Veterinary Immunology and Immunopathology xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

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

Short communication

Saccharomyces cerevisiae decreases inflammatory responses induced by F4+ enterotoxigenic *Escherichia coli* in porcine intestinal epithelial cells

Galliano Zanello[a,b,1], François Meurens[a,1], Mustapha Berri[a], Claire Chevaleyre[a], Sandrine Melo[a], Eric Auclair[b], Henri Salmon[a,*]

[a] Institut National de la Recherche Agronomique (INRA), UR1282, Infectiologie Animale et Santé Publique, F-37380 Nouzilly (Tours), Indre et Loire, France
[b] Société Industrielle Lesaffre, Lesaffre Feed Additives, Marcq-en-Baroeul, France

ARTICLE INFO

Article history:
Received 19 October 2010
Received in revised form
13 December 2010
Accepted 31 January 2011

Keywords:
Saccharomyces cerevisiae
Enterotoxigenic *Escherichia coli*
Pig
Intestinal epithelial cells
Cytokines
Chemokines

ABSTRACT

Probiotic yeasts may provide protection against intestinal inflammation induced by enteric pathogens. In piglets, infection with F4+ enterotoxigenic *Escherichia coli* (ETEC) leads to inflammation, diarrhea and intestinal damage. In this study, we investigated whether the yeast strains *Saccharomyces cerevisiae* (*Sc*, strain CNCM I-3856) and *S. cerevisiae* variety *boulardii* (*Sb*, strain CNCM I-3799) decreased the expression of pro-inflammatory cytokines and chemokines in intestinal epithelial IPI-2I cells cultured with F4+ ETEC. Results showed that viable *Sc* inhibited the ETEC-induced TNF-α gene expression whereas *Sb* did not. In contrast, killed *Sc* failed to inhibit the expression of pro-inflammatory genes. This inhibition was dependent on secreted soluble factors. *Sc* culture supernatant decreased the TNF-α, IL-1α, IL-6, IL-8, CXCL2 and CCL20 ETEC-induced mRNA. Furthermore, *Sc* culture supernatant filtrated fraction <10 kDa displayed the same effects excepted for TNF-α. Thus, our results extended to *Sc* (strain CNCM I-3856) the inhibitory effects of some probiotic yeast strains onto inflammation.

1. Introduction

Enterotoxigenic *Escherichia coli* (ETEC) are pathogenic gram negative bacteria which infect humans and several species of farm animals such as calves and pigs. ETEC interacts with intestinal epithelial cells, colonizes the small intestine and secretes enterotoxins such as the heat-labile enterotoxins (LT), the heat-stable enterotoxins (STa and/or STb), and the enteroaggregative *E. coli* heat-stable enterotoxin 1 (EAST1) (Nagy and Fekete, 2005). In pigs, ETEC infection and enterotoxin secretions can induce intestinal inflammation and diarrhea resulting in reduced growth rate, increased mortality and economic loss (Fairbrother et al., 2005). Moreover, F4+ ETEC strain induce pro-inflammatory response in intestinal epithelial cells (Devriendt et al., 2010). Administration of the yeast *Saccharomyces cerevisiae* variety *boulardii* (*Sb*) has been shown to protect pigs in reducing ETEC translocation (Lessard et al., 2009). *In vitro* studies showed that *Sb* secretes soluble factors that decrease the expression of pro-inflammatory cytokines induced by enteric pathogens (Zanello et al., 2009). However, to our knowledge, there is no *in vitro* data regarding the anti-inflammatory effects of *S. cerevisiae* (*Sc*) secreted soluble factors. *Sc* and *Sb* are members of the same species but they differ genetically, metabolically and physiologically (Edwards-Ingram et al., 2007; Hennequin et al., 2001). Thus, in this study, we assessed if the non-commensal and non-pathogenic yeasts *Sc* (strain CNCM I-3856) and *Sb* (strain CNCM I-3799) secreted factors allowing the down-regulation of pro-inflammatory gene expression in intestinal epithelial cells cultured with F4+ ETEC. *Sc* (strain CNCM I-3856)

* Corresponding author. Tel.: +33 247427331; fax: +33 247427779.
E-mail addresses: salmon@tours.inra.fr, henri.salmon@tours.inra.fr (H. Salmon).
[1] These authors contributed equally to this work.

