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BIOLOGICS

Intestinal toxicity of deoxynivalenol is limited by *Lactobacillus rhamnosus* RC007 in pig jejunum explants

Gisela Romina García[1,2] · Delphine Payros[3] · Philippe Pinton[3] · Cecilia Ana Dogi[1,2] · Joëlle Laffitte[3] · Manon Neves[3] · María Laura González Pereyra[1,2] · Lilia Renée Cavaglieri[1,2] · Isabelle P. Oswald[3]

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Abstract Probiotics have been explored to stimulate gut health in weaned pigs, when they started to consume solid diet where mycotoxins could be present. The aim of this study was to evaluate the effect of *Lactobacillus rhamnosus* RC007 on the intestinal toxicity of deoxynivalenol (DON) in an ex vivo model. Jejunal explants, obtained from 5-week-old crossbred castrated male piglets, were kept as control, exposed for 3 h to 10 µM DON, incubated for 4 h with 10^9 CFU/mL *L. rhamnosus*, or pre-incubated 1 h with 10^9 *L. rhamnosus* and exposed to DON. Histological lesions were observed, para- and transcellular intestinal permeability was measured in Ussing chambers. The expression levels of mRNA encoding six inflammatory cytokines (CCL20, IL-10, IL-1β, TNFα, IL-8 and IL-22) were determined by RT-PCR. The expressions of the phosphorylated MAP kinases p42/p44 and p38 were assessed by immunoblotting. Exposure to DON induced histological changes, significantly increased the expression of CCL20, IL-1β, TNFα, IL-8, IL-22 and IL-10, increased the intestinal paracellular permeability and activated MAP kinases. Incubation with *L. rhamnosus* alone did not have any significant effect. By contrast, the pre-incubation with *L. rhamnosus* reduced all the effects of DON: the histological alterations, the pro-inflammatory response, the paracellular permeability and the phosphorylation of MAP kinases. Of note, *L. rhamnosus* did not adsorb DON and only slightly degrade the toxin. In conclusion, *L. rhamnosus* RC007 is a promising probiotic which, included as feed additive, can decrease the intestinal toxicity of DON.

Keywords Mycotoxin · Deoxynivalenol · Lactobacillus rhamnosus · Intestine · Toxicity

G. R. García and D. Payros equally contributed to this work.

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✉ Isabelle P. Oswald
isabelle.oswald@inra.fr

[1] Departamento de Microbiología e Inmunología, Universidad Nacional de Río Cuarto, Ruta 36 km.601, 5800 Río Cuarto, Córdoba, Argentina

[2] Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Río Cuarto, Argentina

[3] Toxalim (Research Center in Food Toxicology), Université de Toulouse, INRA, ENVT, INP-Purpan, UPS, Toulouse, France

Introduction

The Food and Agricultural Organization estimates that mycotoxins contaminate 25% of the world's agricultural commodities. Mycotoxins have been found in a wide range of food commodities, such as cereals, root crops, nuts and seeds, dairy products and meat products, as well as in animal feeds and ingredients used to produce them. The most significant mycotoxins in naturally contaminated foods and feeds are aflatoxins, ochratoxins, trichothecenes, zearalenone and fumonisins (Bennett and Klich 2003). Among the trichothecenes, deoxynivalenol (DON) is the major mycotoxin produced by *Fusarium* fungi and is commonly found in cereals and grains (Sobrova et al. 2010). Exposure to DON can induce gastrointestinal inflammation and necrosis within the intestinal tract and disturbs the gut barrier function (Cano et al. 2013; Pinton and Oswald 2014). At the molecular level, DON disrupts normal cell function by inhibiting protein synthesis and by activating critical cellular kinases involved in signal transduction related to proliferation, differentiation,

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and apoptosis (Payros et al. 2016). Animal studies showed that acute exposure to high doses of DON results in diarrhea, vomiting, leukocytosis, gastrointestinal hemorrhage and ultimately death, whereas short-term and sub-chronic exposure to DON decreased body weight, weight gain, and feed consumption. Pigs are readily exposed to this toxin through their cereal-rich diet and they are the most sensitive species to this toxin.

Weaning is a stressful event in the pig's life that contributes to intestinal and immune system dysfunctions. It is associated with a dietary change with inclusion of solid diet where mycotoxins can be present. A number of approaches have been reported to control the adverse effects of mycotoxins in animal production systems (Weaver et al. 2013; Gallo et al. 2015). Among the proposed alternatives, probiotics are considered good candidates since they can improve intestinal health by stimulating the immune system and modulating gut microbiota (de Moreno de LeBlanc et al. 2008; Dogi et al. 2008), thereby improving animal health and productivity. For example, previous studies demonstrated that in a mouse model *Lactobacillus rhamnosus* RC007 decreased the gut inflammatory response induced by TNBS (Dogi et al. 2016). In this context, the aim of this study was to evaluate the beneficial effects of *L. rhamnosus* RC007 on the intestinal toxicity of DON in an ex vivo porcine model.

Materials and methods

Toxins

Purified DON was purchased from Sigma-Aldrich (Saint Louis, USA). This toxin was dissolved in water and stored at − 20 °C until use.

Bacterial strains

L. rhamnosus RC007 was previously isolated from maize silage and maintained at the National University of Río Cuarto Collection Centre (Córdoba, Argentina). This lactic acid bacteria strain was identified from both the fermentation pattern (API 50 CHL test) and the 16S rRNA gene sequence. *L. rhamnosus* RC007 was grown at 37 °C for 16 h without agitation in Man, Rogosa and Sharpe (MRS) broth (Sigma-Aldrich), under microaerophilic conditions.

