

Enterotoxigenic *Escherichia coli* and probiotics in swine: what the bleep do we know?

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The concept of certain microorganisms conferring direct benefits to the host relates to the term “probiotic”. Probiotics are microorganisms, bacteria, or yeast that when administered orally in sufficient quantity can counteract the effect of pathogenic microorganisms. The gastrointestinal (GI) tract is the site where probiotics are believed to play the most important role. The proposed effects of probiotics include antagonism of pathogens, interference with adherence, competition for nutrients, enterotoxin inactivation, modulation of the immune response, and strengthening of the intestinal barrier. From birth to postweaning, piglets are very sensitive to gut colonisation by pathogens. Enterotoxigenic *Escherichia coli* represents one of the most common agents of swine diarrhoea. The enterotoxins produced by this *E. coli* virotype are responsible for the loss of electrolytes and water observed following infection. This review addresses more specifically the studies done during the last 10 years deciphering the molecular mechanisms at play between host cell and probiotic interactions in the swine GI tract.

Key words: diarrhoea, pig, probiotic, inflammation, barrier function, tight junction

INTRODUCTION

Gastrointestinal (GI) infection, despite numerous therapeutic alternatives, including antibiotherapy, remains a major clinical issue in man and in animals. Enterotoxigenic *Escherichia coli* (ETEC) strains are one of the causes of GI infections. These pathogens are responsible for diarrhoea by elaboration of exotoxins acting on the small intestine, provoking secretion of fluid and electrolytes and leading to dehydration. Diarrhoea resulting from GI infection corresponds to a decrease in consistency associated with an increased frequency of stools. In swine, ETEC is responsible for neonatal and postweaning diarrhoea, two conditions that leads to growth retardation, require antibiotherapy, and can ultimately result in death of the animals. With the dramatic increase of antibiotic-resistant microbial pathogens, there is a real concern that this condition will be almost impossible to handle in the near future. Nowadays,

there is a renewed interest in the feeding of beneficial microorganisms to animals as an alternative to antibiotics. Furthermore, research devoted to the characterization of the gut microbiota has led to better understanding of their contribution to nutrition and health. In particular, their roles in preventing colonisation by pathogens, stimulation of the immune system, and alteration of intestinal mucosal integrity have been studied. Treating diarrhoea with living microorganisms to restore and/or prevent pathogen effects on the intestinal microflora has been scrutinized by many research groups. Certain published data indicate that probiotics could likely be useful in prevention and treatment of diarrhoea. *In vitro* studies done on the interactions of pathogens with probiotics are central in elucidating the role of individual probiotics in preventing and treating diarrhoea. This review will cover studies done on diarrhoea in swine and in particular will focus on data obtained using cellular models and well-designed clinical studies.

PROBIOTIC, PREBIOTIC, AND SYNBIOTIC

Probiotics are defined as viable microorganisms that when ingested in sufficient amount will reach the intestine in an active state where they can exert positive effects on the host health status. Probiotics can provide benefits to the host gut through a diverse set of mechanisms that include competitive exclusion of

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pathogens (interference), production of antimicrobial compounds, enterotoxin inactivation, modulation of host immune responses, and maintenance of intestinal barrier integrity. A prebiotic is defined as a food supplement that is nondigestible by the host but brings about specific changes, both in composition and/or activity, in selected members of the GI microbiota that confer benefits upon host well-being and health. Prebiotics are not hydrolysed or absorbed in the small intestine but rather are available as substrates for indigenous bacteria in the large intestine. Synbiotics correspond to synergistic combinations of pro- and prebiotics.

Probiotics, prebiotics, and synbiotics aim for the same goal: when ingested, to establish and/or help multiply members of the intestinal microbiota. Probiotics have been used as long as people have eaten fermented foods. While working at the Pasteur Institute in Paris, Ilya Ilitch Metchnikoff suggested that ingestion of lactobacilli-containing yogurt resulted in reduction of toxin-producing bacteria in the gut, promoting health and most probably longevity [1]. Following this conclusion, he promoted yogurt and other fermented foods as beneficial in humans. In fact, sold in pharmacies, the first industrially produced yogurt was developed to aid children suffering from diarrhoea.

The majority of probiotic microorganisms used in humans belong to the genera *Lactobacillus* and *Bifidobacterium*. For monogastric animals besides humans, common probiotics encompass yeasts like *Saccharomyces cerevisiae boulardii* and bacteria from the following genera: *Enterococcus* spp., *Pediococcus* spp., and *Bacillus* spp. [2]. A strain of *E. coli*, strain 1917, was also studied and found to confer a probiotic effect, in humans and animals, under certain conditions.

In animals, probiotics given as feed additives displayed health benefits against intestinal infections. The microorganism strain used as a probiotic should preferably originate from the target host species, as it may more readily assure its survival and colonisation. Commonly, the mode of action of a probiotic is complex and is not fully known.

There is some literature supporting the use of probiotics to prevent or treat intestinal disorders. Probiotics represent an attractive alternative treatment to antibiotics for which no resistance has been reported and considering the deleterious effect of antibiotics on the intestinal microbiota. Thus, probiotics can be seen as useful in the prevention and treatment of GI disorders in humans and animals. Probiotic therapy is believed to increase the numbers and activities of microorganisms that are proposed to possess health-promoting properties,

allowing the normal microbiota to be re-established. The beneficial effect of probiotics is based on the knowledge that the intestinal microbiota protects against infection and that disturbance of it increases susceptibility to infection. A probiotic formula can contain one or more strains of microorganisms belonging to diverse bacterial genera and yeasts. Studies are now conducted on probiotics with sufficient subjects (animals), proper controls, and discerning statistical analysis of results, so proper conclusions can be drawn.

PROBIOTIC CHARACTERISTICS

To be effective as probiotics, bacterial strains must possess some basic characteristics. They must resist gastric acid, bile salts, and pancreatic enzymes. They must also adhere to the intestinal mucosa in order to colonize the intestinal tract. Contrary to bacteria, yeasts like *S. cerevisiae boulardii* have the advantage of being unaffected by antibiotic therapy. This characteristic is important, as antibiotics could be administered for other conditions unrelated to GI infection at the same time as probiotic therapy.

Some proposed health effects of probiotics are relevant to the veterinary field, such as prevention and alleviation of diarrhoea and modulations of the intestinal microbiota and immunomodulatory functions. Most probiotics are of intestinal origin and belong to the lactic acid producing bacteria such as *Bifidobacterium*, *Lactobacillus*, and *Enterococcus*. The efficacy of *E. coli* Nissle 1917 in the pig small intestine of freshly weaned piglets for preventing the deleterious effects of ETEC has been demonstrated [3].