0165-2427/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetimm.2011.01.018

Please cite this article in press as: Zanello, G., et al., *Saccharomyces cerevisiae* decreases inflammatory responses induced by F4+ enterotoxigenic *Escherichia coli* in porcine intestinal epithelial cells. Vet. Immunol. Immunopathol. (2011), doi:10.1016/j.vetimm.2011.01.018

was selected since this yeast has been shown to decrease inflammation in a mouse model of induced colitis (Foligne et al., 2010) suggesting that *Sc* may provide health benefits against enteritis. *Sb* (strain CNCM I-3799) was selected to identify if this yeast presented anti-inflammatory effects as described for the previously studied *Sb* probiotic strain (Dalmasso et al., 2006; Martins et al., 2010).

2. Materials and methods

2.1. Cell, bacteria and yeast cultures

The porcine small intestine epithelial cell line IPI-2I (ECACC93100622) was transformed from epithelial ileal cells of an adult boar (SLAd/d haplotype) (Kaeffer et al., 1993). IPI-2I cells were cultured in DMEM cell culture medium as described previously (Meurens et al., 2009).

ETEC 56190 strain (K88ad, O8: K87: H19) was grown in Luria–Bertani (LB) medium containing 1% tryptone, 0.5% yeast extract, 1% NaCl, pH 7. After overnight incubation at 37 °C with vigorous shaking, bacteria were diluted at 1:400 in fresh LB and grown until midlog phase (~4 h) for all experiments.

Active forms of *Sc* (strain CNCM I-3856) and *Sb* (strain CNCM I-3799) (CNCM: French National deposit Collection of Microorganism Cultures, Institut Pasteur, Paris, France) were provided dried by Lesaffre (Société Industrielle Lesaffre, 147 rue Gabriel Péri, BP 6027, Marcq-en-Baroeul cedex, France) at a concentration of 1×10^{10} yeasts/g. Yeasts were rehydrated in free-DMEM for 45 min, at 30 °C. For the different experiments, *Sc* and *Sb* concentrations were established by yeast coloration with methylene blue buffer (0.3 g/L methylene blue, 20 g/L sodium citrate) and quantification of viable yeasts by coloration exclusion.

2.2. Preparation of killed yeasts and yeast culture supernatants

Sc was killed by ten cycles of freezing/thawing in liquid nitrogen. Viability was controlled by methylene blue coloration and mortality by exclusion. *Sc* freezing resulted in 100% of dead yeasts.

Sc culture supernatant (ScS) and *Sb* culture supernatant (*SbS*) were prepared as described previously with slight modifications (Chen et al., 2006). Yeasts were cultured overnight in DMEM cell culture medium with moderate shaking at 37 °C, and at an initial concentration of 7.5×10^8 yeasts/L. To separate yeasts from the supernatant, the preparation was centrifuged at $9000 \times g$, for 15 min, at 4 °C, and then filtered through a 0.22 μm pore filter (Sartorius Stedim France) resulting in *ScS*. After a further filtration through a 10 kDa cut-off filter (Vivaspin 20 centrifugal concentrator, Sigma–Aldrich), the resulting filtrate was designated as *Sc* ultrafiltrate supernatant <10 kDa (*S* < 10 kDa).

2.3. IPI-2I infection by ETEC

IPI-2I cells were seeded in 6-well plates (Falcon) and cultured for 48 h until confluence ($\sim 1 \times 10^6$ cells/well). After 3 washes with DMEM medium, IPI-2I cells were incubated overnight with *Sc* or *Sb* (3×10^6 yeasts/well) in 4 mL of both antibiotics and FCS-free DMEM. Then, ETEC (1×10^7 CFU/well) was added for 3 h. The yeast concentration was chosen based on preliminary experiments indicating that this concentration was required to inhibit significantly the ETEC-induced pro-inflammatory gene expression. To investigate the effect of *Sc* viability, IPI-2I cells were pre-incubated overnight with killed *Sc* before ETEC addition. To evaluate the effects of supernatants, 4 mL of *ScS*, *S* < 10 kDa or *SbS* were incubated simultaneously with ETEC (1×10^7 CFU/well) for 3 h. The culture supernatant contained the soluble factors produced by an overnight yeast culture at 3×10^6 yeasts/4 mL.

