can be contaminated by *Aspergillus* section *Flavi* species. Lactic acid bacteria (LAB) play a defining role in the preservation and microbial safety of fermented foods. The objective of this study was to study the incidence of lactic acid bacteria, *Aspergillus* section *Flavi* and AFB_1 in brewer's grains and the preliminary antifungal activity of native LAB *in vitro*. LAB and aflatoxigenic *Aspergillus* were found in high counts in brewer's grains used as raw material for pig feedstuff. However, AFB_1 had low AFB_1 natural incidence in samples. *In vitro* antifungal activity of LAB isolated showed that all bacteria tested inhibited two *Aspergillus flavus* strains assayed. The high incidence of LAB could be inhibiting the AFB_1 production in by-products obtained from the beer industry. LAB strains with excellent antimicrobial activity were also found in this substrate.

1. Introduction

Lactic acid bacteria (LAB) are commonly isolated from fermented feed. They play a key role in the preservation and microbial safety of fermented foods. LAB are Gram-positive, nonmotile, nonsporeforming, rod- and coccus-shaped organisms that can ferment carbohydrates and produce, mainly, lactic acid (Singh and Prakash, 2009). They compete with pathogenic microorganisms for nutrients and are potential producers of antimicrobial metabolites such as organic acids, hydrogen peroxide, bacteriocins and antifungal compounds, including fatty acids and phenyllactic acid (Pascual et al., 2008a, b; Ruiz et al., 2009; Dalié et al., 2010).

Since feed represents the major cost in swine production, there is an increasing interest in finding alternative raw materials to those commonly used to develop rations for pigs. By-products from the brewery industry are available in large quantities and have a considerable nutritional potential and a low cost (Mussatto et al., 2006; Gupta et al., 2010). Brewer's grains are the wet materials that remain after the fermentation of barley, during the production of beer. This product contains high protein levels, crude fiber, vitamins and minerals (Mirzaei-Aghsaghali and Maheri-Sis, 2008; Aliyu and Bala, 2011). Nevertheless, the high moisture and content of nutrients in wet by-products lead to a fast decomposition and to a reduction of storage life by bacterial and fungal development (Marston et al., 2009). Among the filamentous fungi, *Aspergillus* section *Flavi* are important spoilage microorganisms of food destined for production as animal feed. These fungi grow on food under favorable conditions of temperature and humidity and some species such as *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus nomius* and *Aspergillus pseudotamarii* may produce aflatoxins (AFs) (Kurtzman et al., 1987; Horn et al., 1996; Samson et al., 2000; Ito et al., 2001; Hedayati et al., 2007). Aflatoxins (AFs) are secondary metabolites that have adverse effects on human and animal health. They are considered as carcinogenic, mutagenic, teratogenic, hepatotoxic and immunosuppressive substances (Robens and Richard, 1992; Eaton and Gallagher, 1994; Pitt, 2000; Sahoo and Mukherjee, 2001). Aflatoxin B_1 (AFB_1) was classified as a human carcinogen by the International Agency for Research on Cancer (IARC, 1993).

There is a growing interest in methods that reduce fungal growth in food. Biological control has focused increasing attention because it is an ecologically friendly alternative to chemical fungicides (El-Kassas and Khairy, 2009; Tsitsigiannis et al., 2012). Several researches have reported on the inhibitory properties of LAB on fungal growth. In a related study, Chang and Kim (2007) showed inhibition of mycelial growth of *A. flavus* by *Lactobacillus casei* KC-324. The inhibitory activity was due to extracellular metabolites as evidenced by the use of cell-free supernatants. Other published studies also demonstrated that three lactobacilli species

* Corresponding author. Departamento de Microbiología e Inmunología, Universidad Nacional de Río Cuarto, Ruta 36 Km. 601, 5800 Río Cuarto, Córdoba, Argentina. Tel.: +54 0358 4676539.
E-mail address: pasurmendi@exa.unrc.edu.ar (P. Asurmendi).