For potential microorganisms, proper *in vitro* studies should precede *in vivo* trials in order to evaluate their probiotic properties. For a better understanding of the interaction between intestinal cells and probiotics, cell models have been useful. The increasing application of probiotics in animal nutrition requires an investigation of the underlying mechanism of their health-promoting effects. One must remember that the gut microbiota is a fairly stable ecosystem and difficult to change without markedly altering the diet [4]. The results of animal experiments evaluating the efficacy of probiotics as alternatives to antibiotics have been inconsistent. A substantial body of evidence suggests that probiotics are able to counteract the pathogenic effects of ETEC. This matter will be discussed in the following sections.

DIARRHOEAGENIC *ESCHERICHIA COLI*

Commensal *E. coli* is a normal inhabitant of the intestine of warm-blooded animals. Genome plasticity of *E. coli* has permitted acquisition of diverse mobile genetic elements. Pathogenic *E. coli* strains are clonal strains that have acquired a characteristic pool of virulence factors. These strains can be grouped together on the basis of these amalgams. ETEC corresponds to one of these groups which are referred to as pathotypes or virotypes. The virulence factors of ETEC can be transferred horizontally between strains. ETEC has been defined as a noninvasive pathogen that does not cause damage to intestinal epithelial cells.

ETEC causes diarrhoea by disrupting intestinal cell homeostasis. The pathogenesis of ETEC starts with bacterial attachment to the host small intestinal epithelial cells followed by production of enterotoxins. Electrolyte imbalance results from exotoxin activity. ETEC infections are associated with acute watery diarrhoea. They are said to be of the secretory type, beginning with a sudden onset of watery stools without blood and with or without vomiting. These symptoms rapidly lead to dehydration. Inflammation and disruption of barrier function are also associated with this *E. coli* virotype.

Early control of intestinal disorders in newborn animals is crucial, as they are highly susceptible to diarrhoea caused by ETEC. The resulting infection can be fatal for young animals, especially postweaning [5]. Probiotics have been widely promoted as alternatives to the use of antibiotics or other feed additives.

ENTEROTOXIGENIC *ESCHERICHIA COLI*

ETEC causes diarrhoea through an electrolyte imbalance mediated by the exotoxins they elaborate. Adherence to the host cells is a fundamental mechanism for targeted delivery of the secreted enterotoxins. Colonisation factors (CFs) and enterotoxins differentiate ETEC from other categories of diarrhoeagenic *E. coli*. Colonisation is mediated by one or more proteinaceous fimbrial or afimbrial CFs. These CFs are exposed on the surface of ETEC and promote attachment to epithelial cells of the small intestine via specific interactions with molecules acting as receptors. In swine, the most frequently encountered fimbrial adhesins are F4 (K88), F5 (K99), F6 (987), F41, and F18 [6]. A non-fimbrial adhesin (AIDA-1, adhesin involved in diffuse adherence) has also been associated with certain ETEC strains isolated from pigs [7].

The ETEC strains implicated in postweaning diarrhoea

in piglets express F4 fimbriae, and the F4ac variant is the most prevalent in weaned piglets (96 to 98%) compared with the F4ab or F4ad variants (0.8 to 4.0%) [8]. Devriendt *et al.* [9] demonstrated that polymeric fimbriae (F4) are essential for adherence to porcine intestinal epithelial cells and provoke the secretion of IL-6 and IL-8. As an F4-negative strain could also stimulate cytokine secretion, it was demonstrated that the strain also expressed flagella. When tested, purified flagellin was shown to induce secretion of the same cytokines. Flagellin was thus recognized as a dominant factor involved in the induction of pro-inflammatory responses in intestinal epithelial cells.

Enterotoxins

ETEC produces two types of enterotoxins, heat-labile (LT) and heat-stable toxins (STs). LT enterotoxin is highly homologous to cholera toxin (CT). It is a heterologous molecule (AB₅) in which A is the enzymatically active moiety and B is responsible for binding to its receptor, the ganglioside GM1 [10]. The A subunit inhibits G_sα GTPase activity through ADP-ribosylation. This modification results in a constitutive activation of adenylate cyclase, the effector of the G protein. An increase of intracellular cAMP levels, in turn, activates the cystic fibrosis transmembrane regulator (CFTR). Ultimately, secretion of Cl⁻ and HCO₃⁻ is observed. Diarrhoea, a frequent loss of watery stools, then results.

STs secreted by ETEC are low molecular weight cysteine-rich molecules. Two heat-stable enterotoxins are responsible for diarrhoea in pigs, STa and STb [11]. STa is a peptide comprising 18 (STaP, porcine origin) or 19 amino acids (STaH, human origin) with six cysteines involved in formation of three disulfide bonds. Both are active in piglets. STa binds to its receptor, the extracellular domain of guanylyl cyclase C (GC-C) found on the apical plasma membrane of the intestinal epithelial cells. This interaction activates the intracellular domain of GC-C, resulting in intracellular accumulation of cGMP. This increase in cGMP activates cGMP-dependent protein kinase II, leading to the phosphorylation of CFTR subsequently resulting in Cl⁻ and HCO₃⁻ secretion. Inhibition of Na⁺ absorption is also noted. These changes lead to a net loss of water due to osmosis.

STb enterotoxin is a 48 amino acid peptide comprising two disulfide bonds. This enterotoxin binds to sulfatide, a glycosphingolipid widely distributed on the surface of intestinal epithelial cells [10]. After internalisation, STb activates a GTP-binding regulatory protein and leads to an increase in Ca⁺⁺ level, which, in turn, activates calmodulin-dependent protein kinase II (CAMKII). It is

also responsible for activating protein kinase C (PKC). These kinases phosphorylate CFTR, and this results in Cl^- and HCO_3^- secretion. At the same time, PKC inhibits Na^+ uptake and CAMKII opens a calcium-activated Cl^- channel. The elevated intracellular Ca^{++} level affects the activities of phospholipases A2 and C, resulting in the formation of prostaglandin E2 from membrane lipids and production of serotonin. These two secretagogues also mediate the transport of H_2O and electrolytes out of the cells contributing to diarrhoea.

These enterotoxins have all been shown to play an active role in tight junction (TJ) opening, contributing to an increase of permeability and leakage [12–14].

PROBIOTIC ACTIVITIES

Inhibition of bacterial adherence

Although probiotic bacteria may protect against ETEC-induced enteric infections, the underlying mechanism is often unknown. Many probiotics have been shown to inhibit pathogen adherence [15]. Probiotics have the capacity to either reduce or prevent colonization of an animal's intestines by pathogens. For example, a live culture of *Enterococcus faecium* 18C23 could efficiently inhibit the adherence of *E. coli* F4ac to the piglet intestinal mucus (F4 corresponds to K88 fimbriae of the previously used classification system). Approximately 9.0% of inoculated *E. faecium* adhered to the small intestinal mucus. The interaction was specific and dose-dependent, with over 90% inhibition when 10^9 CFU or more living bacteria per ml were added simultaneously with *E. coli* F4ac to immobilized mucus. Molecules from both the 18C23 cells and the spent culture contributed to the inhibition of adherence. The inhibition was not solely the result of the pH effect, as a considerable inhibitory effect was observed even after pH neutralization. The inhibition could have been the result of steric hindrance brought about by the molecules produced by *E. faecium* [16].

