Innovative Romanian Food Biotechnology Vol. 18, 2020
2020 by Galaţi University Press Received October 2017/Accepted May 2020

RESEARCH ARTICLE

ANTI-INFECTIOUS ACTIVITY AND PROPHYLACTIC EFFECTS OF *BIFIDOBACTERIUM BIFIDUM* (STRAINS OF HUMAN ORIGIN), PROBIOTIC FEEDING ON ENTEROPATHOGENIC *ESCHERICHIA COLI*, IN RATS (*IN VIVO* ANTAGONISM)

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Abstract: The *Bifidobacterium bifidum* strain was isolated on MRS medium supplemented with 0.5g/L of cysteine hydrochloride, 2 mg/L of nalidixic acid and 0.1 mg/L of mupirocin. This strain was isolated from breastfed infant faeces. The effectiveness of *Bifidobacterium bidum* as a probiotic against enteropathogenic *Escherichia coli* infection was studied using the rat model. After dissection of all rats, macroscopic and microscopic observations of histological sections of the digestive tract were analysed until 2 and 3 weeks postinfection. The pathogenicity of *E. coli*, marked by body weight loss and intestinal histopathological changes in the infected group, was significantly reduced in the *B. bifidum*-treated group. Feeding *B. bifidum* for 7 days before infection resulted in greater post-challenge feed intake and weight gain and lower faecal levels of enteropathogenic *E. coli*. A reduced degree of protection against *E. coli* infection was observed when bifidobacteria were given during the several days after *E. coli* infection.

Keywords: *Escherichia coli* (EPEC); infection; probiotics; protection.

Introduction

Diarrhea remains the second leading cause of death in children younger than 5 years globally, accounting for 1.3 million deaths annually. Enteropathogenic *Escherichia coli* (EPEC), one of the diarrheagenic *E. coli* pathotypes, are among the most important pathogens infecting children worldwide because of their high prevalence in both the community and hospital setting, and

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because they are one of the main causes of persistent diarrhea. Since the diagnosis of these pathogens is now based mainly on molecular diagnosis, there has been an important change in the prevalence and distribution of these pathogens. The purpose of this paper is to review the current epidemiology of EPEC infection in children and the new insights into its physiopathology (Theresa *et al.,* 2011).

These bacteria have been shown to confer enhanced resistance against infection with enteric pathogens and a variety of mechanisms have been proposed to explain the effects. Potential mechanisms to explain the enhanced resistance conferred by bifidobacteria, include competitive adhesion to intestinal mucosa, production of antimicrobial substances and/or stimulation of mucosal immunity factors (Servin, 2004). Inhibition of enteric pathogens by commercial bifidobacteria has been reported in recent studies using in vitro tests. Although the studies of *E. coli* infection using animal models with type culture probiotic strains have been reported (Kim *et al*., 2001; Shu and Gill, 2001;Asahara *et al*., 2004), none have been done using a human fecal bifidobacterial isolate. In fact, probiotic microorganisms should not only be capable of surviving passage through the digestive tract but also have the capability to proliferate in the gut. Probiotics must be able to exert their benefits on the host through growth and activity in the human body. We have previously reported that a human bifidobacterial strain was able to significantly inhibit *E. coli O157:H7* adhesion to Caco-2 cells (Gagnon *et al*., 2004). The present study was conducted to evaluate the anti-infectious activity of this strain, genetically identified as *Bifidobacterium bifidum* Bbf1, against *E. coli* in a rat model.

Materials and Methods

Animals

The mice were housed individually in a temperature controlled environment (22 ± 2°C) with a 12 h light/dark cycle and fed standard rodent chow, with free access to water throughout the experiment (Gagnon *et al.,* 2006).

In vivo feeding

Twenty four albino rats (Wistar strain) aged 5 – 6 weeks. The rats were fed on basal diet, for 1 week *ad libitum* before the treatment. They were assigned randomly to one of the five following experimental group of six rats each:(1) control group: not given bifidobacteria, Not challenged;

(2) infected group: not given bifidobacteria, challenged with *E. coli* (10^8 CFU/mL for one week); (3) pre-infection feeding group: given bifidobacteria on day 7, and challenged with *E. coli* for second week; (4) post-infection feeding group: given *E. coli* on 7 day, then with bifidobacteria (Doumandji, 2007).