Intestinal jejunal explants

Jejunal explants were obtained from 5-week-old crossbred castrated male piglets as described previously (Lucioli et al. 2013; Pierron et al. 2016a). All animal experiments were performed at Toxalim animal facility under the guidelines of the French Minister of Agriculture (agreement APAFiS#6303_2016080314392462). Three authors (I.P.O., D.P, P.P.) have an official agreement with the French Veterinary Services permitting animal experimentation. Explants were treated (1) with complete William's Medium E (Sigma, Saint Quentin Fallavier, France), control group, (2) exposed to 10^9 CFU/mL *L. rhamnosus* in complete medium, (3) pre-treated with complete medium 1 h and then exposed to 10 µM DON in complete medium or (4) pre-incubated 1 h with 10^9 CFU/mL *L. rhamnosus* and then exposed for 3 h to 10 µM DON in complete medium. The explants were incubated at 39 °C.

Histological assessment

For histological assessment, the explants were treated 3 h before the fixation of the jejunal explants with 10% formalin for 24 h. Then, the tissues were dehydrated and embedded in paraffin according to standard histological procedures. Sections of 5 µm were stained with haematoxylin and eosin (Sigma-Aldrich) for histopathological assessment and intestinal morphometry (villi length). Histological findings were evaluated based on histological parameters and the severity of lesions described in Table 1 of the Electronic Supplementary Material. Images were acquired with a Leica DMRB microscope. Analyses were performed using a MOTIC Image Plus 2.0 MLw image analysis system.

Intestinal permeability

Ex vivo paracellular and transcellular permeability were evaluated in jejunal explants after 3-h treatment. Two jejunal fragments for each animal were mounted in a Ussing chamber (Easy Mount, Physiologic Instruments), exposing a surface area measuring 0.1 cm^2. The specimens were bathed on each side with oxygenated thermostated Krebs solution (Sigma) and after equilibration, fluorescein sodium salt (FSS, 376 Da, Sigma-Aldrich) as a marker of paracellular permeability and horseradish peroxidase (HRP, 44 kDa, Sigma-Aldrich) as a marker of transcellular permeability were added in mucosal compartment at 40 µg/mL and 0.4 mg/mL concentrations, respectively. Epithelial permeability to FSS was determined by measuring the fluorescence intensity 485 nm/525 nm using an automatic Infinite M200 microplate reader (Tecan). Epithelial permeability to intact HRP activity was assessed by an enzymatic assay measuring HRP activity with TMB substrate using a Tecan. Permeability was calculated as a ratio of the flux to the initial concentration, as previously described.

Gene expression analysis by RT-qPCR

For the gene expression analysis, jejunal explants were treated for 4 h and stored at − 80 °C before RNA extraction.

Total RNAs were extracted in lysing matrix D tubes (MP Biomedicals, Illkirch, France) containing guanidine thiocyanate–acid phenol (Eurobio). The quantity of these RNAs was assessed (Nanodrop). Reverse transcription and RT-qPCR steps were performed as already described (Alassane-Kpembi et al. 2017; Pierron et al. 2016b). Primers are indicated in Table 1. Amplification efficiency and initial fluorescence were determined by the ΔC_t method. Obtained values were normalized using two reference genes, ribosomal protein L32 (RPL32) and cyclophilin A. Expression levels of mRNA were expressed relative to the mean of the control.

Immunoblotting

Expression of the phosphorylated MAP kinases p38 and p44/p42 and of the junction proteins were assessed on piglet jejunal explants by immunoblotting as previously described (Meissonnier et al. 2008; Pierron et al. 2016a). Explants were treated for 30 min (1) with complete medium (control), (2) exposed to 10^9 CFU/mL *L. rhamnosus* in complete medium, (3) treated with 10 µM DON in complete medium or (4) pre-incubated 1 h with 10^9 UFC/mL *L. rhamnosus* and then exposed for 30 min to 10 µM DON in complete medium. Proteins were extracted, quantified and separated by SDS-PAGE. For the expression of MAPKinase, the membranes were probed with rabbit antibodies (Cell Signaling Technology, Danvers, USA) specific for phospho-p42/p44 or phospho-p38 diluted at 1:500 or p42/p44 and p38 diluted at 1:1000 overnight at 4 °C. For the expression of junction proteins, mousse or rabbit antibodies specific for ZO-1, Claudin-2, Claudin-3 (Invitrogen, Camarillo, USA), JAM-A, Claudin-2 (Santa Cruz, Dallas, USA), and E-Cadherin (Cell Signaling Technology,) diluted at 1:250 or 1:500 (E-Cadherin) were used. After washing, the membranes were incubated with 1:10,000 CF™770 goat anti-rabbit IgG (Biotium, Hayward, USA) 1 h for the detection. Antibody detection was performed using an Odyssey Infrared Imaging Scanner (Li-Cor Science Tec, Les Ulis, France) with the 770 nm channel. The expression of the proteins was estimated after

Table 1 Primer sequences used for RT-qPCR analysis (F: forward; R: reverse)