Probiotics can inhibit pathogen adherence by steric hindrance or competitive exclusion [17]. Modulation of the host immune system is another possible mode of action of probiotics. *Bifidobacterium animalis* MB5 and *Lactobacillus rhamnosus* GG protected cultures of intestinal Caco-2 cells from the inflammation-associated responses by partly reducing adherence and by counteracting neutrophil migration probably through the regulation of chemokine and cytokine expression [17].

Translocation

Translocation of microorganisms from the intestinal lumen to epithelial cells constitutes a pathogenic step

that permits invasion and dissemination of pathogens. *Pediococcus acidilactici* and *S. cerevisiae boulardii* are two probiotics currently used in swine. Administration of one of these two microorganisms modulates the development of porcine mucosal immunity and reduces intestinal bacterial translocation after ETEC challenge [18]. Probiotics reduce bacterial translocation through interactions with the intestinal epithelium and immunocompetent cells that contribute to enhancing the barrier function of the intestine wall and protection from the deleterious effects of pathogens. After ETEC challenge (F4⁺ O149), bacterial translocation to the mesenteric lymph node was reduced in pigs treated with *P. acidilactici*, *S. cerevisiae boulardii*, or a mix of these two strains compared with the control. There was evident protection against an ETEC-induced increase in intestinal permeability [18].

Inhibitory molecules

Probiotics produce a variety of substances that can inhibit both Gram-negative and Gram-positive bacteria. Beneficial effects of probiotics are also mediated via production of microbicidal substances. These inhibitory substances can exert bactericidal or bacteriostatic actions and include organic acids, hydrogen peroxide, and bacteriocins. They can contribute to reduction of the number of “unwanted”/pathogenic viable bacteria but could also affect bacterial metabolism or toxin production.

Krause *et al.* [19] tested the efficacy of *E. coli* probiotics in young pigs challenged with *E. coli* F4. They also tested the symbiotic interaction with raw potato starch, which can be used as prebiotic. The combination of raw potato starch and the probiotic had a beneficial effect on piglet growth and resulted in a reduction of diarrhoea and increased microbial diversity in the gut. In the ileum, colon, and faeces, there was an inverse relationship between the presence of probiotic *E. coli* (UM-2 and UM-7) and pathogenic *E. coli* F4. Their data demonstrated the efficacy of an *E. coli* probiotic selected based on its ability to produce colicins (a bacteriocin produce by *E. coli*) active against pathogenic *E. coli* F4.

Biofilm formation

Bacterial interference is based on the concept that a bacterial strain can interfere with the ability of another strain to colonise (and infect in some instances) the host. Many microorganisms live as sessile communities and adhere to surfaces. The small intestine and colon are the sites most heavily colonised by microorganisms in the GI tract. We now believe that bacterial biofilm formation

plays an important role in intestinal colonisation [20]. *E. coli* strain Nissle 1917 has been used since 1920 as a probiotic against a variety of intestinal disorders. This strain was recently shown to be a good biofilm former. It was significantly better at biofilm formation when in competition with enterotoxin-producing *E. coli* H10407, constituting 92% of biofilm after 16 hr [21]. It was able to outcompete ETEC during biofilm formation but failed to do so in the planktonic growth phase. It is becoming clear that biofilm formation is not solely associated with virulence but rather seems to be a wide fitness-associated trait. Thus, biofilm formation can probably give a probiotic strain an edge over pathogens.

ETEC and inflammation

Noninflammatory diarrhoea is caused by pathogens that affect specifically the small intestine by adhering to the mucosa and disrupting the absorptive and/or secretory processes of the enterocytes. No important mucosal destruction or acute inflammation is observed. Typically, ETEC infections have been shown to correspond to this definition. Noninflammatory diarrhoea generally produces few systemic symptoms, such as abdominal cramps, nausea, vomiting, and fever [22]. ETEC infection causes diarrhoea that is similar but less severe than that caused by cholera. Nevertheless, diarrhoeagenic *E. coli*, including ETEC, secretes toxins and produces virulence factors that exploit host cell functions to facilitate bacterial colonization. Many of these bacterial proteins subvert the inflammatory response of the host cell. These mechanisms include pro- and anti-inflammatory responses that favour bacterial survival and growth.

Besides acting as a barrier, the intestinal epithelium is also active in secreting molecules affecting both microbes and immune cells [23]. The epithelium actively participates in directing the innate immune response to enteric pathogens via secretion of chemokines that regulate immune cells. Overall, infection and inflammation take place in the intestine, which already contains a complex microbiota. The final response is the recruitment of neutrophils and other inflammatory cells induced by chemokines, such as IL-8, which in turn are regulated by several cytokines. Although neutrophils represent the primary line of host defence against microorganism, a massive and prolonged infiltration of neutrophils may perpetuate inflammation and ultimately lead to cell damage, epithelial barrier dysfunction, and the pathophysiology of diarrhoea [24]. A reduction in inflammatory cell infiltration by certain lactobacilli and bifidobacteria in pathogen-induced intestinal inflammation has been reported. However, not

all probiotic species exert the same anti-inflammatory activity in the intestine [17].

Probiotics and inflammation

In recent years, the pathogenesis of ETEC has been linked to epithelial inflammatory responses in the form of cytokine expression [9]. In piglets, infections with this *E. coli* virotype cause inflammatory responses in intestinal epithelial cells and subsequently diarrhoea, leading to reduced growth rate and mortality [6]. These strains display both pro- and anti-inflammatory properties and toxin production influences the outcome of this inflammatory process.

Pro-inflammatory responses involve modulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and mitogen-activated protein kinases (MAPK) signaling pathways primarily dependent upon LT enterotoxin [23]. LT-induced NF- κ B activation depends on the cAMP-dependent activation of the Ras-like GTPase (Rap1), but it is independent of protein kinase A (PKA). In effect, most of the secreted LT is associated with outer membrane vesicles (OMVs). Recent studies indicated that soluble LT and OMV-associated LT activate the NF- κ B and MAPK pathways differently. OMV-associated LT induces high levels of inflammatory cytokines such as IL-6 and TNF- α in T84 cells [25]. This result was confirmed in a mouse model [26].

ETEC STa toxin elicited significant amounts of IL-8, but ETEC isolates synthesising both STa and LT enterotoxins did not. This may be related to specific CFs found on the bacterial strains studied [27]. LT and STa are involved in the pro-inflammatory response by modulating NF- κ B and MAPK pathways.