0022-474X/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jspr.2013.11.002

(*Lactobacillus delbrueckii*, *Lactobacillus fermentum* and *Lactobacillus plantarum*) were able to reduce aspergilli fungal growth (Aryantha and Lunggani, 2007). Previous work carried out by our group has shown that two probiotic bacteria (*L. fermentum* and *Lactobacillus rhamnosus*) have great potential for controlling *Aspergillus* (Gerbaldo et al., 2012). However, the information of aspergilla and lactic acid bacteria incidence in stored brewer's grains and the interaction between them is still limited. Accordingly, this brewery by-product represents an optimal substrate for aspergilla and lactic acid bacteria growth, thus the aim of the present work was to study the incidence of lactic acid bacteria, *Aspergillus* section *Flavi* and AFB_1 in brewer's grains and the preliminary antifungal activity of native LAB *in vitro*.

2. Materials and methods

2.1. Sampling

Samples of brewer's grains were collected in a brewery factory from Villa General Belgrano, Córdoba, Argentina. The substrate was stored in plastic bags and the storage conditions were not controlled. Thirty two samples (3 kg each) were collected at 2-month intervals from July 2010 to June 2012. Primary samples were homogenized, and quartered to get 1 kg samples for laboratory analysis. All samples were immediately tested for LAB and *Aspergillus* count. Physical properties determination (pH and a_W) were carried out according to the methodology described by Asurmendi et al., (2013). Then, they were dried and stored for up to 7 days at 4 °C for aflatoxin analyses.

2.2. Isolation and identification of lactic acid bacteria

Assays were performed following the methodology proposed by The International Commission on Microbiological Specification for Foods (ICMSF, 1996). Ten grams of each sample were homogenized in 90 mL of 0.1% sterile peptone water. After homogenization, serial dilutions (10^{-4}–10^{-6}) were carried out in 1% peptone solution and plated on Man Rogosa and Sharpe agar media (MRS) (bioMérieux®, France), in triplicate. Plates were incubated under a 5% CO_2 atmosphere condition for 48 h at 37 °C, at which time LAB were counted. Plates containing 30–300 colonies were used for counts, and results were expressed as CFU g^{-1}. Different types of colonies were sub-cultured in MRS broth and incubated in micro-aerobiosis for 48 h at 37 °C. LAB isolates were identified using the following tests: Gram stain, production of catalase and cytochrome oxidase, CO_2 production from glucose, growth at different temperatures (10 and 45 °C), growth at different pH values (4.4 and 9.6), growth at different NaCl concentrations (6.5 and 18%) and acid production with different carbon sources (glycerol, erythritol, D-arabinose, L-arabinose, D-xylose, D-adonitol, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, dulcitol, inositol, D-mannitol, D-celobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, D-raffinose and starch.) (Holt et al., 1994). LAB strains were stored at −80 °C in 30% glycerol (v/v).

2.3. Survey of Aspergillus species

Quantitative enumeration of *Aspergillus* section *Flavi* was carried out using the surface-spread method. Ten grams of each brewer's grains sample was homogenized in 90 mL sterile peptone water (0.1%) to obtain a 10^{-1} dilution. Serial dilutions up to 10^{-3} were made with the same diluents. Aliquots of 0.1 mL were inoculated in triplicate onto the following media: dichloran rose bengal chloramphenicol agar (DRBC) and dichloran 18% glycerol agar (DG18) (Pitt and Hocking, 1997). Plates were incubated for 7 days at 25 °C and the results were expressed as CFU g^{-1} of sample. Taxonomic identification of *Aspergillus* species was done according to Pitt and Hocking (1997) and Klich (2002).

2.4. Aflatoxin B_1 analyses

Production of AFs by *Aspergillus* strains isolated from brewer's grains was performed following the methodology proposed by Geisen (1996), with some modifications. Determination of AFB_1 in brewer's grains was carried out using MultiSep® clean-up column (Romer, USA.). The aflatoxin analytical quantification was determined by high performance liquid chromatography (HPLC), following the method proposed by Trucksess et al. (1994). The detailed methodology was described in a previous work (Asurmendi et al., 2013).

