The Novel Porcine *Lactobacillus sobrius* Strain Protects Intestinal Cells from Enterotoxigenic *Escherichia coli* K88 Infection and Prevents Membrane Barrier Damage\(^1,2\)

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Abstract

Lactobacilli have a potential to overcome intestinal disorders; however, the exact mode of action is still largely unknown. In this study, we have used the intestinal porcine intestinal IPEC-J1 epithelial cells as a model to investigate a possible protective activity of a new *Lactobacillus* species, the *L. sobrius* DSM 16698\(^T\), against intestinal injury induced by enterotoxigenic *Escherichia coli* (ETEC) K88 infection and the underlying mechanisms. Treatment of infected cells with *L. sobrius* strongly reduced the pathogen adhesion. *L. sobrius* was also able to prevent the ETEC-induced membrane damage by inhibiting delocalization of zonula occludens (ZO)-1, reduction of occludin amount, rearrangement of F-actin, and dephosphorylation of occludin caused by ETEC. RT-PCR and ELISA experiments showed that *L. sobrius* counteracted the ETEC-induced increase of IL-8 and upregulated the IL-10 expression. The involvement of IL-8 in the deleterious effects of ETEC was proven by neutralization of IL-8 with a specific antibody. A crucial role of IL-10 was indicated by blockage of IL-10 production with neutralization of ETEC, which was likely favored by the leaking barrier. The protective effects were not found with *L. amylovorus* DSM 20531\(^\#\) treatment, a strain derived from cattle waste but phylogenetically closely related to *L. sobrius*. Together, the data indicate that *L. sobrius* exerts protection against the harmful effects of ETEC by different mechanisms, including pathogen adhesion inhibition and maintenance of membrane barrier integrity through IL-10 regulation. J. Nutr. 137: 2709–2716, 2007.

Introduction

The intestinal epithelium constitutes the major barrier that separates the internal from the external environment, providing high resistance to free diffusion of solutes and entry of pathogens and harmful luminal antigens. To establish an efficient barrier, intercellular spaces must be strictly sealed by tight junctions (TJ).\(^5\) These junctions are composed by a complex of proteins, including the zonula occludens (ZO) and occludin proteins, in close apposition to the cytoskeletal actin and myosin ring (1–3). Disruption of the mucosal barrier may be caused by several pathogens that release toxins after the essential step of adhesion to the brush border of intestinal cells, inducing changes to TJ proteins or actin and myosin organization (4). A leaking barrier may favor indiscriminate passage of pathogens and other external antigens, dysregulation of epithelial cell signaling, and impairment of the mucosal immune system, with consequent development of inflammatory reactions, including production of cytokines than can affect the TJ proteins and thus promote further leakiness (3,5,6).

Lactobacilli are probiotics that may confer health benefits to the host (7–10) and there is accumulating evidence that they are effective in preventing intestinal disease in both humans and animals due to their ability to maintain or restore normal microbiota, inhibit pathogen adhesion to the intestinal wall, and prevent inflammatory processes (11–16). Some studies have also shown an ability of lactobacilli to protect against pathogen-induced membrane barrier disruption (17–20). However, up to now, the exact mode of action of lactobacilli is still largely unknown.

A new *Lactobacillus* species, *L. sobrius* strain DSM 16698\(^T\), has been recently isolated from unweaned piglets and found to be abundant in the intestine of healthy piglets (21,22). *L. sobrius*...
has not yet been characterized for potential protective activity against intestinal disease, but there is evidence that feeding *L. sobrius* may reduce diarrhea associated with enterotoxigenic *Escherichia coli* (ETEC) K88 infection (23), decrease colonization of ETEC, and improve weight gain of infected piglets (24). R. Konstantinov, H. Smidt, A. Akkermans, L. Casini, P. Trevisi, M. Mazzoni, S. De Filippi, P. Bosi, and W. M. de Vos, unpublished data. In this study, we used the IPEC-1 (intestinal porcine epithelial cells) cell line derived from the small intestine of a newborn unsuckled piglet (24) as a model to investigate whether *L. sobrius* was able to protect against intestinal injury induced by ETEC K88 infection and the underlying mechanisms. In previous studies, we have found that infection of intestinal cells with ETEC K88 induced an inflammation-associated response (25,26). Other than a role against pathogen adhesion, we have examined whether *L. sobrius* was able to prevent possible perturbations on either TJ proteins and cytoskeletal organization and whether this protection was exerted through modulation of cytokines, which are known to be involved in epithelial barrier function (1). We have also investigated the effects of the non-porcine *L. amylovorus* strain DSM 20531, phylogenetically closely related to *L. sobrius*, based on the 16S ribosomal RNA sequences but with considerable differences in genomic content and ecophysiological properties (21).

