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Screening probiotics from *Lactobacillus* strains according to their abilities to inhibit pathogen adhesion and induction of pro-inflammatory cytokine IL-8

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ABSTRACT

Probiotics can be screened according to their abilities to inhibit pathogen adhesion and inhibit the production of pro-inflammatory cytokines. Eleven *Lactobacillus* strains isolated from traditional fermented dairy foods in Xinjiang, China, were studied for their potential to inhibit adhesion of *Escherichia coli* to intestinal epithelial cells and to inhibit *E. coli*–induced production of interleukin (IL)-8 by intestinal epithelial cells. The results showed that the 11 strains could inhibit adhesion of *E. coli* to Caco-2 cell monolayers and inhibit the induction of IL-8 production by *E. coli* in HT-29 cells. The inhibiting activities of the *Lactobacillus* strains against *E. coli* adhesion and IL-8 induction were strain-specific and not positively correlated, whereas the excluding activity of the strains against *E. coli* adhesion and their coaggregation with *E. coli* were positively correlated. The effector molecules of the strains with probiotic potential should be identified to explain the mechanism behind these observations.
Key words: *Lactobacillus*, adhesion, inflammatory, IL-8

INTRODUCTION

Probiotics are live microorganisms that, when consumed in adequate amounts, produce beneficial effects on host, including restoring gastrointestinal tract flora, immunomodulation, and so on (FAO/WHO, 2001; Fukuda et al., 2011; Sánchez and Urdaci, 2012; Unno et al., 2015).

Specific probiotics should be selected based on their ability to inhibit adhesion or displace pathogens adhering to human intestinal mucosa (Collado et al., 2007). Adhesion and colonization of pathogenic bacteria on the intestinal mucosa are important steps in pathogenic infection. Probiotics are able to adhere to the intestinal mucosa and thus inhibit the binding of pathogens and invasion of intestinal epithelial cells (Faghfoori et al., 2015). As probiotic strains, some *Lactobacillus* strains are reported to adhere to intestinal epithelium, inhibiting invasion by pathogens and improving epithelial barrier function (Chen et al., 2012; Saxami et al., 2016; Yu et al., 2017).

Immunomodulation capacity of *Lactobacillus* strains is considered a criterion for probiotic assessment (Hardy et al., 2013). Lactobacilli could affect pro-inflammatory responses [tumor necrosis factor-α (TNF-α), IL-8] and anti-inflammatory responses (IL-10) of eukaryotic cells (HT-29, Caco-2, or other eukaryotic cells) treated with pathogens, lipoprotein, or other factors (Preising et al., 2010; Stöber et al., 2010; Sun et al., 2012). Some *Lactobacillus* strains can lower secretion of the pro-inflammatory cytokine IL-8 and promote secretion of the anti-inflammatory IL-10 of intestinal epithelial cells (Butel, 2014; Belguesmia et al., 2016).

Some probiotics have been reported to inhibit pathogenic bacteria by adhering to intestinal epithelium and to decrease pro-inflammatory cytokine expression induced by pathogens (Vanderpool et al., 2008; Liu et al., 2010; Nueno-Palop and Narbad, 2011; Sun et al., 2012; Savino et al., 2015). However, the relationship between inhibiting ability of probiotics against pathogens adhering to epithelial cells and their inhibiting ability against pathogens that induce pro-inflammatory cytokine secretion from epithelial cells is not clear.

In our previous study (Tuo et al., 2013), we studied the adhesion ability of 22 strains of *Lactobacillus* isolated from traditional fermented dairy food in Nalati, Xinjiqng, China, and some strains with higher adhering ability were considered potential probiotic strains. In this study, 11 *Lactobacillus* strains with the highest adhesion ability among the 22 *Lactobacillus* strains were selected to assess their ability to inhibit adhesion of *E. coli* to intestinal epithelial cells and inhibit induction by *E. coli* of IL-8 production by intestinal epithelial cells, and the relationship between the 2 abilities.