2.4. Analysis of relative mRNA expression using quantitative real-time PCR

IPI-2I cells were lyzed with Trizol reagent (Invitrogen) and total RNA was isolated using RNeasy Mini Kit (Qiagen). Reverse transcription and quantitative real-time PCR (RT-qPCR) were performed using cDNA synthesized as previously described (Bruel et al., 2010). Real time assays were run on a Bio-Rad Chromo4 (Bio-Rad). To minimize sample variations, we used high quality RNA which was assessed by calculating OD_{260}/OD_{280} (≈2) and by capillary electrophoresis (Agilent 2100 Bioanalyzer, Agilent Technologies, Inc., Santa-Clara, USA). Samples were normalized internally using simultaneously the average cycle quantification (*Cq*) of multiple reference genes. Hypoxanthine PhosphoRibosyl-Transferase 1 (HPRT-1), Ribosomal Protein L 19 (RPL-19) and Hydroxymethylbilane synthase 2 (HMBS2) were used as reference genes given to their stability in porcine intestinal epithelial cells (Bruel et al., 2010; Meurens et al., 2009). Expression data are expressed as relative values after Genex macro analysis (Bio-Rad) (Vandesompele et al., 2002). Primer sequences and annealing temperatures of analyzed genes were reported previously (Bruel et al., 2010).

2.5. Statistical analysis

The comparison of the differences in mRNA relative expression was evaluated by one-way ANOVA and differences tested by non-parametric Dunnett's test (using GraphPad Prism software version 4.00, GraphPad Software Inc., San Diego, CA, USA). Differences were considered significant when $P < 0.05$.

3. Results and discussion

To identify if the yeasts *Sc* and *Sb* inhibit pro-inflammatory cytokine and chemokine expressions in IPI-2I cells, we first assessed their ability to decrease the ETEC-induced TNF-α gene expression as a positive control of inhibition. As shown in Fig. 1, ETEC up-regulated between 5 and 15 folds the TNF-α gene expression in IPI-2I cells ($P < 0.01$). IPI-2I cells were chosen in this study as a model for intestinal epithelial cells but *in vitro* adhesion assay displayed that the $F4^+$ ETEC strain 56190 did not adhere to IPI-2I cells (data not shown). This result sug-

Please cite this article in press as: Zanello, G., et al., *Saccharomyces cerevisiae* decreases inflammatory responses induced by $F4^+$ enterotoxigenic *Escherichia coli* in porcine intestinal epithelial cells. Vet. Immunol. Immunopathol. (2011), doi:10.1016/j.vetimm.2011.01.018