Intestinal epithelial cells (IECs) are central to the activation of innate immunity and subsequently for induction of an adaptive immune response. ETEC F4 strains induce pro-inflammatory responses in porcine IECs. These cells are sensors detecting pathogen-associated molecular patterns (PAMPs) through pathogen recognition receptors (PRRs) such as Toll-like receptors (TLRs). Upon recognition of these PAMPs, IECs secrete several cytokines and chemokines. These then act on the underlying mucosal immune cells, such as dendritic cells, to trigger the innate immune defences and promote adaptive immune responses [9]. Various PRRs are involved in regulating intestinal epithelial cell barrier integrity. For example, IECs express TLR5 and IL-6 and IL-8 secretion is observed. Lipopolysaccharides (LPSs) increase intestinal TJ permeability both *in vitro* and *in vivo* by inducing membrane expression of TLR4 and CD14 [28]. TLR signalling pathways are responsible for

the induction of cytokines and chemokines in response to microbial-associated patterns (MAPs). In subsequent sections, the inhibitory effects of tested yeast and bacterial probiotics strains on the inflammatory process observed through various investigations are discussed.

Saccharomyces

The effect of *Pediococcus acidilactici* and *S. cerevisiae boulardii* on intestinal colonisation of ETEC F4 (LT⁺, STa⁺, STb⁺, and EAST1⁺) was tested [29]. Attachment of ETEC F4 to the intestinal mucosa was significantly reduced in pigs treated with these two probiotic strains. In addition, proinflammatory cytokines such as IL-6 were upregulated in ETEC F4-challenged pigs treated with *L. acidilactici* alone or in combination with *S. cerevisiae boulardii* compared with the control groups. These two probiotics were effective in reducing ETEC F4 attachment to the ileal mucosa, whereas the presence of *P. acidilactici* was required to modulate the expression of intestinal inflammatory cytokines in pigs. A notable finding of the study was a significant increase in IL-8 gene expression in the ileum of the pigs challenged with ETEC F4 compared with non-challenged pigs.

Zanello *et al.* [30] investigated whether the yeasts *S. cerevisiae* (strain CNCM I-3856) and *S. cerevisiae boulardii* (strain CNCM I-3799) decreased the expression of pro-inflammatory cytokines and chemokines in intestinal epithelial (IPI-21) cells cultured with ETEC F4. Viable *S. cerevisiae* inhibited ETEC-induced TNF- α gene expression, whereas *S. cerevisiae boulardii* did not. In contrast, killed *S. cerevisiae* failed to inhibit the expression of pro-inflammatory genes. Inhibition was dependent on secreted soluble factors, as culture supernatant showed full activity. The culture supernatant of *S. cerevisiae* decreased the ETEC-induced mRNA of the cytokines TNF- α , IL-1 α , IL-6, IL-8, CXCL2, and CCL20. Furthermore, the culture supernatant fraction smaller than 10 kDa displayed the same effects except for TNF- α . Thus *S. cerevisiae* secretes factors inhibiting the expression of pro-inflammatory cytokines and chemokines resulting from ETEC infection. This suggests that this strain of *S. cerevisiae* may protect the gut against intestinal damage caused by pathogen-associated inflammation in piglets.

S. cerevisiae (strain CNM I-3856) modulated transcript and protein expressions involved in inflammation, recruitment, and activation of immune cells in differentiated porcine intestinal epithelial (IPEC-1) cells [30, 31]. Viable *S. cerevisiae* inhibited the ETEC-induced expression of pro-inflammatory transcripts IL-6, IL-8, CCL20, CXCL2, and CXCL10 and proteins IL-6 and

IL-8. Measurement of trans-epithelial resistance (TER) indicated that *S. cerevisiae* failed to maintain the barrier integrity in a monolayer exposed to ETEC suggesting that *S. cerevisiae* does not inhibit ETEC enterotoxin activity. This yeast displays multiple immunomodulatory effects at the molecular level in IPEC-1 cells, suggesting that *S. cerevisiae* may influence the intestinal inflammatory reaction.

Lactobacillus

Recently, strain LR1 of *Lactobacillus reuteri* exhibited good adherence on IPEC-1 cells [32]. Pre-incubation of IPEC-1 with *L. reuteri* LR1 before ETEC exposure significantly inhibited ETEC adherence to the cells in culture. It also modulated transcript and protein expression of cytokines involved in inflammation. *L. reuteri* possesses regulatory activity with respect to early immune responses. In fact, this lactobacilli strain inhibited ETEC-induced expression of IL-6 and TNF- α pro-inflammatory transcripts and protein (IL-6) and increased the level of the IL-10 anti-inflammatory cytokine. In effect, ETEC increased the permeation of dextran (4000 Da) but co-incubation with *L. reuteri* reduced the flux of this marker. ETEC disrupted cell-cell contacts affecting ZO-1, resulting in TJ disruption. On the other hand, *L. reuteri* maintained the barrier integrity of IPEC-1 cell monolayers exposed to ETEC. *L. reuteri* could prevent ETEC-induced TJ disruption by maintaining the correct localisation of ZO-1 as well as by inhibiting the destruction of ZO-1 protein. This strain showed desirable probiotic properties modulating the immune response and strengthening barrier function, two important indicators of good health status. This strain could be of potential use in swine production, but animal feeding trials are needed to confirm the above findings.

Another study indicated that co-incubation of ETEC with either *Lactobacillus rhamnosus* GG (LGG) or *Lactobacillus johnsonii* strongly inhibited the attachment of ETEC to IPEC-J2 cells [33]. In contrast, in that study, co-incubation of *L. reuteri* and ETEC did not reduce ETEC attachment. A challenge with ETEC caused TJ openings observed as a loss of cell-cell contact; the ZO-1 lining was weakened and broken by ETEC challenge. An *L. reuteri* strain isolated from a pig significantly attenuated the ETEC-induced disruption of ZO-1 in the vicinity of TJ structures and the loss of intestinal barrier integrity, whereas *L. johnsonii* and LGG provided less protection under ETEC challenge, partly by modulating TJ protein expression and distribution. The relative abundance of *Lactobacillus* spp. is strongly correlated with expression of heat-shock protein 27 (HSP27 having

chaperone properties) in the small intestine of young pigs. HSP carries crucial housekeeping functions to maintain mucosal barrier integrity against various stimuli in the intestinal environment. In particular, HSP27 is associated with cytoskeleton stabilisation [34]. HSP27 has been observed to stimulate overproduction of IL-10 (a major anti-inflammatory cytokine). *L. johnsonii* and *L. reuteri* strains were shown to significantly induce HSP27, whereas LGG did not. HSP27 can bind directly to cytoskeleton protein F-actin and stabilise the TJ complex [34]. Interestingly, ZO-1 can also bind directly to F-actin to regulate cytoskeleton organisation [35].

Inflammation derived from pathogen infection involves the elevation of TLR signalling. The immunomodulatory activities that protect intestinal cells from ETEC F4 infection through cytokine regulation were established for *L. amylovorus* DSM16698TM [36]. Western blot analysis showed that *L. amylovorus* and its cell-free supernatants suppress the activation of different steps of TLR4 signalling in Caco-2/TC7 cells and pig explants by inhibiting the ETEC-induced increase in the level of TLR4 and MyD88 (a universal adapter protein for TLRs (except TLR3) to activate NF- κ B) and the overproduction of inflammatory cytokines IL-8 and IL-1 β . *L. amylovorus* also blocks the upregulation of extracellular heat shock protein Hsp72 and Hsp90, which are critical for TLR4 function.