Strains tested

The strain of *E. coli* used was isolated from the infant diarrhoeal stools of the Oran hospital. We chose this species after analysis of several coprocultures of diarrhoeal infant stools, and then incubated in EMB medium at 44°C/24 h.

Bidobacterium bifidum (Bbf1) strain was isolated from (breastfed infant faeces on MRS medium contened 0.5g/L of cysteine hydrochloride, 2 mg/L of nalidixic acid, we chose this strain by its antagonistic effect against *E.coli* EPEC strain studied, and resistance in the intestinal tract (Biavati *et al*., 1992) and its capacity to survive at high rates.

Bacterial strains and growth conditions

Bifidobacteria are counted by using the MRS_C medium at pH 6.8 supplemented with nalidixic acid (2 mg/L), neomycin sulphate (100 mg/L), paronomycine sulphate (100 mg), lithium chloride (12g) and the addition of (0.1 mg) mupirocin, antibiotics which are resistant bifidobacteria and lactobacilli are many sensitive (Rada *et al.,* 1999). Acetic acid is the second selective agent anaerobic gram-positive bacteria, and incubated under anaerobic conditions using an atmosphere generation system (AnaeroGen, Oxoid) at 37°C.

E. coli EPEC was reactivated in EMB medium and incubated aerobically at 37°C. All strains were subcultured at least three times prior to the experiments (Mahmoudi *et al.,* 2013).

Study of the intestinal flora after dissection rats

Histopathological analysis

After sacrifice, the small intestines of the rats were removed. The organs were fixed in 10% formalin (Silva *et al.,* 1999). The sample has to undergo at first dehydration, a sample soaked with paraffin wax melted in 56°C during 24 h to stiffen to be able to cut it.

Histopathological examinations

The tissue samples from intestines were fixed in buffered 4% formaldehyde and processed for paraffin embedding. The histological sections (3–5 lm) were stained with hematoxylin and eosin (H&E). The slides were coded and examined by optical microscopy by a single pathologist, who was unaware of the experimental conditions of each sample (Hould, 1998).

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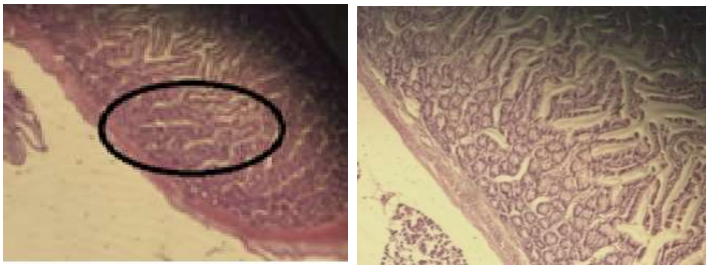
Results and discussion

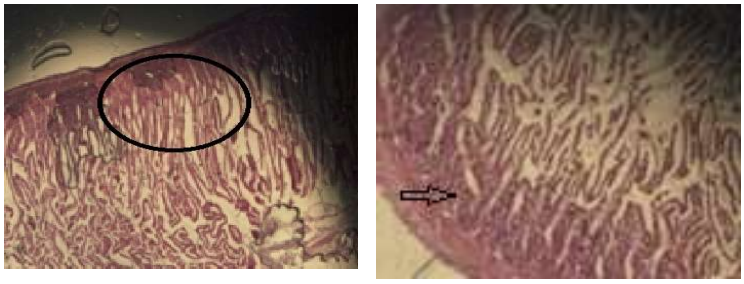
Histological examination of *B.bifidum* (Bbf1) fed rat, hematoxylin and eosin, and PAS-stained sections of intestines 7 days after *E. coli* EPEC challenge revealed that intestinal injuries due to infection were attenuated in rat given bifidobacteria compared to infected untreated rat.

Our results suggest that bifidobacterial feeding influence an intestinal immune reaction against pathogenic invasion. Moreover, our histological analyses have shown that local intestinal inflammation caused by *E. coli* was attenuated in the presence of bifidobacteria.

The lower number of goblet inflammatory cells in rat fed bifidobacteria suggests less intestinal inflammation and hence decreased severity of the infection. (Fig (A)), the lamina propria was completely destroyed lymphocyte infiltration. These observations revealed that these rats suffering from acute diarrhea (diarrhea is a state indicating that the gut is irritated). Regarding the rats that received milk fermented with *B. bifidum*, and after the first dissection (the 1st week), microscopic observation of histological sections showed, no significant changes in the intestinal mucosa compared to control rats (D), the same results were found after the second dissection (after discontinuation of treatment) (B). These results confirm the evidence of the barrier effect of bifidobacteria, and / or protection by *B. bifidum* against the infection caused by the contamination with EPEC.