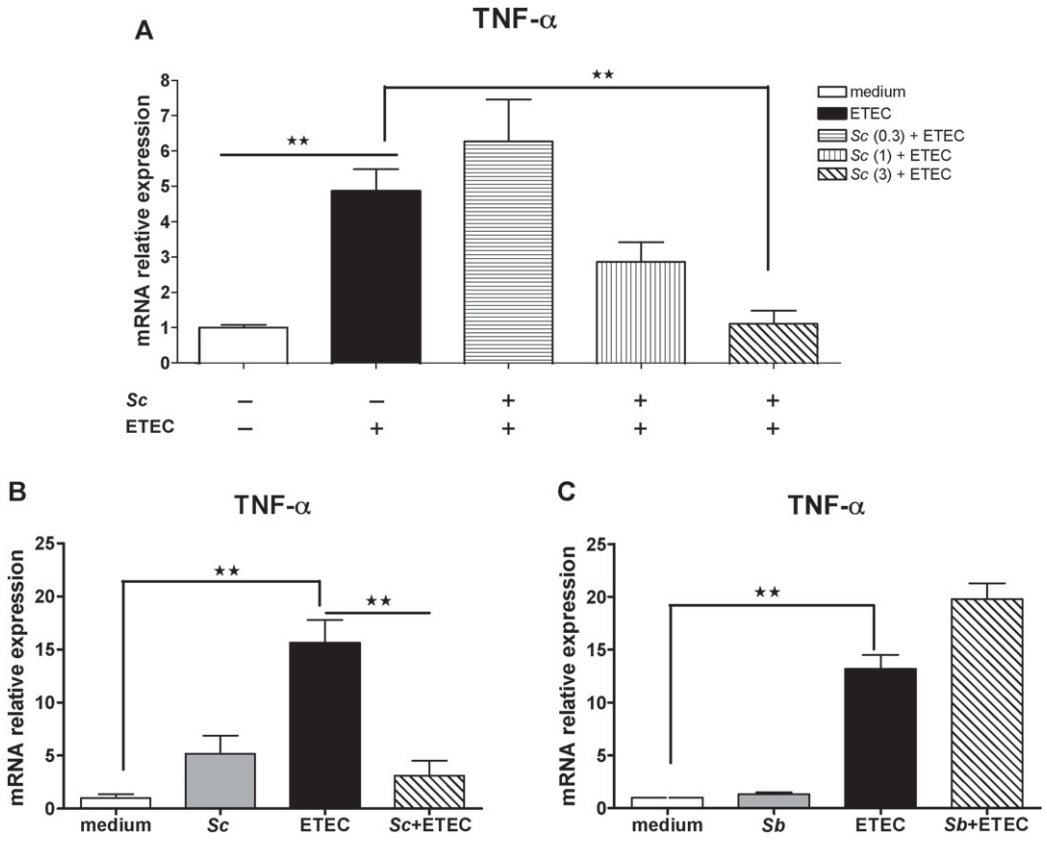

Fig. 1. Effects of *Sc* and *Sb* on TNF-α gene expression in IPI-2I cells cultured with ETEC. (A) IPI-2I cells (1×10^6 cells/well) were pre-incubated overnight with different concentrations of *Sc* (0.3-1-3 $\times 10^6$ yeasts/well) and then ETEC (1×10^7 CFU/well) was added to the co-culture for 3 h in order to identify the yeast inhibitory effect on TNF-α gene expression. (B) and (C) IPI-2I cells (1×10^6 cells/well) were pre-incubated overnight with either *Sc* or *Sb* (3×10^6 yeasts/well) and then ETEC (1×10^7 CFU/well) was added to the co-culture for 3 h in order to compare the inhibitory effect on both yeasts on TNF-α gene expression. Data are presented as means of TNF-α mRNA relative expressions ± SEM ($n = 6$). Results are representative of 3 independent experiments. $^{**}P < 0.01$.

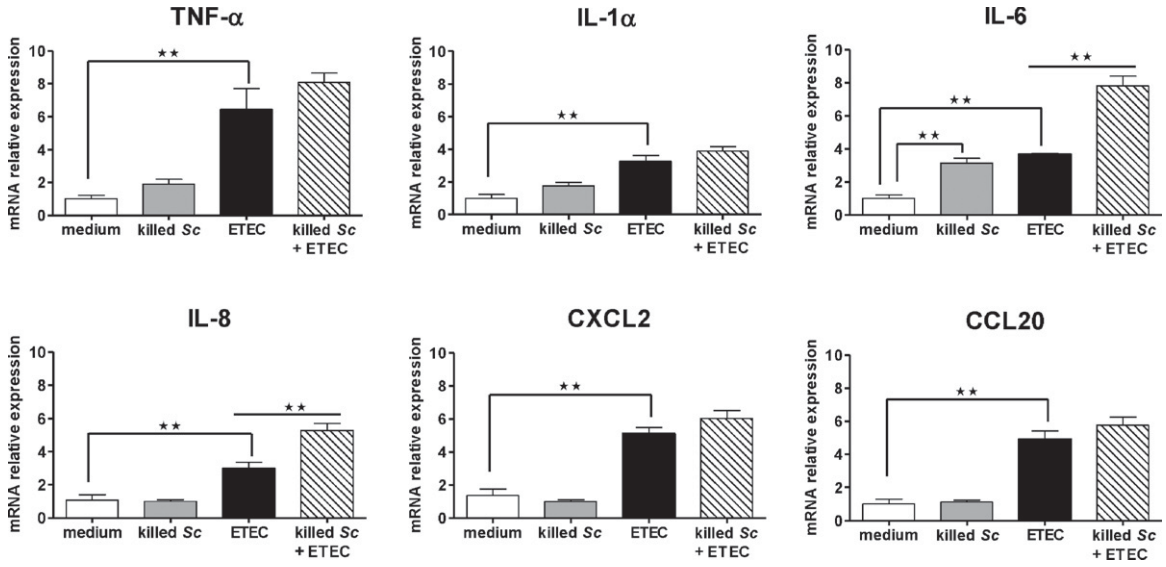

Fig. 2. Effects of *Sc* viability on cytokine and chemokine mRNA expressions in IPI-2I cells cultured with ETEC. IPI-2I cells (1×10^6 cells/well) were pre-incubated overnight with killed *Sc* (3×10^6 yeasts/well) and then ETEC (1×10^7 CFU/well) was added to the co-culture for 3 h. Data are presented as means of mRNA relative expressions ± SEM ($n = 5$). $^{**}P < 0.01$.