**Materials and Methods**

**Epithelial cell culture.** The IPEC-1 cells (provided by D. D. Black, Children's Foundation Research Center of Memphis, Le Bonheur Children's Medical Center, Memphis, TN) were grown in complete DMEM/F-12 medium (Eurobio) supplemented with 5% heat-inactivated fetal calf serum, 2 mmol/L L-glutamine, 15 mmol/L HEPES, 5 μg/mL epidermal growth factor (Becton Dickinson), ITS (10 mg/L insulin, 5.5 mg/L transferrin, 5 mg/L selenium; Sigma), 105 U/L penicillin, and 100 mg/L streptomycin, at 37°C in an atmosphere of 5% CO2 and 95% air at 90% relative humidity (24). According to the different experiments, IPEC-1 cells were grown on Transwell filters, tissue culture plates, or glass coverslips, as described below, and cultured for 10 d after confluence in medium without fetal calf serum to allow differentiation. In this culture condition, the IPEC-1 cells can differentiate and exhibit enterocyte features, including microvilli and TJ, when grown on Transwell filters (24,27). We have also found that these cells develop TJ and microvilli when grown of tissue culture dishes (data not shown). Medium was changed 3 times per week.

**Bacterial growth.** ETEC strain K88 (provided by Istituto Zoono profilattico Sperimentale della Lombardia e dell’Emilia, Reggio Emilia, Italy) was grown in Luria-Bertani (LB) broth containing 1% tryptone and 0.5% yeast extract (both from OXOID) plus 1% NaCl, pH 7.0. After overnight incubation at 37°C in aerobic conditions. We used aliquots of supernatants to detect *L. sobrius* strain DSM 16698*™* and *L. amylovorus* strain DSM 20531, a commercial strain isolated from cattle waste (28). The lactobacilli were grown in DeMan Rogosa Sharp (MRS) medium (Difco) at 37°C under anaerobic conditions. After overnight incubation, they were diluted 1:100 in fresh LB and grown for ~2 h until mid-log phase for all experiments. Two different lactobacilli were used: *L. sobrius* strain DSM 16698*™* and *L. amylovorus* strain DSM 20531, a commercial strain isolated from cattle waste (28). The lactobacilli were grown in DeMan Rogosa Sharp (MRS) medium (Difco) at 37°C under anaerobic conditions. After overnight incubation, they were diluted 1:100 in fresh MRS, grown for ~4 h until mid-log phase, and processed as described for ETEC.

For each experiment, bacterial cells were harvested by centrifugation at 3000 × g for 10 min at 4°C and then resuspended in antibiotic-free DMEM/F-12 medium. The viability of ETEC, *L. sobrius*, and *L. amylovorus* grown on DMEM/F-12 medium did not differ from that of bacteria grown on LB or MRS media, as tested in preliminary experiments by colony-forming units (CFU) counts after agar plating of bacterial inocula in the different media. Bacterial concentrations of both ETEC and lactobacilli were determined in preliminary experiments by densitometry and confirmed by serial dilutions followed by CFU counts of ETEC on LB agar after 16 h incubation and of the 2 lactobacilli on MRS agar after 48-h incubation under anaerobic conditions.

**Bacterial adhesion.** Bacterial adhesion was tested by 2 different methods: agar plating and fluorescent in situ hybridization (FISH) assays. IPEC-1 cells (1 × 10⁶ cells per well) were seeded on 24-well plates for agar plating assay or on glass coverslips in 24-well plates for FISH assay. At d 10, cells were treated with 1 mL of medium containing ETEC (1 × 10¹¹ CFU/L), *L. sobrius*, or *L. amylovorus* (1 × 10¹² or 1 × 10¹³ CFU/L) either alone or simultaneously with ETEC for 1.5 h. We chose the pathogen concentration and time of incubation based on preliminary experiments to allow bacterial adhesion and membrane damages without disruption of the cell monolayer. For agar plating assay, IPEC-1 cells were lysed with 1% Triton-X-100 and adhered ETEC was quantified by plating appropriate serial dilutions of lysates on LB agar. Preliminary experiments confirmed that the 2 lactobacilli were not able to form colonies after overnight incubation on LB agar at 37°C in aerobic conditions.