Gene symbol	Gene name	Primer sequence	References
Cyclo A	Cyclophilin A	F: CCCACCGTCTTCTTCGACAT R: TCTGCTGTCTTTGGAACTTTGTCT	NM_214353 Pinton et al. (2015)
RPL32	Ribosomal Protein 32	F: AGTTCATCCGGCACCAGTCA R: GAACCTTCTCCGCACCCTGT	NM_001001636 Alassane-Kpembi et al. (2017)
IL-22	Interleukin 22	F: AAGCAGGTCCTGAACTTCAC R: CACCCTTAATACGGCATTGG	AY937228 Cano et al. (2013)
CCL20/MIP3α	C–C Chemokine Ligand 20	F: GCTCCTGGCTGCTTTGATGTC R: CATTGGCGAGCTGCTGTGTG	NM_001024589 Cano et al. (2013)
IL-1β	Interleukin 1 beta	F: ATGCTGAAGGCTCTCCACCTC R: TTGTTGCTATCATCTCCTTGCAC	NM_214055 Pierron et al. (2016b)
IL-8	Interleukin 8	F: GCTCTCTGTGAGGCTGCAGTTC R: AAGGTGTGGAATGCGTATTTATGC	NM_213867 Grenier et al. (2012)
IL-10	Interleukin 10	F: GGCCCAGTGAAGAGTTTCTTTC R: CAACAAGTCGCCCATCTGGT	NM_214041 Cano et al. (2013)
TNF-α	Tumor necrosis Factor alpha	F: ACTGCACTTCGAGGTTATCGG R: GGCGACGGGCTTATCTGA	NM_214022 Grenier et al. (2012)
ZO-1	Zonula Occludens 1	F:ATAACATCAGCACAGTGCCTAAAGC R: GTTGCTGTTAAACACGCCTCG	ENSG00000104067 Current article
Claudin-2	Claudin 2	F: CAGCATGAAATTTGAGATCGGA R:GAGGAAATGATGCCCAAGTAGAGA	ENSG00000165376 Current article
Claudin-3	Claudin 3	F: CTGCTCTGCTGCTCGTGCCC R:TCATACGTAGTCCTTGCGGTCGTAG	ENSG00000165215 Current article
Claudin-4	Claudin 4	F: CTGCTTTGCTGCAACTGCC R:TCAACGGTAGCACCTTACACGTAGT	OTTHUMG00000023425 Pinton et al. (2012)
E-Cadherin	E cadherin	F: ACCACCGCCATCAGGACTC R: TGGGAGCTGGGAAACGTG	ENSSSCG00000006369 Current article
JAM	Junctional Adhesion Molecule A	F: CGTGCCTTCATCAACTCTTCCTATA R:CACAAGTGTAATCTCCAGCATCAGA	ENSSSCG00000025652 Current article

normalization with the non-phosphorylated protein signal or β-actin.

Deoxynivalenol adsorption and degradation in vitro

To test the ability of *L. rhamnosus* RC007 to adsorb DON in vitro, a 24-h culture was obtained inoculating 15 mL of MRS broth and incubating in microaerofilia with 5% CO_2 and 37 °C. After incubation 1 mL aliquots containing 10^9 CFU/mL were transferred to sterile microtubes, centrifuged at 3000 rpm for 10 min and supernatants were removed. Pellets were resuspended in 900 µL sterile phosphate-buffered saline (PBS) and added 200 µL of a 52.90 ng/mL DON solution. Tubes were incubated for 30, 60, 120 and 180 min at 37 °C in triplicates. After incubation, the tubes were centrifuged and DON concentration in the supernatants was quantified by HPLC according to Pascale et al. (2014) with some modifications. Briefly, supernatants containing unbound DON were evaporated to dryness under N_2 flow at 50 °C and residues were redissolved in 300 µL methanol:water (15:85, v/v). Fifty microliter aliquots were injected in a Waters e2695 Separations Module (Waters Corp., Milford, MA, USA) connected to a Waters 2998 Photodiode Array Detector (Waters Corp.) set at 220 nm. The column was a C18 RP Phenomenex Luna (150 × 4.60 mm, 5 µm) (Phenomenex Inc., Torrance, CA, USA). The mobile phase was composed by 95% A: methanol:water (15:85, v/v) and 5% B: acetonitrile:methanol (1:1, v/v) + 0.5% acetic acid, pumped at 1.2 mL/min. A calibration curve was performed injecting 50 µL of DON standard solutions equivalent to 10, 25, 50, 100 and 200 ng DON. Controls tubes containing DON + PBS incubated at the same conditions were also included.

DON degradation by *L. rhamnosus* RC007 enzymes was tested using a cell-free culture supernatant. A 24-h culture was centrifuged at 3000 rpm for 10 min and then filtered through a 0.22-µm Millipore cellulose acetate membrane. Eight-hundred microliter aliquots were transferred to microtubes in triplicates and added 200 µL of 52.90 ng/mL DON solution. Tubes were incubated for 48 h at 37 °C in the darkness and DON concentration was quantified by HPLC as previously described. Controls tubes containing DON and MRS broth without *L. rhamnosus* were included.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 4.0. Differences between the experimental groups were evaluated using one-way analysis of variance (ANOVA) followed by Bonferroni post-test (which allows comparison of all pair of groups). All data were expressed as mean ± standard error of the mean (SEM). A p value below 0.05 was considered as significant.