Please cite this article in press as: Zanello, G., et al., *Saccharomyces cerevisiae* decreases inflammatory responses induced by F4+ enterotoxigenic *Escherichia coli* in porcine intestinal epithelial cells. Vet. Immunol. Immunopathol. (2011), doi:10.1016/j.vetimm.2011.01.018

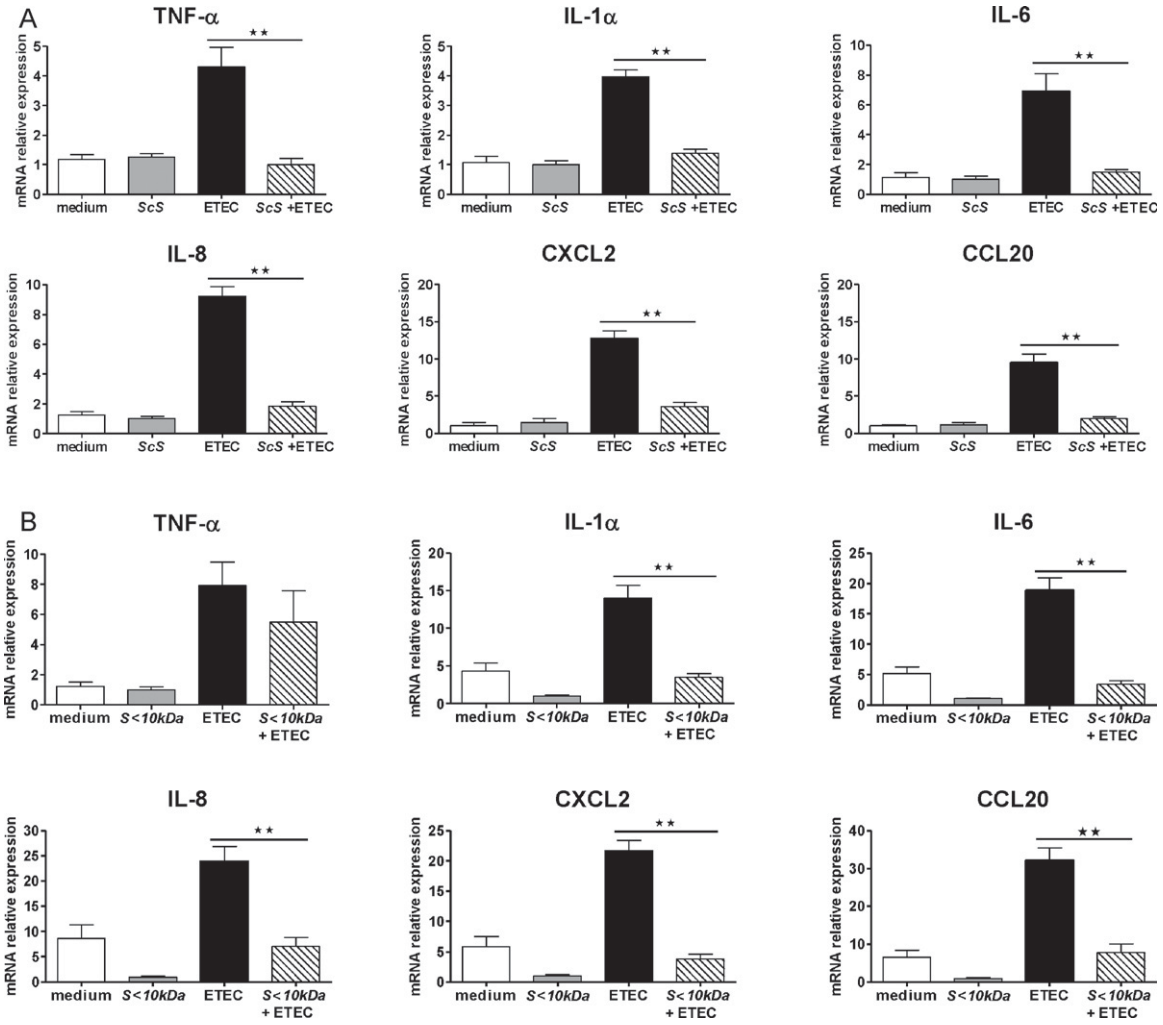

Fig. 3. Effects of the *Sc* culture supernatant (*ScS*) and the ultrafiltrated fraction (*S < 10* kDa) on cytokine and chemokine mRNA expressions in IPI-2I cells cultured with ETEC. IPI-2I cells (1×10^6 cells/well) were cultured with (A) *ScS* or (B) *S* < 10 kDa simultaneously with ETEC (1×10^7 CFU/well) for 3 h. Data are presented as means of mRNA relative expressions ± SEM ($n = 5$). Results are representative of 2 independent experiments. $^{**}P < 0.01$. In each graph, IPI-2I cells exposure to ETEC increased significantly mRNA levels of cytokines and chemokines as compared to medium but no sign of significance (asterisk) was added.