Lactobacillus sobrius is a newly isolated porcine intestinal commensal that is abundant in the porcine GI tract during the neonatal period [37, 38]. Probiotic supplementation with *L. sobrius* DSM16698 significantly reduced the levels of ETEC F4 in the ileum when fed directly to piglets after weaning [39]. In contrast, the number of days when the piglets had an increased faecal water content was significantly higher in the probiotic-treated group (increase in the days of diarrhoea). A significant reduction in ileal ETEC prevalence, an increase in animal body weight gain, and modulation of IgA development were evidenced in the piglets fed *L. sobrius* compared with those fed the control diet.

Zhang *et al.* [40] preincubated IPEC-J2 cells with and without *L. rhamnosus* ATCC7469 and then exposed them to ETEC F4. Increased TLR4 mRNA expression was observed after ETEC challenge, but this increase was attenuated by *L. rhamnosus* treatment. Pretreatment with *L. rhamnosus* counteracted ETEC F4-induced increases in TNF- α concentration. Pretreatment with *L. rhamnosus* increased ZO-1 and occludin protein expression. The findings of their study suggested that *L. rhamnosus* protects intestinal epithelial cells from ETEC F4-induced damage, partly through its anti-inflammatory action. In

addition, *L. rhamnosus* may activate intestinal epithelial cells in response to bacterial infection, in turn increasing TJ integrity and thus enhancing barrier function and restricting pathogen invasion. Preincubation with *L. rhamnosus* was superior to co-incubation in reducing the adherence of ETEC F4 to IPEC-J2 cells and subsequently attenuating ETEC F4-induced mucin layer destruction and suppressing apoptosis. This strain of *L. rhamnosus* interacts with porcine intestinal epithelial cells to maintain the epithelial barrier and promote intestinal cell activation in response to bacterial infection.

Bacillus

The probiotic LSP122 containing viable spores of *Bacillus licheniformis* was tested for its efficacy in controlling postweaning diarrhoea in piglets [41]. *Bacillus toyoi* (Toyocerin[®]) was also tested in that study. This strain is widely used and contains viable spores of *B. toyoi* (which is a strain of *Bacillus cereus*). All animal groups supplemented with a probiotic exhibited a reduced incidence and severity of diarrhoea. No ETEC strains were detected on day 22 in the LSP122 and Toyocerin groups receiving 10⁷ viable spores. All performance parameters taken into account in this study were improved by the use of probiotics in piglet diet during the first four weeks postweaning. At 10⁷ viable spores per gram of feed, this probiotic is a useful agent for the control of postweaning diarrhoea due to ETEC.

Enterococcus

Enterococcus faecium NCIMB10415 is used as a probiotic supplement in farm animals, as it has shown positive effects on diarrhoea incidence in pigs [42, 43]. It is licensed as a feed additive for sows and piglets and has been demonstrated to reduce diarrhoea incidence and severity in weaning piglets [43]. Its effects on GI immunological parameters and barrier function have also been reported [44–47]. Lodemann *et al.* [48] focused on the barrier function of these cells in the presence of ETEC with and without the probiotic strain. This probiotic prevented damage induced by ETEC on epithelial cell integrity when pre- and co-incubation was performed. Preincubation with this *E. faecium* abrogated or reduced all examined effects, on IPEC-J2 and Caco-2 cell lines, induced by an ETEC strain producing LT, STa, STb, and EAST1 toxins [49]. The ETEC strain decreased TER and increased mannitol flux rates in both cell lines and also increased IL-8 mRNA and protein expression. These changes could be prevented by co-incubation with *E. faecium*. *E. faecium* thus positively affected epithelial integrity and could participate in restoration of the

damage to TER induced by ETEC strains. *E. faecium* has now been accredited for piglet nutrition.

Long-term application of probiotic *E. faecium* NCIMB 10415 modifies the intestinal microbiota of sows and hence their faecal microbiota [43]. The actual percentage of piglets with postweaning diarrhea in the probiotic-treated group was 20% compared to 38% in the control group. Genes for STb and STa toxins were most widely distributed between studied isolates in both treatment groups. With weaning, the frequency of isolates carrying pathogenicity genes, especially STb and STa, increased.

ETEC and barrier function

The intestinal mucosal barrier is the largest interface separating the internal and external environments. A critical component of this barrier is the cell junctions between adjacent intestinal epithelial cells, which form a semipermeable diffusion barrier. These intercellular junctions comprise the tight junction, the adherens junction, desmosomes, and the gap junction [50]. The TJ is the most apical structure responsible for controlling the permeability of the paracellular pathway. ETEC can adhere to the brush border of epithelial cells and damage the cell junctions, leading to intestinal inflammation and diarrhoea [51, 52]. The impaired intestinal barrier caused by ETEC infection can allow pathogens and toxins, such as endotoxin from Gram-negative bacterial cell walls, to enter the body.

The maintenance of barrier function is associated with dynamic modulation of the TJ complex, which encloses IECs, protecting them against the uptake of food antigens, gut microbes, and other macromolecules [35]. Occludin, ZO-1, and claudin-1 are the most important and critical components in the structural and functional organisation of the TJ. These proteins play primary roles in membrane function and integrity through interaction of ZO proteins and the membrane-spanning proteins claudin-1 and occludin [53].

Cytokines play important roles in the inflammatory response and participate in regulation of the integrity of the intestinal barrier. Many cytokines have been shown to regulate the cell junctions and cytoskeleton structure and function [54]; in particular, IL-8, a pro-inflammatory cytokine inducer recruiting neutrophils to phagocytose antigens has been associated with pathogen-induced alterations of TJs [55].

Probiotics and barrier function

In vitro studies have shown that ETEC F4 increases TJ permeability in IPEC-J2 cells [56] and reduces occludin abundance in IPEC-1 enterocytes [51]. ETEC F4 induces

a profound increase in IL-8 and TNF- α expression, which may cause disruption of membrane barrier. ETEC inhibited the expression of mitogen-activated protein kinase phosphatase-1 (MKP-1), which can inhibit the transcript abundance of pro-inflammatory cytokine genes by inactivating MAPK [57], which is important in maintaining the integrity of TJ. In cells infected with ETEC, the expression levels of claudin-1, ZO-1, and E-cadherin were reduced. *L. plantarum* partially inhibited F4-triggered expression of inflammatory cytokines IL-8 and TNF- α . Partial protection of the cell barrier was provided by prior exposure to *L. plantarum* [58]. The induction of negative regulators of TLRs by *L. plantarum* in IPEC-J2 cells therefore may be important in inhibiting the inflammatory cytokines and probably does so through the NF-K β and MAPK pathways.