Results

L. rhamnosus RC007 reduced the histological and alterations induced by DON in jejunal explants

To investigate the ability of *L. rhamnosus* to counteract the effect of DON on intestinal tissue, explants were kept as control, treated with DON, *L. rhamnosus* or both. Control explants, as well as explants treated with *L. rhamnosus*, displayed normal villi lined with columnar enterocytes and microvilli at the top of intestinal cells (Fig. 1a(i), (ii), b, c). After 3-h incubation with 10 µM DON, explants displayed severe histological alterations such as multifocal to diffuse villi atrophy, necrosis, loss of apical enterocytes and loss of microvilli on epithelial cells (Fig. 1a (iii), b, c). Conversely, jejunal explants exposed to *L. rhamnosus* prior to DON treatment showed normal villi length (Fig. 1b, c), columnar intestinal epithelial cells with microvilli, without villi fusion and atrophy (Fig. 1a(iv)).

L. rhamnosus RC007 reduced the paracellular permeability induced by DON ex vivo on jejunal explants without altering the junction proteins

To continue the investigations on the intestinal barrier function, transcellular and paracellular permeability was measured on jejunal explants mounted in Ussing chambers. Transcellular permeability was assessed by measuring the intact horseradish peroxidase (44 kDa) activity and paracellular permeability (< 4 kDa) was determined by measuring the passage of fluorescein salt sodium (376 Da) (Fig. 2). Exposure to 10 µM DON did not increase significantly the transcellular permeability to horseradish peroxidase in piglet jejunal explants as compared to control group or groups treated with *L. rhamnosus* (Fig. 2a). However, exposure to 10 µM DON significantly increased the paracellular permeability (17.59 ± 4.9 FSS cm/s 10^{-7} in DON-treated explants versus 7.93 ± 1.75 FSS cm/s 10^{-7} in control group; $p < 0.001$). By contrast, no significant increase of paracellular permeability to fluorescein salt sodium was observed in jejunal explants treated with *L. rhamnosus* and 10 µM DON as compared to control group but a significant decrease was observed in the group treated with *L. rhamnosus* and DON (12.28 ± 5.23 FSS cm/s 10^{-7} in *L. rhamnosus* and DON-treated explant versus 17.59 ± 4.9 FSS cm/s 10^{-7} in DON-treated explants, $p < 0.05$) (Fig. 2b).

The next step was to characterize the effect of the probiotic strain *L. rhamnosus* on the expression of junction proteins in jejunal explants treated or not with DON (Table 2). Exposure to *L. rhamnosus*, DON and the co-exposure to *L. rhamnosus* and DON both did not affect the expressions of the 6 junction proteins tested (E-cadherin, ZO-1,