2.5. In vitro antifungal activity of isolated LAB

LAB strains were streaked in one third of plates containing MRS broth with 1.2% of agar. A well was done at the opposite side of the plate and 100 μl of a 10^2 conidia mL^{-1} suspension of each strain were inoculated. Plates were incubated for 7 days at 25 °C. Plates with an aflatoxigenic fungus on one side that were not inoculated with bacterial isolates served as controls. The aspergilli suspensions were obtained from 7 day-old-cultures of actively growing *Aspergillus* strains in malt extract agar. Conidial suspensions were homogenized in PBS solution added with 0.1% of Tween 80. The mixture was centrifuged 15 min at 3000 rpm. The conidia counts were made in a Neubauer chamber and the conidial suspensions were adjusted with dilutions in PBS added with 0.1% of Tween 80. The inhibition areas were examined after the incubation time.

2.6. Statistical analyses

Data analyses were performed by analysis of variance. Fungal and bacterial counts were transformed to $\log_{10}(x+1)$ to obtain the homogeneity of variance. pH, a_W and microorganisms counts means were compared using Fisher's protected Least Significant Difference (LSD) test. The analysis was conducted using the GLM Procedure of the Statistical Analysis System (version 9.2) (Quinn and Keough, 2002).

3. Results

3.1. Physical properties characterization

The mean values of pH and a_W of brewer's grain samples at different sampling periods are shown in Table 1. The pH mean values ranged between 3.94 and 4.55. The highest pH level was found in January–February 2011 sampling period ($P < 0.05$). The moisture content (a_W) in brewer's grains samples ranged from 0.99

Table 1
Values of pH and a_W from brewer's grain at different sampling periods.

Periods	pH	a_W	Periods	pH	a_W
Jul–Aug 2010	4.03^{ab*}	0.993^{ab**}	Jul–Aug 2011	4.32^{ab}	0.994^{ab}
Sep–Oct 2010	4.01^{ab}	0.990^{a}	Sep–Oct 2011	4.07^{ab}	0.992^{ab}
Nov–Dec 2010	3.94^{a}	0.995^{b}	Nov–Dec 2011	4.31^{ab}	0.996^{b}
Jan–Feb 2011	4.55^{b}	0.993^{ab}	Jan–Feb 2012	4.33^{ab}	0.994^{ab}
Mar–Apr 2011	4.35^{ab}	0.992^{ab}	Mar–Apr 2012	4.25^{ab}	0.995^{b}
May–Jun 2011	4.00^{ab}	0.991^{ab}	May–Jun 2012	4.32^{ab}	0.996^{b}

* The mean of pH values with different letters indicate significant difference according to LSD test ($P < 0.05$).
** The mean of a_W levels with different letters indicate significant difference according to LSD test ($P < 0.05$).

to 0.996. November–December 2010, November–December 2011, March–April 2012, May–June 2012 showed the highest a_W levels ($P < 0.05$).

3.2. Lactic acid bacteria determination

Figure 1 shows the mean counts of LAB obtained from brewer's grains at different sampling periods. LAB were isolated from all samples, most of the LAB counts were in the order of 10^8 UFC g^{-1}. The LSD test showed statistically significant differences among sampling periods. The highest count was observed in a sample obtained in November–December 2011 with a value of 1.4×10^9 CFU g^{-1} and the lowest in November–December 2010 and May–June 2012 with levels of 1.5×10^8 and 4.8×10^7 CFU g^{-1}, respectively. No relation was found between these sampling periods and a_W variation; however, the November–December 2010 period which had a low LAB count also showed the lowest pH value. A total of 178 LAB strains were isolated from brewer's grains samples. All of the strains were Gram positive and catalase and oxidase negative. The frequency of isolated LAB showed that 60.7% ($n = 108$) were rod-shaped bacteria and 39.7% ($n = 70$) cocci bacteria.