FISH assay was performed as previously described (29). The hybridization was carried out using Cy3-labeled probe L. *sobrius*-Otu171-0088-a-A-18 (CCG TTT CCC AAC GTC ATT) to specifically target the 16S ribosomal RNA of *L. sobrius* and *L. amylovorus*, and fluorescein isothiocyanate (FITC)-labeled universal bacterial specific probe, S-O-Bact-0338-a-A-17 (GCT GCC TCC CGT AGG AGT), at 50°C for 16 h. 4',6-Diamino-2-phenylindole was also used to stain nucleic acids of bacterial cells. The digital images were analyzed and the fluorescence positive cells were recorded using Qwin image analysis software (Leica Microsystems).

**Localization of TJ and cytoskeletal proteins.** IPEC-1 cells (1 × 10⁶ cells per filter) differentiated on Transwell filters were untreated or apically treated with 1 mL of medium containing ETEC (1 × 10¹¹ CFU/L), *L. sobrius*, or *L. amylovorus* (1 × 10¹² CFU/L) either alone or simultaneously with ETEC for 1.5 h. In some experiments, neutralizing anti-IL-8 or IL-1β antibodies were apically added to the culture medium of cells infected with ETEC (5 mg/mL; R&D System) and neutralizing anti-IL-10 antibody was added to the culture medium of cells treated with *L. sobrius* simultaneously with ETEC (10 mg/mL; R&D System). Preliminary experiments demonstrated that the addition of the neutralizing antibodies to the cells did not affect protein distribution (data not shown). Localization of ZO-1, occludin, and F-actin was analyzed by Western blot of immunoprecipitated proteins to the cells did not affect protein distribution (data not shown). Localization of ZO-1, occludin, and F-actin was analyzed by Western blot of immunoprecipitated proteins. Other than a role against pathogen adhesion, we have examined whether *L. sobrius* was able to prevent possible perturbations on either TJ proteins and cytoskeletal organization and whether this protection was exerted through modulation of cytokines, which are known to be involved in epithelial barrier function (1). We have also investigated the effects of the non-porcine *L. amylovorus* strain DSM 20531, phylogenetically closely related to *L. sobrius*, based on the 16S ribosomal RNA sequences but with considerable differences in genomic content and ecophysiological properties (21).

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IL-8 and IL-10 production was used to evaluate cytokine mRNA expression in response to bacterial treatment. The PCR products were analyzed on 2% agarose gel and the absence of genomic DNA contamination. Primer pairs were discarded if the alignment of both primer sequences resulted in the amplification of nonspecific sequences. Each cytokine was coamplified with glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) as a housekeeping internal control, because preliminary experiments showed that this gene expression was unaffected by bacterial treatment. The primers were provided by MWG Biotech. The PCR products were analyzed on 2% agarose gel and the intensity of the bands was measured by Scion image software. The ratio of cytokine-GAPDH mRNA intensities was used to evaluate cytokine mRNA level.

**Measurement of cytokine production.** IL-8 and IL-10 production was measured in culture supernatants of IPEC-1 cells (4 × 10⁶ cells per well in 6-well plates) untreated or treated with 4 mL of medium containing ETEC (1 × 10¹³ CFU/L) or *L. sobrius* (1 × 10¹² CFU/L) either alone or simultaneously with ETEC for 3 h. Cytokine level was measured by ELISA using a commercial kit (R&D System).

**ETEC invasion.** Internalization of viable bacteria was assayed by gentamicin protection assay, as previously described (25). IPEC-1 cells (1 × 10⁶ cells per well on 24-well plates) were treated with 1 mL of medium containing ETEC (1 × 10¹¹ CFU/L) or *L. sobrius* (1 × 10¹² CFU/L) either alone or simultaneously with ETEC for 3 h. After extensive washes with PBS, cells were incubated with culture medium containing 50 µg/L gentamicin sulfate (Sigma) for 2.5 h to kill residual viable extracellular bacteria. Cells were lysed with 1% Triton-X-100 and viable intracellular bacteria were quantified by agar plating. As control for ETEC killing, we added gentamicin to some wells together with ETEC at the beginning of the experiment.

**Statistical analysis.** The significance of the differences was evaluated by 1-way ANOVA followed by Fisher’s test. Significance was set at P < 0.05. All statistical analyses were performed with SPSS software program (version 8.0; SPSS).