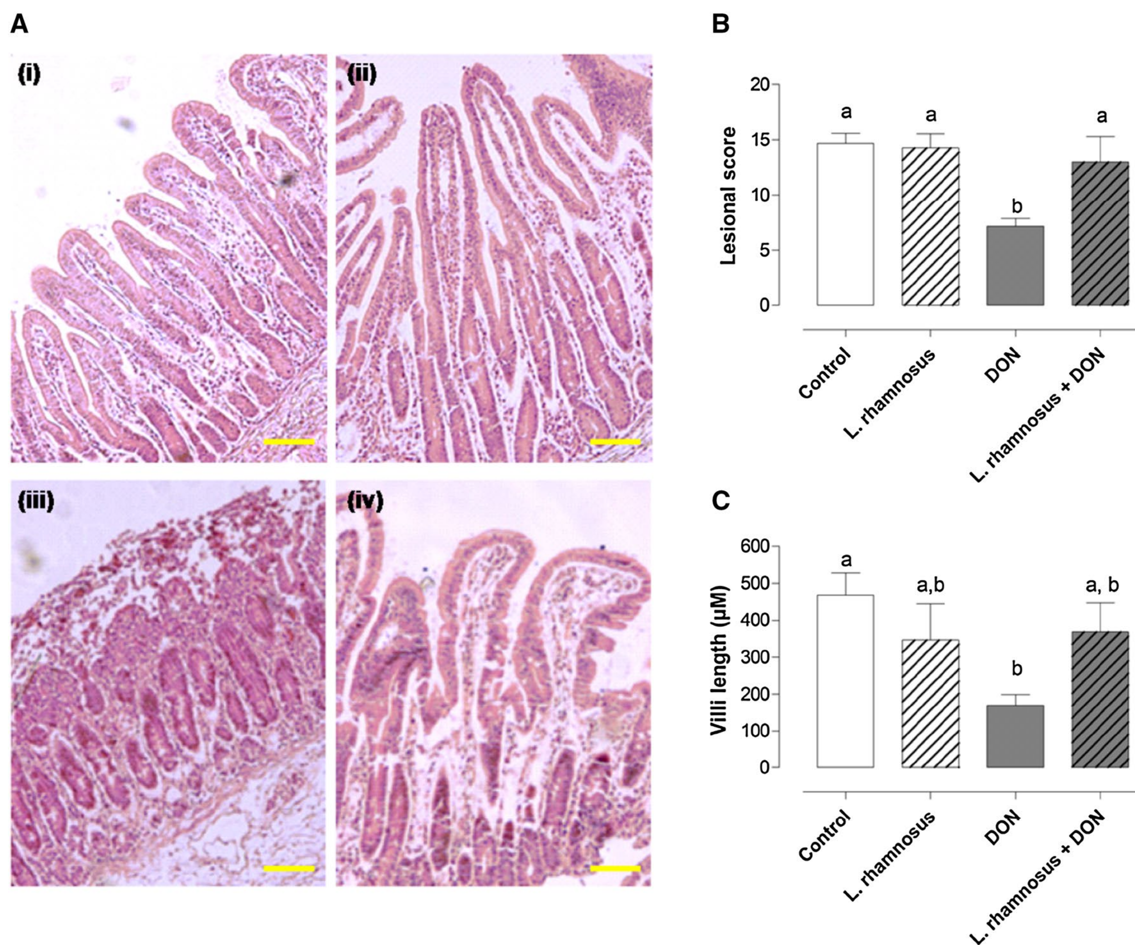

Fig. 1 Effects of *L. rhamnosus* and DON on intestinal explants. **a** Morphology, **b** lesional score and **c** villi length. Jejunal explants were exposed (i) with complete medium (control), (ii) with 10^9 CFU/mL *L. rhamnosus* in complete medium, (iii) pre-treated with complete medium 1 h and then exposed to 10 µM DON in complete medium or (iv) pre-incubated 1 h with 10^9 CFU/mL *L. rhamnosus* and then exposed 3 h to 10 µM DON in complete medium. Yellow bars represented 10 µm. Results represent mean and SEM of explants from six animals. One-way ANOVA with Bonferroni's multiple comparisons

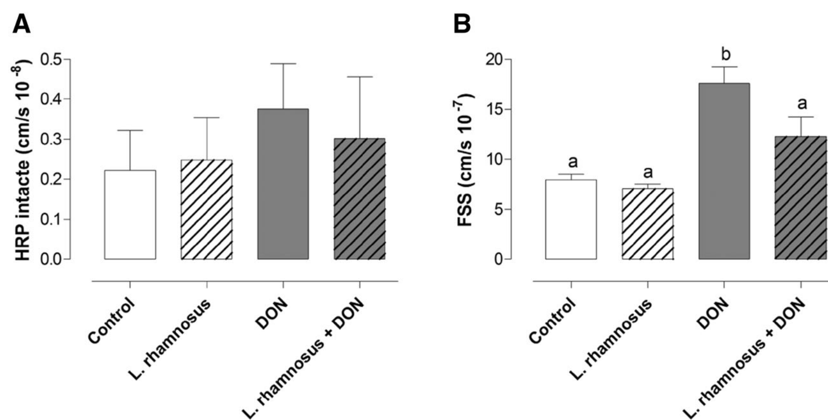

Fig. 2 Effects of *L. rhamnosus* and DON on intestinal transcellular (**a**) and paracellular (**b**) permeability in jejunal explants. Jejunal explants were exposed (i) with complete medium (control), (ii) with 10^9 CFU/mL *L. rhamnosus* in complete medium, (iii) pre-treated with complete medium 1 h and then exposed to 10 µM DON in complete medium or (iv) pre-incubated 1 h with 10^9 CFU/mL *L. rhamnosus* and then exposed to 10 µM DON in complete medium. After 3 h of incubation, transcellular (HRP) and paracellular (FSS) permeabilities were quantified. Results represent mean and SEM of explants from six animals. One-way ANOVA with Bonferroni's multiple comparisons

Table 2 Effect of DON and *L. rhamnosus* on the expressions of the genes and proteins of tight junctions in jejunal explants

	Jejunal treatment			
	Control	*L. rhamnosus*	DON	*L. rhamnosus* + DON
Gene expression ($2\Delta C_t$)				
Claudin-2	0.09 ± 0.01[a]	0.17 ± 0.04[ab]	0.13 ± 0.02[ab]	0.25 ± 0.04[b]
Claudin-3	0.02 ± 0.004[a]	0.06 ± 0.01[ab]	0.02 ± 0.005[a]	0.07 ± 0.02[b]
Claudin-4	0.08 ± 0.02[a]	0.11 ± 0.02[a]	0.13 ± 0.02[a]	0.14 ± 0.02[a]
E-cadherin	1.7 ± 0.7[a]	1.5 ± 0.32[a]	2.0 ± 0.77[a]	1.32 ± 0.56[a]
ZO-1	0.02 ± 0.005[a]	0.02 ± 0.002[a]	0.02 ± 0.005[a]	0.02 ± 0.004[a]
JAM	0.10 ± 0.008[ab]	0.06 ± 0.01[b]	0.13 ± 0.01[a]	0.08 ± 0.01[b]
Protein expression (A. U.)				
Claudin-2	0.21 ± 0.02[a]	0.24 ± 0.04[a]	0.22 ± 0.02[a]	0.19 ± 0.02[a]
Claudin-3	0.30 ± 0.05[ab]	0.28 ± 0.06[ab]	0.47 ± 0.08[a]	0.19 ± 0.05[b]
Claudin-4	4.01 ± 0.74[a]	2.61 ± 0.34[a]	3.21 ± 0.40[a]	2.45 ± 0.40[a]
E-cadherin	0.07 ± 0.01[a]	0.06 ± 0.01[a]	0.08 ± 0.02[a]	0.04 ± 0.01[a]
ZO-1	0.04 ± 0.005[a]	0.03 ± 0.01[a]	0.03 ± 0.004[a]	0.02 ± 0.01[a]
JAM	0.04 ± 0.01[a]	0.05 ± 0.02[a]	0.05 ± 0.01[a]	0.04 ± 0.01[a]

Data are mean ± SEM. [a, b] Within a line, values not sharing a common superscript are significantly different ($p < 0.05$)

JAM, Claudin-2, -3 and -4) in jejunal explants. This lack of effect was observed both at the mRNA and the protein levels.