gested that the up-regulation of TNF-α mRNA level could be due to bacterial constituents like LPS or flagellin (Devriendt et al., 2010). TNF-α up-regulation was significantly inhibited (÷4.4, $P < 0.01$, Fig. 1A) by *Sc* at the ratio of 3 yeasts/cell. Comparison of yeast effects at this ratio indicated that *Sc* inhibited TNF-α mRNA expression (÷5, $P < 0.01$, Fig. 1B) whereas *Sb* did not (Fig. 1C). These results were reminiscent of a similar TNF-α reduction by *Sb* probiotic strain in T84 colonocytes infected with an enterohemorrhagic *E. coli* (EHEC) (Dalmasso et al., 2006).

Then, we investigated if *Sc* inhibitory effects on pro-inflammatory gene expression were due to yeast-secreted factors. We assessed the effect of yeast viability and IPI-2I cells were co-cultured with both killed *Sc* and ETEC (Fig. 2). The levels of TNF-α, IL-1α, IL-6, IL-8, CXCL2 and CCL20 transcripts were analyzed. Co-culture of IPI-2I cells with ETEC alone for 3 h up-regulated the mRNA levels of TNF-α (×6), CCL20 (×5), IL-6 and CXCL2 (×3.7), IL-1α (×3.2) and IL-8 (×3) (Fig. 2) whereas adjunction of killed *Sc* failed to inhibit the expression of these genes (Fig. 2). Furthermore, IL-6 and IL-8 mRNA levels were up-regulated in presence of killed *Sc* (×2.1 and ×1.8 respectively), probably due to yeast wall structures such as β-glucans (Sonck et al., 2010).

IPI-2I cells were next cultured with both yeast culture supernatants and ETEC in order to identify if *Sc*-secreted factors had inhibitory effects on pro-inflammatory cytokine and chemokine expressions. *ScS* inhibited the ETEC-induced mRNA levels of IL-8 (÷5), CCL20 (÷4.9), IL-6 (÷4.7), TNF-α (÷4.3), CXCL2 (÷3.5) and IL-1α (÷2.8) suggesting the presence of one or several anti-inflammatory factors (Fig. 3A). To identify if *Sc*-secreted anti-inflammatory factors were smaller than 10 kDa as described for *Sb* probiotic strain (Sougioultzis et al., 2006), IPI-2I cells were co-cultured with both the *S* < 10 kDa

Please cite this article in press as: Zanello, G., et al., *Saccharomyces cerevisiae* decreases inflammatory responses induced by F4+ enterotoxigenic *Escherichia coli* in porcine intestinal epithelial cells. Vet. Immunol. Immunopathol. (2011), doi:10.1016/j.vetimm.2011.01.018

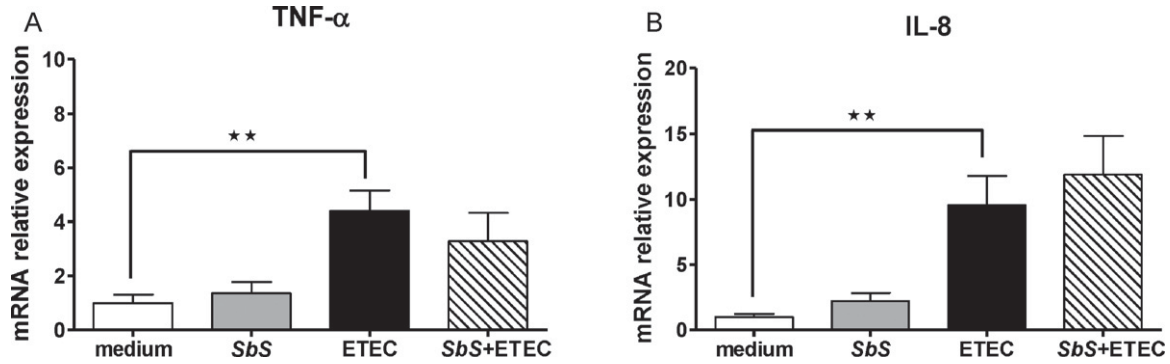

Fig. 4. Effects of *Sb* culture supernatant (*SbS*) on cytokine and chemokine mRNA expressions in IPI-2I cells cultured with ETEC. IPI-2I cells (1×10^6 cells/well) were cultured with *SbS* simultaneously with ETEC (1×10^7 CFU/well) for 3 h. Data are presented as means of mRNA relative expressions ± SEM ($n = 6$). $^{**}P < 0.01$.

