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Zearalenone adsorption capacity of lactic acid bacteria isolated from pigs

María F. Vega[a,*], *Susana N. Dieguez*[a,b,d], *Belén Riccio*[b], *Sandra Aranguren*[b], *Antonio Giordano*[b], *Laura Denzoin*[b], *Alejandro L. Soraci*[b], *María O. Tapia*[b], *Romina Ross*[c], *Ana Apás*[c], *Silvia N. González*[c]

[a] *Departamento de Tecnología y Calidad de los Alimentos, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, Tandil, Buenos Aires, Argentina*
[b] *Laboratorio de Toxicología CIVETAN – CONICET, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, Tandil, Buenos Aires, Argentina*
[c] *Departamento de Salud Pública, Facultad de Bioquímica Química y Farmacia, Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina*
[d] *Comisión de Investigaciones Científicas de la Provincia de Buenos Aires CICPBA*

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ABSTRACT

The ability to adsorb zearalenone by five strain of lactic acid bacteria was evaluated: four strains of *Lactobacillus spp*. isolated from pig rectal swabs and one commercial strain (*Lactobacillus rhamnosus*). Several factors affecting the adsorption capacity were evaluated in order to improve the adsorption of the mycotoxin by bacteria. The stability of the zearalenone–bacteria complex was analyzed. In every case, bacterial adsorption capacity was higher than 40.0%. The strain showing the highest adsorption (68.2%) was selected for the following steps of this research. The adsorption percentages obtained after processing 6.5 and 7.5 mL MRS broth were 57.40% + 3.53 and 64.46% + 0.76, respectively. The stability of zearalenone–bacteria complex was evaluated by successively rinsing. In the first rinsing step 42.26% + 0.414 was still bound. In the second rinsing step 25.12% + 0.664 was still bound, whereas 15.82% + 0.675 remained in the pellet after the third rinse. Results obtained demonstrated that Lactic Acid Bacteria has capacity to adsorb zearalenone. Finally adsorption was increased using a higher volume of initial broth. These results could be used to design a new lyophilized powder for detoxification, using lactic acid bacteria as potential zearalenone adsorbents.

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* *Corresponding author*.
E-mail: mfvega@vet.unicen.edu.ar (M.F. Vega).
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Introduction

Zearalenone (ZEA) is a worldwide distributed mycotoxin as indicated by the International Agency for Research on Cancer (IARC).[1–3] Its toxicity and incidence was confirmed by recent reports.[4,5] This resorcylic lactone is produced mainly by *Fusarium graminearum* and is one of the most important toxins causing serious reproductive failures in pig production, due to its ability to couple 17-β-estradiol receptors. This interference with cytosolic estrogen receptors of target cells makes it an endocrine disruptor. When the dangerousness of this mycotoxin is considered, two factors are important: its stability to heat, milling, storage and processing of feedingstuffs,[6] and the severity of the intoxication, even if the ingestion takes place only for a few days as indicated by Anadón et al.[7]

Several physicochemical methods for detoxification have been widely used, but the tendency is to use biological methods that do not cause nutritional and palatability changes in feed.[8] There are several food additives for decontamination and/or detoxification of animal feedstuffs, such as the commercial adsorbent Mycosorb® (Alltech Inc.), in which the structure of yeast glucan layer is modified to increase its affinity and binding rate of mycotoxins above that of the native cell wall product.[9] In spite of its greater efficacy, the use of Mycosorb is limited because of its high price.

Interactions between *Fusarium* mycotoxins, ZEA, its derivative α-zearalenol (α-ZOL) and food-grade strains of *Lactobacillus* have been reported by several researchers.[10–17] El-Nezami et al.[13] demonstrated that, after co-incubation of ZEA and *Lactobacillus*, a considerable proportion (38.0%–46.0%) of these mycotoxins were recovered from bacterial pellets. Bacteria showed capacity to adsorb toxins and, as expected, results demonstrated that the binding depended on bacterial concentration. Co-incubation of ZEA and α-ZOL with bacteria significantly affected the percentage of toxin bound, suggesting that these toxins may share the same binding site. Recently Tinyiro et al.[18] using some *Bacillus* strains, showed that the adsorption percentage was high (78.0% and 95.0%) and variation depended on the strain used. ZEA adsorption ability by *Planococcus* spp has also been demonstrated by Lu et al.[19]