L. rhamnosus RC007 reduced the expression of pro-inflammatory cytokines induced by DON

To deeper investigate the protective effect of *L. rhamnosus*, the expression of several pro-inflammatory genes was assessed by qPCR (Fig. 3). As expected, DON increased significantly the expression of IL-1β, TNFα, IL-22 and IL-8 pro-inflammatory cytokine genes as compared to control group. An increase of the chemokine gene expression CCL20 and the anti-inflammatory cytokine IL-10 was also observed. By contrast, treatment of jejunal explants with *L. rhamnosus* and DON did not induce an increase in the expression of inflammatory genes.

L. rhamnosus RC007 reduced the phosphorylation of MAP kinases p42/p44 and p38 induced by DON

The ability of *L. rhamnosus* to counteract the effects of DON on the activation of the MAP kinase was then examined. After 30 min of exposure to 10 µM of DON, MAP kinases were activated in jejunal explants (Fig. 4). DON significantly increased phosphorylated p42/p44 and phosphorylated p38 compared to control condition. By contrast, pre-exposure of jejunal explants to *L. rhamnosus*, did not activate these MAP kinases, even in the presence of DON.

Deoxynivalenol adsorption and degradation in vitro

When the ability of *L. rhamnosus* RC007 to adsorb DON was tested, no significant effect was observed at any of time point. Of note, cell-free supernatant from *L. rhamnosus* RC007 culture was able to degrade 13% DON when incubated for 48 h at 37 °C (Table 3).

Discussion

The epithelial barrier of the gastrointestinal tract is the initial line of defense against orally ingested bacterial and viral pathogens, as well as food- and feed-derived toxins. After ingestion, DON induces several adverse effects including an alteration of gut integrity and the induction of an inflammatory response (Akbari et al. 2014; Pinton and Oswald 2014). One of the many strategies to counteract the harmful effect of mycotoxins is the addition of probiotic to animal diet. Probiotics added in feed improve performance, promote metabolic processes of digestion and nutrient utilization, and modulate intestinal barrier and immune responses (Hardy et al. 2013). Nevertheless, the magnitude of the effect seems to be strain dependent. In the present work, the effects of the probiotic strain *L. rhamnosus* RC007 on DON intestinal toxicity using a jejunal explants model were investigated. In the context of reducing the number of experimental animals, intestinal explants represent a powerful model and have been introduced as a model to test intestinal integrity since allows to preserving normal histological structure in vitro (Nietfeld et al. 1991). This model was used to investigate the effects of food contaminants (Lucioli et al. 2013; Alassane-Kpembi