**Results**

**L. sobrius inhibits bacterial adhesion.** We report that ETEC adhered to the IPEC-1 cells, as indicated by both the FISH (Fig. 1A) and agar plating assays (Fig. 1D). *L. sobrius* and *L. amylovorus* were able to adhere to the cells (Fig. 1B,C). The FISH assay also showed a coaggregation of *L. sobrius*, but not of *L. amylovorus*, with ETEC (data not shown). The agar plating indicated that both the *Lactobacillus* spp. strains had a similar ability to adhere to the cells (1.2 × 10¹⁰ CFU/L with the higher concentration). Treatment of IPEC-1 cells with *L. sobrius* was able to strongly reduce the number of adhered ETEC and this effect was achieved already with the lower concentration (Fig. 1D). In contrast, *L. amylovorus* did not protect the cells against ETEC adhesion, even at higher concentrations (Fig. 1D).

**Impact of ETEC on TJ and cytoskeletal proteins and protection by *L. sobrius* but not by *L. amylovorus*.** To investigate whether ETEC caused membrane barrier disruption and whether the 2 lactobacilli could inhibit such damage, we performed an immunolocalization of ZO-1 and occludin (Fig. 2) and of F-actin (Fig. 3), which is part of the cytoskeleton proteins anchored to the TJ (2,3). Uninfected IPEC-1 cells showed uniform distribution of ZO-1 and occludin around the cell boundaries. Moreover, long, thick fibers with end points reaching cell periphery and an even distribution of perijunctional F-actin were apparent in these cells. Treatment of IPEC-1 cells with *L. sobrius* did not modify ZO-1 and occludin localization and F-actin organization, whereas treatment with *L. amylovorus* induced an uneven distribution of ZO-1 and occludin, as well a partial rearrangement of F-actin. Infection with ETEC caused a TJ opening, as indicated by the ZO-1 delocalization, showing loss of cell-cell contact (15.2 ± 1.7 damaged cells over 32 ± 2.5 total cells per vision field) and by occludin dissociation from the membrane with scattered distribution inside the cells. Disorganization of F-actin was also induced by ETEC. When the cells were treated with ETEC simultaneously with *L. sobrius*, a correct distribution and organization of TJ and cytoskeletal proteins was observed. In contrast, after *L. amylovorus* cotreatment with ETEC, delocalization of ZO-1 and occludin, loss of cell-cell contact (11.3 ± 2.2 damaged cells over 28 ± 3.2 total cells per vision field), and disorganization of F-actin were still present.
**L. sobrius** completely inhibits the ETEC induced occludin reduction and dephosphorylation. The strong perturbations of occludin localization caused by ETEC were associated with a strong decrease of the protein, as indicated by Western blot analysis (Fig. 4). The protein reduction did not occur when the cells were cotreated with ETEC and **L. sobrius**. Because occludin phosphorylation is correlated with TJ assembly and function (33), we also investigated whether ETEC infection caused a dephosphorylation of occludin and whether this alteration occurred in the presence of the *Lactobacillus* (Fig. 4). The infected cells showed a reduced phosphorylation of occludin tyrosine residues, whereas the level of phosphorylation in the cells cotreated with ETEC and **L. sobrius** was similar to that of untreated cells.

**L. sobrius** counteracts the ETEC-induced dysregulation of cytokine gene expression. To evaluate the possibility that **L. sobrius** can counteract ETEC-induced dysregulation of cytokines involved in membrane barrier function that was counteracted by **L. sobrius**, we analyzed the gene expressions of IL-1β, IL-8, and IL-10 (Fig. 5). Infection with ETEC caused an upregulation of IL-1β and IL-8 and a downregulation of IL-10 compared with untreated cells. Treatment of cells with **L. sobrius** did not modify the cytokine expression compared with untreated cells. Treatment of cells with **L. sobrius** simultaneously with ETEC abrogated the increase of IL-1β and IL-8 and induced an upregulation of IL-10.

**Membrane damage is mediated by IL-8 production and protection by **L. sobrius** is exerted by IL-10 activity.** To investigate whether IL-1β, IL-8, and IL-10 were effectively involved in the membrane barrier disruption and in the protective activity of **L. sobrius**, we analyzed the immunolocalization of ZO-1, occludin, and F-actin of cells treated with neutralizing anti-IL-1β, anti-IL-8, or anti-IL-10 antibodies. Although the damages of TJ and cytoskeleton proteins remained after anti-IL-1β antibody treatment of cells infected with ETEC (data not shown), most of the alterations were not induced when the infected cells were treated with anti-IL-8 antibody and only sporadic alterations of occludin distribution were found (Fig. 6). Blocking IL-10 production with neutralizing anti-IL-10 antibody in cells treated with **L. sobrius** simultaneously with ETEC abrogated **L. sobrius** protection, as indicated by loss of cell-cell contact (13 ± 1.7 damaged cells over 31.2 ± 3.7 total cells per vision field), delocalization of occludin, and disorganization of F-actin (Fig. 6).