fraction and ETEC. The $S < 10$ kDa fraction inhibited the ETEC-induced CXCL2 (÷5.6), IL-6 (÷5.5), CCL20 (÷4.1), IL-1α (÷4) and IL-8 (÷3.4) expressions showing that soluble factors <10 kDa inhibited pro-inflammatory gene expressions, excepted for TNF-α (Fig. 3B). In contrast, *SbS* was used as control and did not inhibit the ETEC-induced pro-inflammatory gene expression as described for TNF-α and IL-8 (Fig. 4). In the current work, *Sc* anti-inflammatory effects have not been evaluated at the protein level. However, the inhibition of transcript expression observed for a cluster of pro-inflammatory genes strongly suggests *Sc* effectiveness in the inhibition of the related protein productions. Indeed, the yeast inhibition of IL-8 transcript has been previously shown to correlate with the decrease of protein production (Martins et al., 2010). These inhibitory effects were in accordance with several studies reporting that the culture supernatant of *Sb* probiotic strain down-regulated the expression of pro-inflammatory cytokine such as IL-8 (Chen et al., 2006; Mumy et al., 2007). Moreover, this down-regulation was dependent of mitogen-activated protein kinase (ERK1/2, p38, JNK) and/or NF-κB inhibition (Chen et al., 2006; Martins et al., 2010; Mumy et al., 2007) suggesting that *Sc*-secreted factors could interfere with pro-inflammatory signalling pathways in IPI-2I cells. In addition, the anti-inflammatory effects of *Sb* probiotic strain were correlated with a decrease in intestinal damage caused by enteric pathogen such as *Salmonella enterica* serovar Typhimurium (Martins et al., 2010). Overall, these data suggested that *Sc* (strain CNCM I-3856) may be administrated as probiotic yeast to prevent intestinal damage caused by ETEC inflammation. In contrast, *Sb* (strain CNCM I-3799) did not display inhibitory effects on cytokine and chemokine expressions. These results were different from those observed with the previously studied *Sb* probiotic strain and indicated that anti-inflammatory effects onto intestinal epithelial cells were strain dependent. Thus, this study showed that regulation of inflammatory gene expression was not a mechanism specific of all *Sb* strains and displayed that *Sc* (non *boulardii*) yeasts can induce this effect despite their genetic, metabolic and physiologic differences.

In conclusion, we reported in this study that *Sc*-secreted factors inhibit the expression of pro-inflammatory cytokines and chemokines induced by ETEC as described for *Sb* probiotic strain. These data suggest that *Sc* may protect the gut against intestinal damage caused by pathogen-associated inflammation in piglets and represent a first step in the better understanding of *Sc* immuno-regulatory effects in infectious and inflammatory diseases.

Acknowledgements

G. Zanello was supported by a Ph.D. grant from Société Industrielle Lesaffre and the French Government. We gratefully acknowledge P. Vandekerckove and G. Pignède (Lesaffre) for their assistance in this work. We thank Dr. C. Martin (Institut National de la Recherche Agronomique, Clermont-Theix, France) for kindly providing the F4+ ETEC strain 56190.

References

Bruel, T., Guibon, R., Melo, S., Guillen, N., Salmon, H., Girard-Misguich, F., Meurens, F., 2010. Epithelial induction of porcine suppressor of cytokine signaling 2 (SOCS2) gene expression in response to *Entamoeba histolytica*. Dev. Comp. Immunol. 34, 562–571.

Chen, X., Kokkotou, E.G., Mustafa, N., Bhaskar, K.R., Sougioultzis, S., O'Brien, M., Pothoulakis, C., Kelly, C.P., 2006. *Saccharomyces boulardii* inhibits ERK1/2 mitogen-activated protein kinase activation both *in vitro* and *in vivo* and protects against *Clostridium difficile* toxin A-induced enteritis. J. Biol. Chem. 281, 24449–24454.

Dalmasso, G., Loubat, A., Dahan, S., Calle, G., Rampal, P., Czerucka, D., 2006. *Saccharomyces boulardii* prevents TNF-alpha-induced apoptosis in EHEC-infected T84 cells. Res. Microbiol. 157, 456–465.