ETEC may activate myosin light chain kinase, leading to contraction of the circumferential actomyosin ring and thus causing damage to cell junctions and alteration of permeability [56]. Treatment with *Lactobacillus fructosus* C2 attenuated the pathogen-induced alterations in epithelial barrier function. In fact, Yu *et al.* [59] found that disruption of the membrane barrier by ETEC was associated with strong increase of IL-8 expression. Treatment with *L. fructosus* C2 reduced IL-8 expression, suggesting it could prevent the occurrence of inflammation. Probiotics can inhibit epithelial barrier disruption by MAPK-dependent mechanisms [60, 61].

Infection of Caco-2 cells with ETEC F4 stimulated high expression of IL-8. At the same time, the expression levels of claudin-1, ZO-1, and E-cadherin were decreased significantly. *Lactobacillus amylophilus* D14 provoked a reduction in dextran permeability, increased TER in Caco-2 cells [62], and decreased the secretion of IL-8. In Caco-2 cells, it increased the expression and distribution of TJ proteins such as ZO-1, claudin-1, and E-cadherin when co-cultured with ETEC. This bacterial strain may thus influence the mitogen-activated protein kinase pathway to regulate the correct assembly of TJs and adherens junctions. It could protect cell junctions and mucosal barrier from damage caused by ETEC.

Many *in vitro* and *in vivo* studies demonstrated that *L. plantarum* can protect against dysfunction of the intestinal epithelial barrier by restoring the structure and function of TJ and reducing paracellular permeability [63, 64]. *L. plantarum* reportedly also protects the intestinal barrier function of early-weaned piglets against ETEC F4 challenge [58]. However, the underlying cellular mechanism of this protection was unclear. Using IPEC-J2, Wu *et al.* [58] showed that pretreatment with *L. plantarum* prevented the reduction in TER, inhibited

the increase transcript abundances of IL-8 and TNF- α , decreased expression of claudin-1, occludin, and ZO-1, and decreased the protein expression of occludin provoked by ETEC F4. A recent study showed that *L. reuteri* effectively improved the intestinal mucosal barrier in formula-fed pigs [65]. An earlier study investigated the preventive effect of *L. plantarum* on diarrhoea in relation to intestinal barrier function in young piglets challenged with ETEC F4 [66]. Pigs challenge with ETEC F4 had decreased ZO-1 and occludin mRNA together with a reduced protein expression in the jejunum. The above suggest that *L. plantarum* given to piglets in early life can effectively prevent diarrhoea induced by ETEC F4 challenge in young piglets by improving the function of the intestinal barrier by protecting the morphology and permeability of the intestine and expression of genes for TJ proteins (ZO-1 and occludin). No changes were detected in claudin-1 mRNA abundance as well as protein content in agreement with Johnson *et al.* [56]. The beneficial effect of *L. plantarum* was most obvious in the jejunum of young piglets. *L. plantarum* clearly prevented the damage to intestinal morphology caused by ETEC F4 infection.

Dose effect

Li *et al.* [67] evaluated the dose effect of the probiotic *L. rhamnosus* ATCC7469 in weaned piglets when orally administered at low (10^{10} CFU per day) and high doses (10^{12} CFU per day) for one week before ETEC F4 challenge. High-dose administration of the probiotic increased the incidence of diarrhoea before ETEC F4 challenge despite the fact that both doses ameliorated ETEC F4-induced diarrhoea, with increased *Lactobacillus* and *Bifidobacterium* counts accompanied by reduced coliform shedding in faeces. Interestingly, *L. rhamnosus* administration reduced the *Lactobacillus* and *Bifidobacterium* counts in the colonic contents, and piglets receiving the high dose also had lower *Lactobacillus* and *Bifidobacterium* counts in their ileal contents. The data showed that *L. rhamnosus* shapes the composition of the gut microbiota, attenuates acute inflammatory responses (decreases TLR4 expression and TNF- α , IL-8, and porcine defensin 2 mRNA expression) induced by ETEC F4, and slows the progression of acute infectious diarrhoea. These results contradict the assumption that the beneficial effects of probiotics in preventing infectious diarrhoea are augmented with increasing dose. Instead, it was observed that pretreatment with a low dose of *L. rhamnosus* might be more effective in ameliorating diarrhoea symptoms.

Another research team tested low (10^9 CFU/ml) and

high (10^{11} CFU/ml) doses of the same probiotic strain (*L. rhamnosus* ATCC7469) administered orally to piglets for one week before ETEC F4 challenge [68]. Notably, transiently increased serum concentrations of interleukin-17A were observed after ETEC F4 challenge in pigs pretreated with the high dose but not with the low dose of *L. rhamnosus*. Expression of jejunal IL-2, ileal transforming growth factor β 1 (TGF- β 1), and ileal IL-10 was upregulated in the piglets receiving the low dose of the probiotic after ETEC F4 challenge. However, high doses of *L. rhamnosus* may increase levels of serum IL-17A after ETEC F4 challenge, thus eliciting a strong pro-inflammatory response.

Yang *et al.* [69] administered a low (3.9×10^8 CFU/day) and high (7.8×10^8 CFU/day) dose of a *B. licheniformis* and *B. subtilis* spore mixture (BLS-mix) orally to mucin 4 (MUC4)-resistant pigs for one week before ETEC F4/VTEC/EPEC challenge. MUC4-resistant pigs are not absolutely F4ab/ac receptor-negative pigs, as previous studies found that more than 30% showed positive adherence with F4ab/ac ETEC and more receptors for F4 fimbriae have been discovered [70, 71]. Administration of the probiotic BLS-mix partially ameliorates *E. coli*-induced enteritis by facilitating upregulation of intestinal IL-22 and IkBa (an inhibitor of the NF- κ B transcription factor) expression and by preventing loss of intestinal epithelial barrier integrity via elevation of ZO-1 expression. The above suggest that a low dose of the probiotic BLS-mix may allow for better protection of the host against enteric pathogens in clinical practice.

The abovementioned studies highlight the fact that high doses of certain probiotics may negate the preventive effects, at least in part, by distributing the established microbial ecosystem and by interfering with mucosal immune responses against potential enteric pathogens.

Negative effects

LGG is a probiotic used in humans and is normally not found in pigs. Dietary administration of LGG did not prevent or reduce the detrimental effect of ETEC F4 infection on growth performance and health status of weaned piglets [72]. In fact, after oral stimulation with ETEC, the inclusion of LGG in the diet of weaned piglets impaired growth performance, feed intake, and health status as compared with the standard diet. The counts of lactic acid bacteria, enterobacteria, and yeasts in the colon were not affected, but a trend toward increased ETEC excretion was observed. It was also reported that high doses of LGG (10^{10} CFU/L) can be pro-inflammatory (increase in IL-8). The overall humoral response (secretory IgA) was depressed by the probiotic

treatment.