3.3. Isolation of Aspergillus section Flavi and determination of AFB_1

Aspergillus section *Flavi* counts (CFU g^{-1}) at different sampling periods are shown in Fig. 1. *Aspergillus* genus was present in 84.4% of brewer's grains samples. *Aspergillus* section *Flavi* was isolated from 71.9% samples. No section *Flavi* species were observed from the samples obtained during the May–June 2011 sampling period. The statistical analyses showed that there were significant differences among mean counts of *Aspergillus* section *Flavi*. The November–December 2010 period had the highest mean counts of *Aspergillus* section *Flavi* species. Also, this sampling period showed the lowest pH and the highest a_W values. Whereas, May–June 2011, November–December 2011 and January–February 2012 had the lowest counts ($P < 0.05$). A total of 82 strains of *Aspergillus* section *Flavi* species were isolated from brewer's grains samples and they were identified as *A. flavus* ($n = 70$) and *A. parasiticus* ($n = 12$). The TLC screening showed that 41.4% of the *A. flavus* strains were able to produce AFB_1 and that all of the *A. parasiticus* strains produced AFB_1 and AFG_1. The average level of AFB_1 within the producing isolates was 2105.2 ng g^{-1} and 2217.4 ng g^{-1} for the *A. flavus* and the *A. parasiticus* strains, respectively. The highest level of AFB_1 observed was 11,458.2 ng g^{-1} and it was produced by an *A. flavus* strain isolated during March–April 2011 (Table 2). Analysis of AFB_1 in brewer's grains revealed a low contamination with this mycotoxin. Five (15.6%) of the 32 samples analyzed were positive for AFB_1, with mean levels ranging between 2.5 and 50.35 ng g^{-1}. Two samples showed AFB_1 levels exceeding the maximum allowed for pig feedstuffs (20 ng g^{-1}). These samples were obtained during November–December 2010 and January–February 2011 (data not shown). The a_W levels corresponding to sampling period mentioned above were of 0.995 and 0.993, respectively.

3.4. Antimicrobial activity of lactic acid bacteria

Fourteen LAB isolated from brewer's grains and identified as described above (*Lactobacillus brevis* B20, *L. plantarum* B29, *Lactobacillus paracasei* B38, *L. plantarum* B54, *L. plantarum* B57, *Pediococcus pentosaceus* B65, *L. brevis* B72, *Pediococcus acidilactici B82*, *P. acidilactici B83*, *P. pentosaceus* B86, *Lactococcus lactis* subsp. *lactis* B87, *L. brevis* B131, *L. brevis* B133, *Lactobacillus* sp. B144, *L. brevis* B146, *Lactobacillus cellobiosus B149*) were screened for antifungal activity using two fungal strains, *A. flavus* 21 (AF21) and *A. flavus* 54 (AF54). The aspergilli (AF21 and AF54) were selected for producing high concentrations of AFB_1 in previous tests, 2467.6 ng g^{-1} and 11,458.2 ng g^{-1}, respectively.

The obtained results demonstrated that all of the tested LAB strains inhibited the development of the *Aspergillus* strains studied, as compared to controls (Table 3). When fungal and LAB strains were tested on the same plate, the fungal growth decreased compared with the fungal control. This qualitatively assay indicated the synthesis of compounds with antimicrobial activity by all of the tested LAB strains. It was also observed that, in the presence of the antagonistic strains, the macroscopic appearance of the fungal colonies changed. The colonies were floccose and showed a