Increased IL-10 and decreased IL-8 production by **L. sobrius**. The ELISA further confirmed the involvement of IL-10 in the protective effect of **L. sobrius**, because a strong production of this cytokine was observed in the cells treated with **L. sobrius** simultaneously with ETEC. In addition, **L. sobrius** was able to inhibit the increased IL-8 secretion induced by ETEC (Fig. 7).

**Reduction of ETEC invasion by **L. sobrius**.** Infection of IPEC-1 cells with ETEC resulted in pathogen internalization into the cells (6.7 ± 1.4 × 10⁶ CFU/L). **L. sobrius** was able to reduce the pathogen invasion already at a concentration of 1 × 10¹² CFU/L (4.2 ± 1.1 × 10⁶ CFU/L) and it was more effective at a concentration of 1 × 10¹³ CFU/L (2.0 ± 0.8 × 10⁵ CFU/L).

**Discussion**

In this study, the ability of the novel isolate **L. sobrius** strain to protect against ETEC infection through reduction of ETEC adhesion and maintenance of membrane barrier integrity is demonstrated. Inhibition of pathogen adhesion is an activity exerted by several lactobacilli (14,20,34) and we show that **L. sobrius** and not **L. amylovorus** was able to reduce the ETEC adhesion. The coaggregation of **L. sobrius** with ETEC suggests that the direct interaction with ETEC may have favored pathogen removal, as previously shown for some strains of *Lactobacillus* (35). In addition to inhibition of adhesion, the maintenance of intestinal epithelial barrier integrity is fundamental to prevent...
bacterial infections and consequent development of inflammatory intestinal diseases (5,36). The TJ plays a fundamental role in the membrane barrier function and integrity through interaction of the ZO proteins with the transmembrane protein occludin and the apical perijunctional actomyosin ring (2–4). A direct association of occludin with ZO-1 has been shown to be crucial for maintenance of TJ structure (2). We report that ETEC caused widespread defect of cell-cell contact, delocalization of ZO-1, dramatic dissociation of occludin from intercellular junctions associated with a decrease of the protein level, and rearrangement of F-actin protein. All these data indicate disruption of the TJ structure. We show that \textit{L. sobrius} was able to counteract the membrane barrier disruption caused by ETEC by maintaining the correct localization of ZO-1, occludin, and F-actin, and by blocking the reduction of occludin amount as well, whereas \textit{L. amylovorus} was unable to protect the cells from the membrane damages. Our results are consistent with those of previous studies reporting a protection against pathogen-induced membrane damage by various \textit{Lactobacillus} strains (17–20). Tyrosine phosphorylation of occludin has been shown to be necessary for the establishment of this protein on the TJ (33). Previous studies have demonstrated that enteropathogenic \textit{E. coli} caused dephosphorylation and dissociation of occludin from TJ of intestinal cells (37). In agreement with these findings, our results show that the ETEC-induced decrease of occludin was associated with a reduction of phosphorylation of tyrosine residues, likely causing occludin to dissociate and translocate to intracellular compartments. We show that treatment of the infected cells with \textit{L. sobrius} prevented occludin dephosphorylation, allowing the interaction of this protein with the other TJ and cytoskeleton proteins and thus the correct TJ assembly and function. However, it is also possible that \textit{L. sobrius} acted directly on ZO-1 and F-actin. The cell wall component

![FIGURE 3](https://academic.oup.com/jn/article-abstract/137/12/2709/4670063/fig3)

\textit{L. sobrius} counteracts the F-actin rearrangement caused by ETEC. IPEC-1 cells were untreated (C), infected with ETEC, or treated with \textit{L. sobrius} or \textit{L. amylovorus} (\textit{L. amy}) either alone or simultaneously with ETEC for 1.5 h and analyzed by immunofluorescence. Each figure is representative of 5 independent assays.