Devriendt, B., Stuyven, E., Verdonck, F., Goddeeris, B.M., Cox, E., 2010. Enterotoxigenic *Escherichia coli* (K88) induce proinflammatory responses in porcine intestinal epithelial cells. Dev. Comp. Immunol. 34, 1175–1182.

Edwards-Ingram, L., Gitsham, P., Burton, N., Warhurst, G., Clarke, I., Hoyle, D., Oliver, S.G., Stateva, L., 2007. Genotypic and physiological characterization of *Saccharomyces boulardii*, the probiotic strain of *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 73, 2458–2467.

Fairbrother, J.M., Nadeau, E., Gyles, C.L., 2005. *Escherichia coli* in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Anim. Health Res. Rev. 6, 17–39.

Foligne, B., Dewulf, J., Vandekerckove, P., Pignede, G., Pot, B., 2010. Probiotic yeasts: anti-inflammatory potential of various non-pathogenic strains in experimental colitis in mice. World J. Gastroenterol. 16, 2134–2145.

Hennequin, C., Thierry, A., Richard, G.F., Lecointre, G., Nguyen, H.V., Gaillardin, C., Dujon, B., 2001. Microsatellite typing as a new tool for identification of *Saccharomyces cerevisiae* strains. J. Clin. Microbiol. 39, 551–559.

Kaeffer, B., Bottreau, E., Velge, P., Pardon, P., 1993. Epithelioid and fibroblastic cell lines derived from the ileum of an adult histocompatible

Please cite this article in press as: Zanello, G., et al., *Saccharomyces cerevisiae* decreases inflammatory responses induced by F4+ enterotoxigenic *Escherichia coli* in porcine intestinal epithelial cells. Vet. Immunol. Immunopathol. (2011), doi:10.1016/j.vetimm.2011.01.018

miniature boar (d/d haplotype) and immortalized by SV40 plasmid. Eur. J. Cell Biol. 62, 152–162.
Lessard, M., Dupuis, M., Gagnon, N., Nadeau, E., Matte, J.J., Goulet, J., Fairbrother, J.M., 2009. Administration of *Pediococcus acidilactici* or *Saccharomyces cerevisiae* boulardii modulates development of porcine mucosal immunity and reduces intestinal bacterial translocation after *Escherichia coli* challenge. J. Anim. Sci. 87, 922–934.
Martins, F.S., Dalmasso, G., Arantes, R.M., Doye, A., Lemichez, E., Lagadec, P., Imbert, V., Peyron, J.F., Rampal, P., Nicoli, J.R., Czerucka, D., 2010. Interaction of *Saccharomyces boulardii* with *Salmonella enterica* serovar Typhimurium protects mice and modifies T84 cell response to the infection. PLOS One 5, e8925.
Meurens, F., Girard-Misguich, F., Melo, S., Grave, A., Salmon, H., Guillen, N., 2009. Broad early immune response of porcine epithelial jejunal IPI-2I cells to *Entamoeba histolytica*. Mol. Immunol. 46, 927–936.
Mumy, K.L., Chen, X., Kelly, C.P., McCormick, B.A., 2007. *Saccharomyces boulardii* interferes with Shigella pathogenesis by post-invasion signaling events. Am. J. Physiol. Gastrointest. Liver Physiol..
Nagy, B., Fekete, P.Z., 2005. Enterotoxigenic *Escherichia coli* in veterinary medicine. Int. J. Med. Microbiol. 295, 443–454.
Sonck, E., Stuyven, E., Goddeeris, B., Cox, E., 2010. The effect of beta-glucans on porcine leukocytes. Vet. Immunol. Immunopathol. 135, 199–207.
Sougioultzis, S., Simeonidis, S., Bhaskar, K.R., Chen, X., Anton, P.M., Keates, S., Pothoulakis, C., Kelly, C.P., 2006. *Saccharomyces boulardii* produces a soluble anti-inflammatory factor that inhibits NF-kappaB-mediated IL-8 gene expression. Biochem. Biophys. Res. Commun. 343, 69–76.
Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., Speleman, F., 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3, RESEARCH0034.
Zanello, G., Meurens, F., Berri, M., Salmon, H., 2009. *Saccharomyces boulardii* effects on gastrointestinal diseases. Curr. Issues Mol. Biol. 11, 47–58.

Please cite this article in press as: Zanello, G., et al., *Saccharomyces cerevisiae* decreases inflammatory responses induced by $F4^+$ enterotoxigenic *Escherichia coli* in porcine intestinal epithelial cells. Vet. Immunol. Immunopathol. (2011), doi:10.1016/j.vetimm.2011.01.018