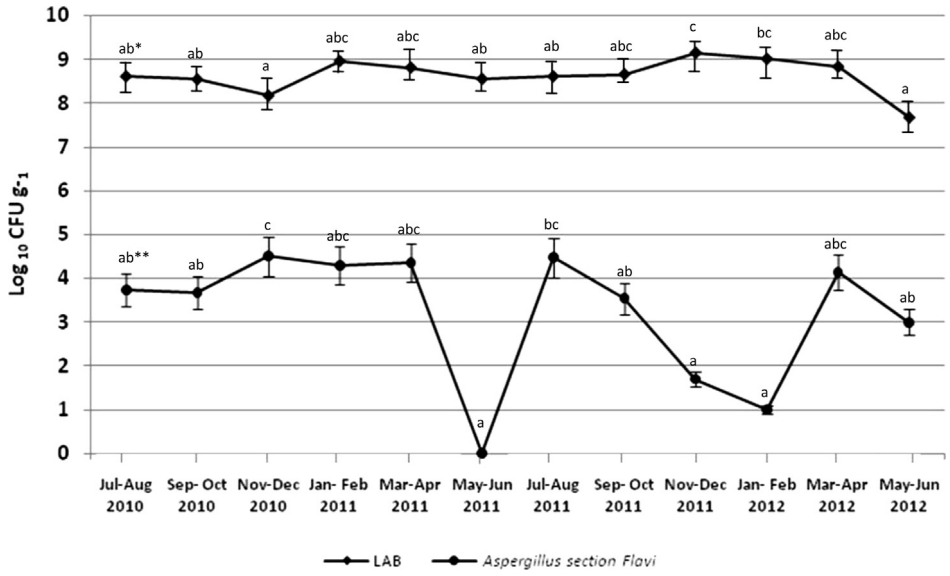

Fig. 1. Lactic acid bacteria and *Aspergillus* section *Flavi* mean counts (CFU g^{-1}) at different sampling periods. *The mean LAB counts with common letters indicate no significant difference according to LSD test ($P \geq 0.05$). **The mean *Aspergillus* section *Flavi* counts with common letters indicate no significant difference according to LSD test ($P \geq 0.05$).

Table 2
Aflatoxin B_1 *in vitro* production by *Aspergillus* section *Flavi* strains isolated from brewer's grains.

	Proportion of positive strains	%	Range AFB_1 (ng g^{-1} of media)[a]	Media concentration of AFB_1 ± SD (ng g^{-1} of media)[b]
A. flavus	29/70	41.4%	5.6–11,458.2	2105.2 ± 2398.9
A. parasiticus	12/12	100%	11.6–6521.5	2217.4 ± 2601.5

[a] Minimum and maximum value of AFB_1 production.
[b] Media concentration of AFB_1 ± standard deviation.

decreased conidiogenic activity. In addition, conidia were usually more distant to the bacterial growth.

4. Discussion

The present study shows that LAB, aflatoxigenic *Aspergillus*, and AFB_1 are present in brewer's grains used as raw material for pig feedstuff. LAB isolation from fermented feed is possible because of their ability to produce high levels of lactic acid as well as being able to survive under highly acidic conditions. The highest LAB counts were found in samples from November–December 2011. However, the LAB counts proved to be high (around 10^8 CFU g^{-1}) during all of the sampling periods. Our results partially agree with those of Wang and Nishino (2008), who reported LAB counts in the order of 10^7 CFU g^{-1} when studying wet brewer's grains in Japan.

The presence of *Aspergillus* section *Flavi* species in brewer's grains was evaluated. The samples showed a high prevalence of species of this section. These microorganisms are a major problem in stored feed due to their production of toxins, mostly during inadequate storage conditions. By comparison, Medina et al. (2006) obtained a lower percentage of samples with *A. flavus* and *A. parasiticus* (64%) in malting barley, the main raw material in brewery industry. In addition, Fraga et al. (2007) and González Pereyra et al. (2008) found a lower contamination of samples by *Aspergillus* section *Flavi* when analyzing feeds intended for poultry and pigs, respectively.

In this work, a high number of *A. flavus* strains from brewer's grains and all of the *A. parasiticus* strains were able to produce AFB_1 *in vitro*. These results do not agree with those reported by Campos et al. (2008), who isolated more aflatoxigenic strains (57%–77%) and no *A. parasiticus* strains from pet food. The production of AFB_1 *in vitro* indicates that the strains have active metabolic pathways to synthesize this mycotoxin. However, the production of AFs may change under *in situ* conditions because it depends on physical (temperature and feed moisture), biological, biochemical and environmental factors. The low natural incidence of AFB_1 in brewer's grains samples reveals that some of the factors mentioned above are interfering with production of this mycotoxin. Moreover, two of the samples exceeded the limit of AFB_1 (20 ng g^{-1}) proposed by the Good Manufacturing Practices for any material intended to be used directly or as an ingredient in feed for pigs. Indeed, one of these samples corresponded to one of the sampling periods that showed low counts of LAB. Research reports indicate that biological factors such as the presence of LAB inhibit aspergilli growth and aflatoxin production. During the November–December 2011 sampling period, the highest LAB count as well as the lowest *Aspergillus* section *Flavi* count ($P < 0.05$) were observed. On the contrary, November–December 2010 period showed a low count of LAB and a high count of *Aspergillus*. This fact might suggest some interaction between both microorganisms under the substrate's conditions. LAB could have inhibited the development of *Aspergillus*. Several authors proposed some inhibitory mechanisms used by LAB strains, including: production of antimicrobial substances such as bacteriocins, phenylacetic acid, 4-hydroxyphenylacetic acid, short chain fatty acids and low molecular weight compounds such as benzoic acid, methylhydantoin, mevalonolactone and dipeptides, other mechanisms such as competition for nutrients, lowering of pH or a combination of mechanisms (Ghonaimy et al., 2007; Muñóz et al., 2010).