![FIGURE 4](https://academic.oup.com/jn/article-abstract/137/12/2709/4670063/fig4)

\textit{L. sobrius} inhibits the occludin reduction and dephosphorylation caused by ETEC. IPEC-1 cells were untreated (Control), infected with ETEC, or treated with \textit{L. sobrius} either alone or simultaneously with ETEC for 1.5 h. The figure shows a Western blot (A) of immunoprecipitated occludin and its phosphotyrosine (P-Y) level, representative of 4 independent assays, and the densitometric values (B) of occludin normalized to \beta-actin and P-Y-occludin normalized to occludin. Values are means ± SEM. Within each ratio, values without a common letter differ, \textit{P} < 0.01.

![FIGURE 5](https://academic.oup.com/jn/article-abstract/137/12/2709/4670063/fig5)

\textit{L. sobrius} counteracts the alterations of IL-1\(\beta\), IL-8, and IL-10 gene expression induced by ETEC. IPEC-1 cells were untreated (Control), infected with ETEC, or treated with \textit{L. sobrius} either alone or together with ETEC for 1.5 h. Cytokine expression was analyzed by RT-PCR and is reported as densitometric values of each cytokine mRNA normalized to GAPDH mRNA. Values represent means ± SEM of 6 independent assays. Within each cytokine, values without a common letter differ, \textit{P} < 0.05.
lipoteichoic acids could have played a key role, because they are ligands for the toll-like receptor-2 (38), which has been shown to enhance ZO-1-associated intestinal epithelial barrier integrity through activation of protein kinase C (39). Together, our results are consistent with the possibility that \textit{L. sobrius} may prevent disruption of membrane barrier by signaling through host cell pathways involving the phosphorylation of occludin and the regulation of TJ and cytoskeleton proteins.

Many cytokines have been shown to regulate the TJ and cytoskeleton structure and function (1). IL-8, a well-known pro-inflammatory cytokine inducer (40), has been associated with pathogen-induced alterations of TJ (41). In agreement with these findings, we found that disruption of the membrane barrier by ETEC was associated with a strong increase of IL-8 expression. Blocking the IL-8 activity with a specific antibody resulted in the disappearance of most of the membrane damages, confirming that this cytokine was implicated in the deleterious effects of ETEC on the gut membrane barrier. On the other hand, we show that blockage of IL-1\(\beta\) activity did not prevent the pathogen-induced membrane damages. This result is in contrast with some previous findings reporting that IL-1\(\beta\) may be involved in membrane dysfunction (42,43). However, other studies have reported an alteration of ion permeability but not of membrane barrier or even an increase of occludin induced by IL-1\(\beta\) (44,45). Treatment with \textit{L. sobrius} prevented the increase of IL-8 and induced a strong upregulation of IL-10. These results are consistent with previous studies indicating that probiotics may reduce intestinal disorders through reduced IL-8 and increased IL-10 production (41,46,47). Neutralization experiments indicated that IL-10 was essential for the protection exerted by \textit{L. sobrius}, because the protective effect was completely abolished when the cells were treated with ETEC together with \textit{L. sobrius} and anti-IL-10 antibody. The importance of IL-10 in the maintenance of the membrane barrier has been indicated also by previous studies showing an increased membrane permeability or necrosis of intestinal cells in IL-10-deficient mice that existed prior to the development of mucosal inflammation (48,49).

The TJ may play a role in preventing entrance of pathogens inside the cells. Indeed, although pathogens gain access into the cells usually via the transcellular route by entering through the apical surface of the cells, it has become apparent that several pathogens may use the paracellular route through the TJ and adherent junctions (50). Although not considered invasive pathogens, several \textit{E. coli} strains have been shown to invade cultured epithelial cells derived from various tissues, particularly those from ileocecum and colon (51–53) and extraintestinal tissues of piglets (54). In agreement with the results of our previous study...
showing an ability of ETEC to invade intestinal cells (25), we report here that a certain amount of ETEC was internalized into the IPEC-1 cells. We could show that L. sobrius was able to inhibit the internalization of the pathogen, presumably by preserving the TJ structure and thus preventing the entry of the pathogen through the damaged membrane barrier.

In conclusion, the results reported here indicate that the new isolate L. sobrius may reduce the ETEC adhesion and prevent the membrane barrier disruption caused by the pathogen and consequently the pathogen invasion. The maintenance of barrier integrity was achieved by IL-10-mediated signaling involving downregulation of IL-8, regulation of TJ and cytoskeleton proteins, and phosphorylation of occludin. These results provide new insights into the protective activity of lactobacilli, supporting the view that they may act through diverse mechanisms.

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Literature Cited