Zhou *et al.* [73] administered orally various doses of a BLS-mix to pigs for one week before ETEC F4/VTEC/EPEC challenge. Their data indicate that oral administration of the BLS-mix to newly weaned F4 pigs ameliorates enteritis symptoms and excessive generation of CD4⁺ IL-10 T cells following consumption of the BLS-mix during episodes of intestinal inflammation caused by enteric pathogens but might prohibit clearance of the pathogen. Induction of IL-10-producing cells by the BLS-mix cannot account for protection of the newly weaned pigs.

Crowding stress and ETEC F4 challenge were also shown to have a negative influence on some performance and immunological measures in pigs, and probiotic feeding (*E. coli* UM-2 and UM-7) had few positive effects on the measured parameters [74]. In addition, it should be noted that no-response studies are probably not published in many cases.

Feed fermentation

As fermented feeds may benefit gut health and improve performance in pigs, some of these feeds were tested to determine their contribution to the health status of animals. The effects of feed fermentation with *L. reuteri* TMWL.656 on growth performance and the abundance of ETEC in weaning piglets was studied [75]. *L. reuteri* strains produce reuteran or levan exopolysaccharides that inhibit ETEC adherence to the mucosa. Feed intake was reduced in pigs fed diets containing exopolysaccharides; however, feed efficiencies did not differ among the diets. *L. reuteri* reduced the level of ETEC colonisation in weaned piglets, and feed fermentation supplied concentrations of reuteran that may specifically contribute to the effect observed on ETEC. All fermented feeds significantly reduced the abundance of *E. coli* and STb toxin in the colonic digesta. Genes coding for *E. coli* and STb were detected in the gut digesta of pigs fed unfermented diets; in contrast, the copy numbers of the genes for *E. coli* and STb were below the detection limit for all samples from pigs fed a reuteran-containing diet fermented with *L. reuteri*. Other ETEC virulence factors, including LT, STa, and F4 fimbriae, were not detected in any of the samples from gut digesta tested (ileum, cecum, and colon).

Probiotic and flaxseed oil association

Chytilova *et al.* [76] determined the immune response after preventive administration of flax-seed oil (rich in n-3 polyunsaturated fatty acids) or probiotic *Lactobacillus plantarum*-Biocenol™ LP96 or their combination in the jejunum of ETEC-challenged gnotobiotic pigs.

Combined treatment downregulated pro-inflammatory cytokine IL-1 α and IL-8 gene expression, upregulated TNF- α , and tended to regulate inflammation induced by ETEC through cytokine IL-10. The authors also observed that the combined treatment stimulated anti-inflammatory properties of Th-1-mediated cell immunity and phagocytosis and tended to regulate the inflammatory response induced by ETEC F4. These findings suggest that immunomodulators can regulate the immune response in different ways and therefore effectively suppress pathogen-induced inflammation at the cellular and molecular levels. A marked anti-inflammatory and immunoregulatory effect of *L. plantarum* alone was confirmed in this study, and moreover flaxseed oil was shown to play an important role in potentiation of the immunomodulatory effect of probiotic microorganisms.

Probiotics interfering with enterotoxin production

Caenorhabditis elegans, a nematode worm currently used as a model, was established as a lifespan assay to preselect probiotic bacteria for controlling ETEC F4 [77]. Lactic acid producing bacteria (13 strains) varied in their ability to protect *C. elegans* from death induced by ETEC strain JG280, which possesses genes coding for LT, STa, and STb; *Lactobacillus zae* LB1 offered the highest protection (86%). Treatment with *Lactobacillus* did not reduce ETEC JG280 colonisation in the nematode intestine. Feeding of *E. coli* strain JFF4, an F4 strain lacking the genes for LT, STa, and STb, did not cause death of the worms. There was a significant increase in gene expression of the three enterotoxin genes during ETEC JG280 infection, which was remarkably inhibited by the LB1 isolate. The clone with either the *estA* (STa) or *estB* (STb) gene expressed in *E. coli* DH5 α killed approximately 40% of the worms compared with the ETEC JG280 strain. Killing by the clones could also be prevented by the LB1 isolate, but this isolate only partially inhibited gene expression of enterotoxins in both ETEC JG 280 and *E. coli* DH5 α *in vitro*. Heat-stable enterotoxins appeared to be the main factors responsible for the observed death of *C. elegans*. Inhibition of ETEC enterotoxin production, rather than interference of intestinal colonisation, appears to be the mechanism of protection provided by *Lactobacillus*.

A strategy to treat GI infections based on molecular mimicry of host receptors for bacterial toxins was also investigated. A recombinant probiotic was developed in which glycosyltransferase genes from *Neisseria meningitidis* or *Campylobacter jejuni* were expressed in an *E. coli* strain (CWG308) [78]. This resulted in the production of a chimeric LPS capable of binding LT

enterotoxin with high avidity. A construct expressing a mimic of lacto-N-neotetraose (CWG308:pLNT) neutralized approximately 94% of LT activity in culture lysates of diverse ETEC strains of human and porcine origin. Pre-absorption with or co-administration of CWG308:pLNT also resulted in significant *in vivo* protection from LT-induced fluid secretion in rabbit ligated ileal loops. A particularly attractive feature of the toxin-receptor blockade strategy is that it does not apply selective pressure for evolution of resistance by the targeted pathogen.

Synbiotic evaluation

The efficiency of *E. coli* probiotics together with the synbiotic interaction with raw potato starch as a prebiotic was tested in young pigs challenged with *E. coli* F4 [19]. Selected probiotics could ferment starch or at least the by-product of starch. The combination of raw potato starch and the probiotic had a beneficial effect on piglet growth performance and resulted in reduction of diarrhoea. In the ileum, colon, and faeces, there was an inverse relationship between the presence of *E. coli* probiotic (UM-2 and UM-7) and pathogenic *E. coli* F4, suggesting they had an inhibitory effect. An increase in gut microbial diversity was also noted. A reduction in microbial number and diversity is related to poor gut health, so an increase in gut microbial diversity can be related to health [79]. The tested synbiotic was effective in reducing the negative effects of ETEC in a piglet challenge model. However, there is a disadvantage of using *E. coli* as a probiotic, as it is not generally considered safe due to the plasticity of its genome (Canadian Food Inspection Agency, 2009).

The potential of the prebiotic oligosaccharide lactulose, a probiotic strain of *Lactobacillus plantarum*, or their synbiotic combination to control postweaning colibacillosis in pigs was evaluated using an oral challenge with an ETEC F4 strain [80]. Inclusion of the probiotic in the feed increased the number of *L. plantarum* bacteria in the ileum and colon and the total number of lactobacilli in the colon and showed a trend to reduce diarrhoea. A decrease of plasmatic TNF- α was also noted. The positive effects of the two additives were combined in the synbiotic treatment. The study showed a potential prebiotic effect for lactulose in piglets along with increases in average daily gain. This improved response was also confirmed by reductions in the plasma pig-MAP (pig major acute-phase protein in serum) concentration and could be due to the observed promotion of lactobacilli and improvements in epithelium architecture. Positive effects of *L. plantarum* and lactulose were additive in their synbiotic combination despite the fact that lactulose

was not able to promote specific growth of *L. plantarum*.