This work on *in vitro* antifungal activity of LAB isolated from brewer's grains showed that all of the tested bacteria inhibited two *A. flavus* strains. Similar results were reported by Onilude et al. (2005), who isolated LAB strains from fermented cereal and reported that all of the strains inhibited the growth of at least one strain of aflatoxigenic *Aspergillus in vitro*. Other authors showed results that differ from those found in our work. For instance, Muhialdin and Hassan (2011) showed that only 45% of the screened LAB strains inhibited fungal growth when evaluated against a single strain of *Aspergillus* section *Flavi*. Also, Hassan and Bullerman (2008) showed that two strains of *L. paracasei* did not inhibit the development of *A. flavus* and *A. parasiticus*.

In conclusion, this study demonstrated the presence of LAB and aflatoxigenic *Aspergillus* in this alternative feedstuff for swine production. The high incidence of LAB could be inhibiting the production of AFB_1 due to the prevalence of these bacteria in by-products obtained from the beer industry. Also, LAB strains with excellent antimicrobial activity on the growth of two aflatoxigenic *A. flavus* strains were isolated. Further studies on the beneficial properties of these LAB, such as the production of secondary metabolites, will contribute to the selection of strains which could be used as biological control agents against pathogenic fungi in feeds intended for swine production.

Table 3
Inhibitory activity of lactic acid bacteria isolated from brewer's grains on *Aspergillus flavus*.

LAB strain	*A. flavus* AF21	*A. flavus* AF54
B20	++	++
B29	+	+
B38	+	+
B54	+	+
B57	+	+
B65	++	+
B72	+	+
B82	++	+
B83	++	+
B86	++	++
B87	++	++
B131	++	++
B133	++	+
B144	+	++

(+) Inhibition zone ≤ 5 mm.
(++) Inhibition zone > 5 mm.

Acknowledgments

This work was supported by the Secretaría de Ciencia y Técnica, Universidad Nacional de Río Cuarto, Córdoba, Argentina. P. Asurmendi has a doctoral fellowship from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), Argentina.

References

Aliyu, S., Bala, M., 2011. Brewer's spent grain: a review of its potentials and application. Afr. J. Biotechnol. 10, 324–331.
Aryantha, I.N.P., Lunggani, A.T., 2007. Suppression on the aflatoxin-B production and the growth of *Aspergillus flavus* by lactic acid bacteria (*Lactobacillus delbrueckii, Lactobacillus fermentum* and *Lactobacillus plantarum*). Biotechnology 6, 257–262.

Asurmendi, P., Barberis, C., Dalcero, A., Pascual, L., Barberis, L., 2013. Survey of *Aspergillus* section *Flavi* and aflatoxin B_1 in brewer's grain used as pig feedstuff in Córdoba, Argentina. Mycotoxin Res. 29, 3–7.

Campos, S.G., Cavaglieri, L.R., Fernádez Juri, M.G., Dalcero, A.M., Krüger, C., Keller, L.A.M., Magnoli, C.E., Rosa, C.A.R., 2008. Mycobiota and aflatoxins in raw materials and pet food in Brazil. J. Anim. Physiol. Anim. Nutr. 92, 377–383.

Chang, I., Kim, J.D., 2007. Inhibition of aflatoxin production of *Aspergillus flavus* by *Lactobacillus casei*. Mycobiology 35, 76–81.

Dalié, D.K.D., Deschamps, A.M., Richard-Forget, F., 2010. Lactic acid bacteria – potencial for control of mould growth and mycotoxins: a review. Food Control 21, 370–380.

Eaton, D.L., Gallagher, E.P., 1994. Mechanisms of aflatoxin carcinogenesis. Annu. Rev. Pharmacol. Toxicol. 34, 135–172.