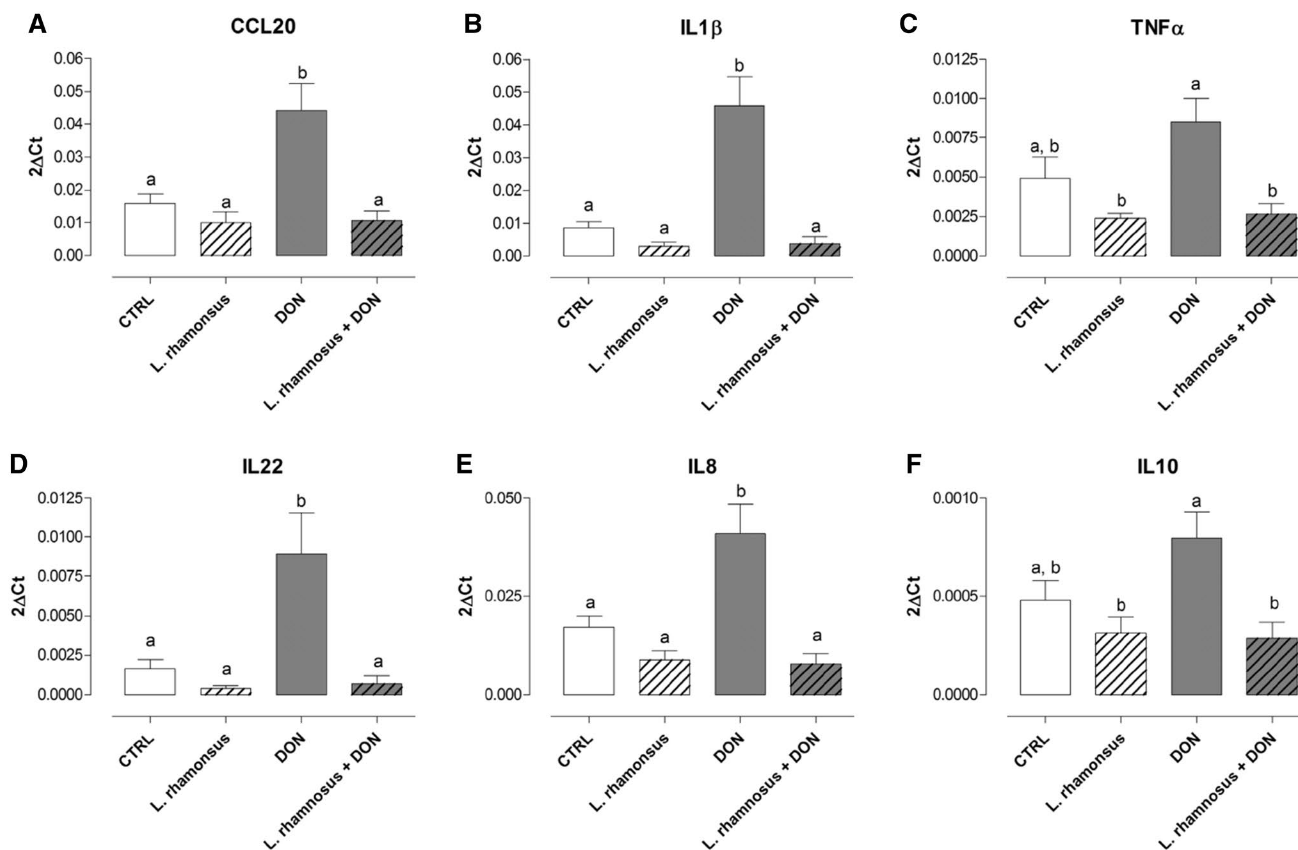

Fig. 3 Effects of *L. rhamnosus* and DON on intestinal inflammation. Jejunal explants were exposed (i) with complete medium (control), (ii) with 10^9 CFU/mL *L. rhamnosus* in complete medium, (iii) pre-treated with complete medium 1 h and then exposed to 10 µM DON in complete medium or (iv) pre-incubated 1 h with 10^9 CFU/mL *L. rhamnosus* and then exposed to 10 µM DON in complete medium. After 3 h of incubation, gene expression was assessed. Relative expression of mRNA encoding for pro-inflammatory cytokines and chemokines was measured by RT-qPCR. Data are normalized to housekeeping gene RPL32 and expressed, in arbitrary unit, as mean and SEM of explants from six animals. One-way ANOVA with Bonferroni's multiple comparisons

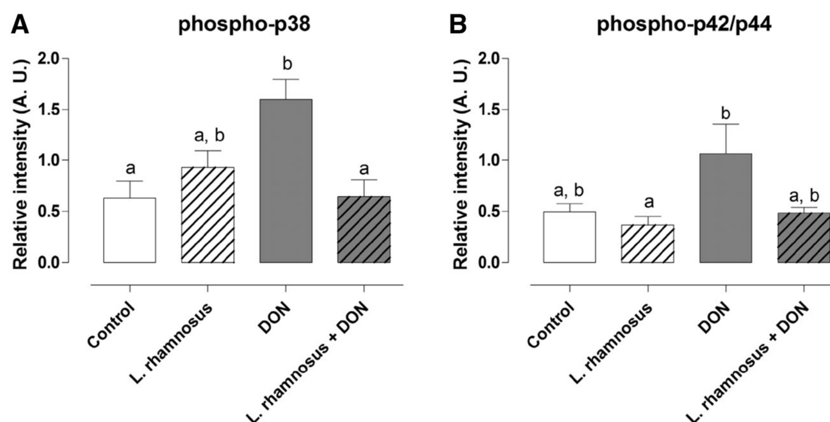

Fig. 4 Effects of *L. rhamnosus* and DON on activation of MAP kinases in jejunal explants. Samples were pre-exposed with *L. rhamnosus* 1 h before treatment 30 min with 10 µM of DON and analyzed by western blot for expression of phosphorylated p42/p44 (**a**), phosphorylated p38 (**b**) or non-phosphorylated forms of p42/p44 and p38, used as a protein loading control. Normalized expression graphs were represented. Results as expressed as mean and SEM of explants from six animals. One-way ANOVA with Bonferroni's multiple comparisons

Table 3 Biological degradation of DON by *Lactobacillus rhamnosus* RC007

Treatment	Final DON level (ng)	% of degradation
Control	4691.07 ± 25.6[a]	0
L. rhamnosus RC007	4065.84 ± 26.16[b]	13.33

Deoxynivalenol degradation was tested using a cell-free culture supernatant. DON concentration was quantified by HPLC

Values with different letter are significantly different according to Fisher's protected LSD test ($p < 0.05$)

et al. 2017). In the present study, as previously observed, the treatment of jejunal explants with 10 µM DON caused important changes on the intestinal barrier function (Lucioli et al. 2013; Ghareeb et al. 2015). The mycotoxin not only altered the intestinal histomorphology but also increased the intestinal permeability, mainly at the paracellular level. The observed effects of DON on the impairment of the intestinal morphology may have consequences on absorption and nutrient intake by intestinal epithelial cells. Consequently, energy and nutrient uptake can be adversely affected leading to growth deficiencies in animals. Intestinal epithelial cells form a monolayer that constitutes a dynamic and selective barrier, and mediates the transport of molecules in two ways, either across the cells (i.e., the transcellular pathway) or between them (i.e., the paracellular pathway). Effects in the paracellular pathway suggest an adverse effect on tight junctions and could result in an increased translocation of luminal antigens and pathogens and an excessive activation of the immune system (Grenier and Applegate 2013).

The *Lactobacillus* concentration used in this study correspond to a typical dose of probiotic administration in human and animals (Yang et al. 2015), and this dose was previously demonstrated in vivo to produce beneficial effects in mice (Dogi et al., 2016). The dose of 10 µM DON was chosen as it causes important changes on the intestinal barrier function (Lucioli et al. 2013; Ghareeb et al. 2015; Pierron et al. 2016b). This concentration is realistic when considering human and animal exposure to DON. In human gut, concentrations of 0.16–2 µg DON/mL (0.5–7 µM) can be considered as realistic. The lower concentration value corresponds to the mean estimated daily intake of French adult consumers on a chronic basis. The higher concentration value simulates level that can be reached after the consumption of heavily contaminated food, as can be occasionally encountered (Alassane-Kpembi et al. 2013). Animal, especially pigs can be exposed higher concentrations of DON. Assuming that DON is ingested in one meal, diluted in 1 L of gastrointestinal fluid and is totally bioaccessible, the in vitro concentration of 10 µM used in this study correspond to feed contaminated with 3 mg DON/kg (Pinton et al. 2009). In a recent scientific opinion, EFSA reported levels of DON in feed grains up to 9.5 mg/kg (EFSA 2017).