Two fundamental rules are considered when bacterial strains are isolated in order to be administered to animals. The first is the host specificity, it is essential to achieve a good adaptation.[20,21] The second is the proximity of the ecosystem, it is vital that the microorganisms are isolated in the same place where it acts on the host.[22,23]

For all these reasons, the purpose of the present work was to study the ZEA adsorption capacity of lactic acid bacteria isolated from pig rectal swabs under *in vitro* conditions, in order to broaden scientific knowledge on feed additives based on the use of lactic acid bacteria as ZEA binders.

Materials and methods

Bacterial strains and culture conditions

Lactobacillus rhamnosus (R1) (Lyofast LR B Sacco) was purchased from Sacco SRL (Cadorago, Italy). This strain was used as reference because of its demonstrated ability to adsorb ZEA.[12,13,24]

The tested *Lactobacillus* strains (L1, L2, L3, and L4) were isolated from 7 piglets kept on a single farm. The piglets, aged 21–60 days, were clinically healthy and no antibiotics had been administered during their lives. All samples were obtained within the same day. The strains were collected with a kit containing a swab and a transport medium (LAPTg semisolid agar without sugars). After being delivered to the laboratory, the swabs were broken off into peptone water, the dilutions were made and inoculated onto solid medium: LAPTg Agar,[25–27] then the plates were incubated in microaerophilia (candle jar system) at 37 °C for 48 h.

Strains of lactic acid bacteria (LAB): L1, L2, L3 and L4 were isolated at the "Laboratorio de Toxicología" at the "Universidad Nacional del Centro de la Provincia de Buenos Aires" Tandil, Buenos Aires, Argentina. LAB strains were kept at −20 °C in LAPTg broth (1.5% peptone, 1% tryptone, 1% glucose, 1% yeast extract, and 0.1% Tween 80), pH 6.8[26] containing 15% glycerol (v/v) until use.

Reactivation process

The strains were defrosted at 37 °C for 15 min, then they were inoculated and incubated in LAPTg broth for 24 h at 37 °C in microaerophilia before genotypic an species identification. Before adsorption process two reactivation times were tested: 24 h (t_1) and 72 h (t_2).

Genotypic identification

The strains isolated for this work L1, L2, L3 and L4, were characterized as lactobacilli by PCR, according to the methodology described by Dubernet et al.[28] Industrial strains provided by Sacco S.A.: *L. rhamnosus* (R1), *Lactobacillus delbrueckii* (R2), *Lactobacillus casei* (R3) and *Lactobacillus helveticus* (R4) were used only for genotypic identification as positive control. Identification was made by amplifying specific region of DNA. The amplification products were visualized by electrophoresis in agarose gels stained with ethidium bromide.[29] General primers for lactobacilli LbLMA1-rev (5′-CTC AAA ACT TTC AAA CAA AGT-3′ and R16-1 5′-CTT GTA CAC ACC GCC CGT CA-3′) were used.[28]

Species identification

Genotypic identification was carried out by partial 16S rRNA gene sequencing. Genomic DNA was extracted according to Pospiech and Neumann.[30] Oligonucleotide primers (PLB16, 5-AGAGTTTGATCCTGGCTCAG-3, and MLB16, 5-GGCTGCTGGCACGTAGTTAG-3) were used to amplify the variable (V1) region of the 16S ribosomal RNA gene according to the protocol described by several authors.[31–33] PCR products were electrophoresed in 1% (wt/vol) agarose gels, stained and visualized as described above. Amplicons were excised from the gel and purified using a GFX PCR DNA gel band purification kit (GE Healthcare, UK). Purified PCR products were sequenced at CERELA-CONICET, Tucumán, Argentina, by using an ABI 3130 DNA sequencer (Applied Biosystems, Foster, CA). rRNA gene sequence alignments were performed using the multiple sequence alignment method[34,35] and

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