Beta-galactomannan had a similar protective role as *S. cerevisiae boulardii* in the control of ETEC infection (F4⁺, LT⁺, STa⁺, and STb⁺) [81]. The strain and the compound inhibited adherence of ETEC on the cell surface of porcine intestinal cells (IPI-2I). Both treatments decreased the ETEC-induced mRNA expression of pro-inflammatory cytokines TNF- α , IL-6, and granulocyte macrophage-colony stimulating factor (GM-CSF) and chemokines CCL2, CCL20, and CXCL8. Similar to the probiotic *S. cerevisiae boulardii*, prebiotic beta-galactomannan may protect intestinal epithelial cells against intestinal pathogens.

Lactobacillus delbrueckii subsp. *delbrueckii* TUA4408L (Ld) or its extracellular polysaccharides (EPSs), acidic EPS (APS) and neutral EPS (NPS), modulated the response of porcine intestinal epitheliocyte cells (PIE) against ETEC 987P [82]. ETEC-induced inflammatory cytokines were downregulated when PIE cells were prestimulated with both Ld or EPSs. Ld, APS, and NPS inhibited ETEC-mediated MAPK and NF- κ B activation by upregulating TLR negative regulators. TLR2 was not shown to play a major role in the immunomodulatory action of Ld, while the activity of APS is mediated by TLR4. Ld and its EPSs have the potential to be used for the development of anti-inflammatory functional foods to prevent intestinal diseases both in humans and animals.

Probiotics and vaccine

E. coli Nissle 1917, a recognized probiotic strain in humans, was exploited as a carrier for targeted delivery of recombinant molecules to the intestinal mucosa [83]. A recombinant strain that expressed the determinant for the F4 fimbriae was constructed. Oral immunization of mice showed that the recombinant strain could only be detected for 7 days in faecal samples after the last feeding, indicating that the strain could transiently colonise the murine intestine. Significant IgG serum titres against F4 were detected as early as 7 days after initial feeding, but no IgA was observed. In contrast, no specific T cell response toward F4 was detected in the spleen and mesenteric nodes. Although dendritic cells readily upregulated maturation and activation of markers in response to F4 stimulation, accompanied by secretion of IL-12, IL-6, IL-10, and TNF- α , restimulation of T cells of mice that had received the construct did not result in detectable T cell proliferation and IL-12 secretion but rather induced an IL-10 bias. This issue prompted the authors to recommend that great care be taken when using probiotic bacteria for oral vaccination, as they

Table 1. Probiotics and their effects on (A) cellular models and (B) in piglets

	Antimicrobial compounds production	Increase microbial diversity	Enterotoxin production inhibition	Inhibition of attachment	Modulating immune response	Strengthening barrier integrity	References
A) Probiotic strain / Cellular model							
<i>Bifidobacterium animalis</i> MB5 / Caco-2					x		[17]
<i>Lactobacillus rhamnosus</i> / Caco-2					x		[17]
<i>S. cerevisiae</i> CNCM I-3856 * / IPI-21, IPEC-1					x		[30, 31]
<i>S. cerevisiae boulardii</i> CNCM I-3799 / IPI-21					x		[30]
<i>Lactobacillus reuteri</i> LR-1 / IPEC-1				x	x	x	[32]
<i>Lactobacillus rhamnosus</i> GG / IPEC-J2				x		x	[33]
<i>Lactobacillus johnsonii</i> / IPEC-J2				x		x	[33]
<i>Lactobacillus amylovorus</i> DSM 16698 / Caco-2					x		[36]
<i>Lactobacillus rhamnosus</i> ATCC 7469 / IPEC-J2					x	x	[40]
<i>Enterococcus faecium</i> NCIMB 10415 / Caco-2		x			x	x	[44–49]
<i>Enterococcus faecium</i> NCIMB 10415 / IPEC-J2		x			x	x	[44–49]
<i>Lactobacillus plantarum</i> / IPEC-J2					x	x	[58]
<i>Lactobacillus fructosus</i> C2 / Caco-2					x	x	[59]
<i>Lactobacillus amylovorus</i> D14 / Caco-2					x	x	[62]
<i>Lactobacillus plantarum</i> / Caco-2				x		x	[63, 64]
<i>Lactobacillus zeae</i> LB1 / <i>C. elegans</i> **			x				[77]
B) Probiotic strain							
<i>Enterococcus faecium</i> 18C23 (piglet intestinal mucus)				x			[16]
<i>S. cerevisiae boulardii</i>						x	[18]
<i>Pediococcus acidilactici</i>						x	[18]
<i>Escherichia coli</i> UM-2	x	x					[19]
<i>Escherichia coli</i> UM-7	x	x					[19]
<i>Pediococcus acidilactici</i>				x	x		[29]
<i>S. cerevisiae boulardii</i>				x	x		[29]
<i>Lactobacillus sobrius</i> DSM 16698				x			[39]
<i>Lactobacillus reuteri</i>						x	[65]
<i>Lactobacillus plantarum</i>						x	[66]
<i>Lactobacillus rhamnosus</i> ATCC 7469		x			x	x	[67, 68]
<i>Bacillus licheniformis</i>					x	x	[69]
<i>Bacillus subtilis</i>					x	x	[69]
<i>Lactobacillus reuteri</i> TMWI.656			x	x			[75]

Saccharomyces cerevisiae**Caenorhabditis elegans*

can be capable of creating an adverse anti-inflammatory environment in the gut.

Conclusions

From the recent studies performed using cell models and in the best cases confirmed using piglets, we can conclude that probiotics have the potential to play a positive role on ETEC (F4⁺strain)-induced diarrhoea (Table 1). Nevertheless, some inconsistencies in the results obtained in various studies constitute a bias that makes these data sometimes irreconcilable. We know that many variables influence the outcome of probiotic treatments, and this could be part of the explanation for the discrepancies observed. For example, the choice of

the probiotic strain, the age of the animal, the time of administration, and the dosage are all relevant for the favourable outcome of a treatment.

Probiotics can act on various steps of the pathogenesis process. Inhibition of adherence to target cells was described many years ago. Blockade of this process is significant, as it represents the first step in the pathogenesis process of ETEC. Also, the bacteriostatic or bactericidal effect of a probiotic represents a desirable issue, as it prevents colonisation of the intestine. Inhibition of enterotoxin production or inhibition of its toxicity has been debated for many years but has only recently been proven. The beneficial effects of probiotics on the inflammation process provoked by ETEC infection and

the restoration or maintenance of barrier function seem now central aspects of probiotics action. Prebiotic and synbiotic approaches could also improve the success rate of the performed treatment.

Overall, considering the potential of probiotics to treat diarrhoeal disease in swine, it is now fair to say that they represent an attractive alternative to antibiotherapy. In the upcoming years, some of the tested probiotics should be used on a regular basis to treat ETEC-induced diarrhoea in swine.

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