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Table 1. *Lactobacillus* strains evaluated in this study

Strain	Species	Origin[1]
22	*Lactobacillus plantarum*	Traditional fermented dairy food, Nalati, Xinjiang, China
23	*L. plantarum*	Traditional fermented dairy food, Nalati, Xinjiang, China
39	*Lactobacillus fermentum*	Traditional fermented dairy food, Nalati, Xinjiang, China
57	*L. plantarum*	Traditional fermented dairy food, Nalati, Xinjiang, China
66	*L. plantarum*	Traditional fermented dairy food, Nalati, Xinjiang, China
67	*L. plantarum*	Traditional fermented dairy food, Nalati, Xinjiang, China
89	*L. plantarum*	Traditional fermented dairy food, Nalati, Xinjiang, China
130	*L. plantarum*	Traditional fermented dairy food, Nalati, Xinjiang, China
196	*Lactobacillus casei*	Traditional fermented dairy food, Nalati, Xinjiang, China
m2.3	*L. casei*	Traditional fermented dairy food, Nalati, Xinjiang, China
f12	*L. plantarum*	Traditional fermented dairy food, Nalati, Xinjiang, China
LGG	*Lactobacillus rhamnosus* GG	Harbin Institute of Technology, Harbin, China

[1]The fermented foods were made by local people using traditional methods under local climate and environmental conditions. Traditional fermented dairy foods from different pastures were sampled and strains were isolated from different pastures.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

Lactobacillus strains tested in this study were isolated from traditional fermented dairy foods in Nalati, Xinjiqng, China, stored in our stock culture collection (Tuo et al., 2013). Detailed information about the strains is listed in Table 1. *Escherichia coli* ATCC43889 was used from our stock culture collection. *Lactobacillus rhamnosus* GG (**LGG**) was a gift from L. Zhang from the Harbin Institute of Technology (Harbin, China). Lactobacilli were cultured anaerobically in de Man, Rogosa, and Sharpe (MRS) broth (Merck KGaA, Darmstadt, Germany) at 37°C for 18 h, and *E. coli* was cultured aerobically in brain-heart infusion (BHI) broth (Difco Laboratories Inc., Detroit, MI) at 37°C for 18 h.

Caco-2 and HT-29 Cell Culture

The human colonic cell line Caco-2 was provided by X. Chen (Ruijin Hospital, Shanghai, China). The Caco-2 cells were grown routinely in Dulbecco's modified Eagle medium (**DMEM**; Gibco/Life Technologies Corp., Grand Island, NY), supplemented with 10% (vol/vol) heat-inactivated (56°C for 30 min) fetal bovine serum (Sijiqing; Zhejiang Tianhang Biological Technology Co. Ltd., Hangzhou, China), penicillin (100 U/mL), and streptomycin (0.1 g/L; Gibco/Life Technologies Corp.) at 37°C in 5% CO_2 in 95% air. The culture medium was replaced every 48 h to maintain the cells. Caco-2 cells were used as intestinal epithelial cell model to study the adhering ability of *Lactobacillus* strains.

Human colonic cancer cell line HT-29 was obtained from the Chinese Academy of Science (Shanghai, China). The HT-29 cells were cultured in McCoy's medium (Gibco/Life Technologies Corp.) supplemented with 10% (vol/vol) inactivated (56°C, 30 min) fetal calf serum (Sijiqing Co. Ltd.) in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. The HT-29 cells were cultured until they were fully differentiated. The HT-29 cells were used as intestinal cell model to study IL-8 cytokine production induced by *E. coli*.

Effect of Lactobacillus Strains on Adhesion of E. coli to Caco-2 Monolayers

The effect of *Lactobacillus* strains on *E. coli* adhesion was assessed by using Caco-2 cells as an intestinal epithelial cell model according to Ramiah et al. (2008). Caco-2 cells were seeded on 12-well cell culture plates (Greiner Bio-One GmbH, Frickenhausen, Germany) at 1×10^5 cells per well. The plates were cultured at 37°C in a humidified atmosphere of 5% CO_2 and 95% air until a confluent monolayer was obtained.

Caco-2 cell monolayers on the 12-well plates were washed twice with sterile PBS (pH 7.2) before the adhesion assay. Then, 18-h cultures of *Lactobacillus* strains and *E. coli* were harvested by centrifugation at 10,000 $\times$ *g* for 10 min at 4°C (Avanti J30I, Beckman Coulter, Brea, CA), washed twice with sterile PBS (pH 7.2), and then resuspended in DMEM (without antibiotic and fetal bovine serum) and adjusted to 1×10^9 cfu/mL.

To study the ability of *Lactobacillus* strains to prevent *E. coli* from adhering to Caco-2 cells by exclusion, 0.5 mL of each *Lactobacillus* strain in DMEM suspension was added into wells with Caco-2 cell monolayers and supplemented with 0.5 mL of DMEM. After 1 h of incubation at 37°C, 0.5 mL of *E. coli*-DMEM suspension was added into the same wells. After 1 h of incubation, each well of the plates was washed 4 times with PBS (pH 7.2) to remove free, nonattached bacterial cells. Then, 1 mL of 1% (vol/vol) Triton X-100 (HFH10, Invitrogen, Carlsbad, CA) was added to each well, and the suspension was stirred to detach the bac-

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