The pre-incubation of explants with *L. rhamnosus* was able to counteract the adverse effects of DON on the intestine as seen by the restored villi length, the columnar intestinal epithelial cells with microvilli and the restored paracellular activity. These results indicate a protective effect of this bacterium. It is believed that a probiotic improves the metabolic processes due to improved development of the gut and increased microvilli height leading to the enlargement of the microvilli's absorptive surface and enables the optimal utilization of nutrients (Ezema 2013). A previous study demonstrated a beneficial effect of *L. rhamnosus* RC007on TNBS-induced colitis with less macroscopic and histologic damages in the large intestine of mice (Dogi et al. 2016). Other studies have reported a beneficial effect of probiotics on intestinal paracellular permeability in different chemically induced colitis models (Osman et al. 2008; Southcott et al. 2008).

The health-promoting properties of probiotics are multiple and include their capacity to activate/modulate the host immune system. Following consumption of probiotic products, the interaction of these bacteria with intestinal enterocytes initiates a host response, since intestinal cells produce various immunomodulatory molecules when stimulated by bacteria in a strain-dependent manner (Galdeano et al. 2007; de Moreno de LeBlanc et al. 2008; Dogi et al. 2008, 2010). In the present study, we observed, as was previously described, a significant expression of pro-inflammatory cytokines in DON-treated intestinal explants (Pierron et al. 2016a; Cano et al. 2013). The pre-incubation of the explants with *L. rhamnosus* RC007 was able to counteract this inflammatory effect. Immunomodulatory properties of this strain were already described; it stimulates the immune system in a healthy host and modulates the inflammatory immune response in a mice model of TNBS colitis (Dogi et al. 2016).

Mitogen-activated protein kinases (MAPKs) mediate a wide variety of cellular behaviors in response to extracellular stimuli (Widmann et al. 1999). The p38 MAP kinases, such as cell proliferation, cell differentiation, cell death, cell migration and invasion, has been implicated in several complex biologic processes (Koul et al. 2013). The p44/42 ERK is of particular interest because it can be involved in intestinal epithelial cell morphology and in the structure of tight junctions. Upon DON exposure, a ribotoxic stress response is induced that results in the activation of MAPKs. This mediates apoptosis, aberrant up-regulation of pro-inflammatory cytokines and chemokines, the disruption of the intestinal barrier and an alteration of the tight junction network (Pestka 2010; Pan et al. 2013; Pinton et al. 2015; Springler et al. 2016). The pre-incubation with *L. rhamnosus* RC007 was able to inhibit the signaling pathway and to decrease MAPKs activation. Numerous evidence demonstrate that probiotics bacteria

communicate with the host by modulating key signaling pathways, such as NFκB and MAPK, to either enhance or suppress activation and influence downstream pathways (Kelly et al. 2004; Resta-Lenert and Barrett 2006; Kim et al. 2008; Watanabe et al. 2009). Similar mode of action was demonstrated with probiotic yeast (Chang et al. 2017).

Barrier property of the intestine is achieved by intercellular junctions that include apical tight junction proteins, such as claudins, ZO and JAM, and subjacent adherens junctions such as E-cadherin (Groschwitz and Hogan 2009). The protective effect of *L. rhamnosus* was not mediated by these molecules as demonstrated by the absence of their regulation whatever the treatment. Previous studies have observed an alteration of claudin, cadherin and ZO-1 in rats or pigs fed DON contaminated diet as well as in intestinal cells treated with the mycotoxin (Pinton et al. 2012; Pinton and Oswald 2014; Akbari et al. 2014). In the present study, we did not observe any of these effects in explants treated with of DON. The difference might be due to reduced exposure length, 4 h, in explants when compared to feeding trials or prolonged exposure of intestinal epithelial cells. Alternatively, as described for other mycotoxins, DON might only relocalize the junction, which could then be restored by *L. rhamnosus* (Lambert et al. 2007; Menningen et al. 2009). The western blot does not allow investigating this hypothesis.

The mechanism by which *L. rhamnosus* transduces its health benefits to the host is still under study. The present paper indicates that this strain does not absorb DON. Moreover, the minimal ability of this strain to degrade DON is too low to explain the observed effect. Previous studies reported microbial transformation of DON into DOM-1 in several animal species as well as in humans (Döll and Dänicke 2004; Turner et al. 2010). In the present study, the modified form of DON obtained after *L. rhamnosus* degradation was not determined. Our data suggest that *L. rhamnosus* interact with MAPK proteins helping to maintain normal function of the gut mucosa, not only enhancing barrier function but also modulating the gut immune response, since MAPKs are involved in regulation of multiple biological processes including development, apoptosis and immunity (Yan and Polk 2002; Jijon et al. 2004; Joshi and Platanias 2012). DON is known to alter the intestinal microbiota (Waché et al. 2009; Robert et al. 2017). Although it was not the objective of this study, we can hypothesis that *L. rhamnosus* restores the gut microbiota, known for its importance for human and animal health.

In conclusion, our results demonstrated that *L. rhamnosus* counteracts the intestinal toxic effects of DON and helps to maintain a healthy gastrointestinal tract. This is crucial as it ensures that nutrients are absorbed at an optimum rate, it provides efficient protection against pathogens, and maintains the indigenous microbiota in adequate numbers and confined to their natural niches.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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