A)



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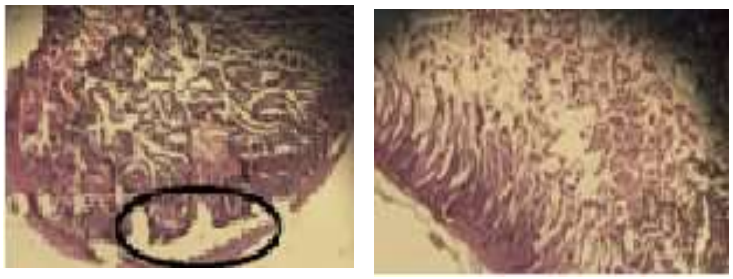

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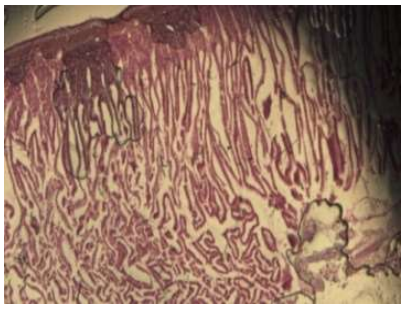

D)

Figure 1. (A) Microscopic observations of histological sections of the small intestine of rats group 2 which displayed pathological anomaly lymphocyte infiltration (A); microscopic observations of histological sections of the small intestine of rats in group 3 lymphocyte infiltration, after the first and second dissection (B); microscopic observations of histological sections of the small intestine of rats in group 4, after the first and second dissection (C); microscopic observations of histological sections of the small intestine of control rats which displayed no pathological anomaly (gr x 10) (D)

Rats in group (fig 1 (C)) of therapeutic, and after the first dissection, signs of infection were less severe but significant and small intestine was less affected, and the lining seems less affected.

These findings suggest that *B.bifidum* represents a good candidate to be used as a probiotic for preventing enteric infection in humans. Fernandez (2000) showed that EPEC could induce lesions (fixing / erasing) in the intestinal epithelium. However, after the end of treatment (15 days after the first dissection), there was complete recovery of the mucosa, with a return to its normal appearance (macroscopic observations) (Mahmoudi *et al.,* 2015). The *in vitro* and *in vivo* study showed that taking probiotics (*Bifidobacterium*) reduced colonization of the digestive tract by pathogenic bacteria and stimulates specific immunity defence response of the host by activating lymphocytes (Amrouche, 2005).The results show a probiotic effect on the

implementation of the strains studied. This colonization by beneficial bacteria contributes significantly to the modification of the intestinal microbiota.

Many mechanisms have been postulated by which bifidobacteria could inhibit enteropathogens. Suppressing intestinal pathogens by bifidobacteria has been attributed to their ability to modify the intestinal microenvironment by secreting organic acids, principally acetic and lactic acids, leading to lower gut pH and by the production of antibacterial compounds.

Conclusions

The Bifidobacteia have been shown to confer enhanced resistance against infection with enteric pathogens and a variety of mechanisms have been proposed to explain the effects. Potential mechanisms to explain the enhanced resistance conferred by bifidobacteria, include competitive adhesion to intestinal mucosa, production of antimicrobial substances and/or stimulation of mucosal immunity factors.

We wanted to confirm *in vivo* anti *E.coli* activity observed bacteriocinogenic of bifidobacteria *In vitro* using an infection model in rats and *E. coli* infections were caused by the oral route in rats and Bbf1 strain was administered preventively and as treatment still orally. Rats given preventive treatment with *B.bifidum* strain showed signs of infection less important than those who had received no treatment. Our results showed that the number of cells and the length of the survival period of *B. infantis* in the digestive tract during ingestion and until the 6^{th} day after ingestion ended were sufficient to enable *B. difidum* to exert its probiotic effect.

Several roles are assigned to the presence of bifidobacteria. One of the most important roles of bifidobacteria is the adhesion there of to the epithelial cells of the intestine thereby create an ecological niche and thus to prevent the invasion of pathogenic bacteria. Some studies have been done on the effect of the administration of bifidobacteria tablets to people with diarrhea and it has been noticed that the intake of these pills helped reduce symptoms.

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