El-Kassas, H.Y., Khairy, H.M., 2009. A trial for biological control of a pathogenic fungus (*Fusarium solani*) by some marine microorganisms. Am.-Eur. J. Agric. Environ. Sci. 5, 434–440.

Fraga, M.E., Curvello, F., Gatti, M.J., Cavaglieri, L.R., Dalcero, A.M., da Rocha Rosa, C.A., 2007. Potential aflatoxin and ochratoxin A production by *Aspergillus* species in poultry feed processing. Vet. Res. Commun. 31, 343–353.

Geisen, R., 1996. Multiplex polymerase chain reaction for the detection of potential aflatoxin and sterigmatocystin producing fungi. Syst. Appl. Microbiol. 19, 388–392.

Gerbaldo, G.A., Barberis, C., Pascual, L., Dalcero, A., Barberis, L., 2012. Antifungal activity of two *Lactobacillus* strains with potential probiotic properties. FEMS Microbiol. Lett. 32, 27–33.

Ghonaimy, G.A., Yonis, A.A.M., Abol-Ela, M.F., 2007. Inhibition of *Aspergillus flavus* and *A. parasiticus* fungal growth and its aflatoxins (B_1, B_2, G_1 and G_2) production by *Latobacillus acidophilus*. J. Egypt. Soc. Toxicol. 37, 53–60.

González Pereyra, M.L., Pereyra, C.M., Ramirez, M.L., Rosa, C.A.R., Dalcero, A.M., Cavaglieri, L.R., 2008. Determination of mycobiota and mycotoxins in pig feed in central Argentine. Lett. Appl. Microbiol. 46, 555–561.

Gupta, M., Abu-Ghannam, N., Gallaghar, E., 2010. Barley for brewing: characteristic changes during malting, brewing and applications of its by-products. Compr. Rev. Food Sci. Food Saf. 9, 318–328.

Hassan, Y.I., Bullerman, L.B., 2008. Antifungal activity of *Lactobacillus paracasei* ssp. *tolerans* isolated from a sourdough bread culture. Int. J. Food Microbiol. 121, 112–115.

Hedayati, M.T., Pasqualotto, A.C., Warn, P.A., Bower, P., Denning, D.W., 2007. *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. Microbiology 153, 1677–1692.

Holt, J., Krieg, N., Sneath, P., Staley, J., Williams, S. (Eds.), 1994. Bergey's Manual of Determinative Bacteriology. Williams & Wilkins, Maryland, pp. 565–570.

Horn, B.W., Greene, R.L., Sobolev, V.S., Dorner, J.W., Powell, J.H., Layton, R.C., 1996. Association of morphology and mycotoxin production with vegetative compatibility groups in *Aspergillus flavus, A. parasiticus*, and *A. tamarii*. Mycologia 88, 574–587.

IARC (International Agency for Research on Cancer), 1993. Monograph on the Evaluation of Carcinogenic Risk to Human, vol. 56, pp. 257–263.

ICMSF (International Commission on Microbiological Specification for Foods), 1996. Microbiología de los Alimentos. Editorial Acribia, Zaragoza.

Ito, Y., Peterson, S.W., Wicklow, D.T., Goto, T., 2001. *Aspergillus pseudotamarii*, a new aflatoxin producing species in *Aspergillus* section *Flavi*. Mycol. Res. 105, 233–239.

Klich, M.A., 2002. Identification of Common *Aspergillus* Species. Centraal Bureau Voor Schimmelcultures, Utrecht, The Netherlands.

Kurtzman, C.P., Horn, B.W., Hesseltine, C.W., 1987. *Aspergillus nomius*, a new aflatoxin-producing species related to *Aspergillus flavus* and *Aspergillus tamarii*. Antonie Van Leeuwenhoek 53, 147–158.

Marston, S.P., Spangler, D.A., Whitehouse, N.L., Erickson, P.S., 2009. Case study: addition of a silage preservative reduces spoilage in wet brewers grain. Prof. Anim. Sci. 25, 388–392.

Medina, A., Valle-Algarra, F.M., Mateo, R., Gimemo-Adelantado, J.V., Mateo, F., Jimenez, M., 2006. Survey of the mycobiota of Spanish malting barley and evaluation of the mycotoxin producing potential of species of *Alternaria, Aspergillus* and *Fusarium*. Int. J. Food Microbiol. 108, 196–203.

Mirzaei-Aghsaghali, A., Maheri-Sis, N., 2008. Nutritive value of some agro-industrial by-products for ruminants - a review. World J. Zool. 3, 40–46.

Muhialdin, B.J., Hassan, Z., 2011. Screening of lactic acid bacteria for antifungal activity against *Aspergillus oryzae*. Am. J. Appl. Sci. 8, 447–451.

Muñóz, R., Arena, M.E., Silva, J., González, S.N., 2010. Inhibition of mycotoxin-producing *Aspergillus nomius* VSC 23 by lactic acid bacteria and *Saccharomyces cerevisiae*. Braz. J. Microbiol. 41, 1019–1026.

Mussatto, S.I., Dragone, G., Roberto, I.C., 2006. Brewer's spent grain: generation, characteristics and potential applications. J. Cereal Sci. 43, 1–14.

Onilude, A.A., Fagade, O.E., Bello, M.M., Fadahunsi, I.F., 2005. Inhibition of aflatoxin-producing aspergilli by lactic acid bacteria isolates from indigenously fermented cereal gruels. Afr. J. Biotechnol. 4, 1404–1408.

Pascual, L.M., Daniele, M.B., Giordano, W., Pájaro, M.C., Barberis, I.L., 2008a. Purification and partial characterization of novel bacteriocin L23 produced by *Lactobacillus fermentum* L23. Curr. Microbiol. 56, 397–402.

Pascual, L.M., Daniele, M.B., Ruiz, F., Giordano, W., Pájaro, C., Barberis, L., 2008b. *Lactobacillus rhamnosus* L60, a potential probiotic isolated from human vagina. J. Gen. Appl. Microbiol. 54, 141–148.

Pitt, J.I., Hocking, A.D., 1997. Fungi and Food Spoilage, second ed. Blackie Academic Press, London.

Pitt, J.I., 2000. Toxigenic fungi and mycotoxin. Br. Med. Bull. 56, 184–192.

Quinn, G.P., Keough, M.J., 2002. Experimental Design Data Analysis for Biologists. Cambridge University Press, Cambridge.

Robens, J.F., Richard, J.L., 1992. Aflatoxins in animal and human health. Rev. Environ. Contam. Toxicol. 27, 69–94.

Ruiz, F.O., Gerbaldo, G., Asurmendi, P., Pascual, L.M., Giordano, W., Barberis, I.L., 2009. Antimicrobial activity, inhibition of urogenital pathogens, and synergistic interactions between *Lactobacillus* strains. Curr. Microbiol. 59, 497–501.

Sahoo, P.K., Mukherjee, S.C., 2001. Immunosuppressive effects of aflatoxin B_1 in Indian major carp (Labeo rohita). Comp. Immunol. Microbiol. Infect. Dis. 24, 143–149.

Samson, R.A., Hoeckstra, E.S., Frisvad, J.C., Filtenborg, O., 2000. Introduction to Food- and Airborne Fungi, sixth ed. Centraalbureau Voor Schimmelcultures, Utrecht, The Netherlands.

Singh, P., Prakash, A., 2009. Screening of lactic acid bacteria for antimicrobial properties against *Listeria monocytogenes* isolated from milk products at Agra region. Internet J. Food Saf. 11, 81–87.

Trucksess, M.W., Stack, M.E., Nesheim, S., Albert, R.H., Romer, T.R., 1994. Multifunctional column coupled with liquid chromatography for determination of aflatoxins B_1, B_2, G_1, G_2 in corn, almonds, Brazil nuts, peanuts and pistachio nuts: collaborative study. J. AOAC Int. 6, 1512–1521.

Tsitsigiannis, D.I., Dimakopoulou, M., Antoniou, P.P., Tjamos, E.C., 2012. Biological control strategies of mycotoxigenic fungi and associated mycotoxins in Mediterranean basin crops. Phytopathol. Mediterr. 51, 158–174.

Wang, F., Nishino, N., 2008. Ensiling of soybean curd residue and wet brewers grains with or without other feeds as a total mixed ration. J. Dairy Sci. 91, 2380–2387.