Searching for Human-derived *Bifidobacterium* that Enhances Intestinal Barrier Function

การตรวจหาบิฟิโดแบคทีเรียมจากมนุษย์ที่เพิ่มความสามารถการทำหน้าที่กีดขวางของลำไส้

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ABSTRACT

Commensal bacteria in human intestinal tract promote intestinal integrity and prevent pathogen-induced damage of epithelial barrier function. This study aimed to find indigenous *Bifidobacterium* with the ability to enhance the integrity of epithelial tight junctions. Seventeen *Bifidobacterium* isolates from infant feces and breast milk were tested by transepithelial electrical resistance (TER) assay in Caco-2 cells. Two *Bifidobacterium* spp. including *B. psedocatenulatum* NB48 and *B. bifidum* NB42 increased TER significantly as compared with control. Their abilities to prevent or improve the intestinal integrity destroyed by important bacterial pathogens are being investigated. These bifidobacteria are potential probiotics for the enhancement of intestinal barrier function.

บทคัดย่อ

แบคทีเรียประจำถิ่นในทางเดินอาหารของมนุษย์ช่วยส่งเสริมความสมบูรณ์แข็งแรงของลำไส้และป้องกันการทำลายการทำหน้าที่กีดขวางของเยื่อบุผิว จุดประสงค์ของงานวิจัยนี้เพื่อหาบิฟิโดแบคทีเรียมที่สามารถเพิ่มความสมบูรณ์แข็งแรงของส่วนเชื่อมติดกันแน่นของเยื่อบุเซลล์ โดยทำการทดสอบด้วยวิธีการวัดค่าความต้านทานกระแสไฟฟ้าของเยื่อบุผิวในเซลล์คาโค-2 ผลการทดสอบบิฟิโดแบคทีเรียม 17 สายพันธุ์ที่แยกได้จากอุจจาระของเด็กทารกและน้ำนมมารดาพบว่ามี บิฟิโดแบคทีเรียม 2 สายพันธุ์คือ บิฟิโดแบคทีเรียม ซูโดแคทีนูลาทัม เอ็นบี48 และบิฟิโดแบคทีเรียม บิฟิดุม เอ็นบี42 เพิ่มค่าความต้านทานเยื่อบุผิวอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับตัวควบคุม ความสามารถของเชื้อเหล่านี้ในการป้องกันหรือแก้ไขความสมบูรณ์แข็งแรงของลำไส้ซึ่งถูกทำลายโดยเชื้อก่อโรคสำคัญกำลังอยู่ในการทดลอง บิฟิโดแบคทีเรียมเหล่านี้มีศักยภาพเป็นเชื้อโพรไบโอติกที่เพิ่มความสามารถการทำหน้าที่กีดขวางของลำไส้

Key Words: *Bifidobacterium*, Epithelial tight junctions**,** Intestinal barrier function

คำสำคัญ: บิฟิโดแบคทีเรียม ส่วนเชื่อมติดกันแน่นของเยื่อบุผิว การทำหน้าที่กีดขวางของลำไส้

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Introduction

The intestinal epithelium is a single layer of cells which has one of key functions as barrier to protect foreign agents and bacterial pathogens (Takahashi et al., 2012). The epithelial cells are attached together by junctional complexes including tight junctions (TJs), adherens junctions and desmosome (Guttman & Finlay, 2009; Viswanathan et al., 2009). TJs encircle epithelial cells at the apical site of the lateral membrane and play crucial role in the maintenance of intestinal barrier function. Epithelial TJs are composed of transmembrane proteins, such as occludin, claudin, junctional adhesion molecules (JAM) and zonula occludens (ZO) (Farkas et al., 2012; Kosińska & Andlauer, 2013). ZO is adaptor protein which links transmembrane proteins with actin cytoskeleton (Ulluwishewa et al., 2011). This structure is important in the regulation of TJs' integrity. Pathogens and other factors such as inflammatory cytokines can damage intestinal barrier leading to the gut leak and various diseases (Fasano & Nataro, 2004; Turner, 2009; Sawada, 2013).

Commensal bacteria such as *Lactobacillus* and *Bifidobacterium* can enhance the integrity of intestinal barrier function and prevent pathogen-induced damage in epithelial barrier function. For examples, *Lactobacillus plantarum* MB452 and *Bifidobacterium infantis* increase the integrity of TJs determined by the increased transepithelial electrical resistance (TER)(Ewaschuk et al., 2008; Anderson et al., 2010)and *B. lactis* 420 cell-free supernatant prevents *Escherichia coli* O157:H7-induced damage of TJs (Putaala et al., 2008). This study aimed to find indigenous *Bifidobacterium* that enhance intestinal barrier function by the ability to improve integrity of epithelial tight junctions.

Objectives of the study

Test the ability to enhance the integrity of epithelial tight junctions of *Bifidobacterium* by transepithelial electrical resistance (TER) assay.

Methodology

Bacterial strains and culture condition

Seventeen *Bifidobacterium* spp. previously isolated from infant feces (Jittaprasatsin, 2008) and breast milk (Chaodong, 2011) were used in this study (Table1). They were inoculated on Modified Columbia agar (MC, Oxoid, Oxoid Ltd., Basingstoke, Hampshire, England) and incubated at 37 ^{0}C in anaerobic condition (The AnaeroPack system, Mitsubishi Gas Chemical, H_2: 5%, CO_2: 10%, N_2: 85%) for 24 hours. After incubation, they were suspended in Dulbecco's modified eagle media (DMEM; containing 20% fetal bovine serum and 2.5% HEPES) to obtain a final concentration of $1.0x10^9$ CFU/mL for further use in the experiment.

Cell culture

Caco-2 human colorectal adenocarcinoma cell line (ATCC HTB-37, Manassas, VA, USA) were grown in 75 cm^2 flasks at 37 ^{0}C and 5% CO_2 for 48 hours. Cells were seeded on transwell insert (6.5 mm diameter, 0.4 µm pore size, 0.33 cm^2 surface area, Collagen membrane insert, Costar/Corning, NY, U.S.A.) at a density of $5x10^4$ cells/well and incubated in DMEM supplemented with 20% fetal bovine serum and 2.5% HEPES at 37 ^{0}C in a humidified atmosphere with 5% CO_2. Culture medium was changed every second day. After incubation for 18-20

days, cells were confluence, polarized and ready to use in the experiment.

Transepithelial electrical resistance (TER) assay

Polarized Caco-2 cells grown on transwell insert were added at the apical side with 100 µL of $1.0x10^9$ CFU/mL of *Bifidobacterium* spp. After incubation for 24 h, transepithelial electrical resistance (TER) was measured by using a voltohmmeter (EVOM^2 Epithelial Tissue Voltohmmeter, WorldPrecision Instruments, FL). Blank control contained only Caco-2 cells and media. The electrical resistance was recorded and calculated by the following formula:

TER (Ω.cm^2) = (Total resistance – Blank resistance) (Ω) x Area (cm^2).

Statistical analysis

All experiments were done in duplicate and data represented standard error. The data were analyzed using the Student's t-test with one-tailed distribution.

Results

The results of TER measurement were shown in Figure 1 as percentage of control. Five isolates of *Bifidobacterium* spp. increased TER as compared with control. Only two isolates of *Bifidobacterium* spp. including *B. psedocatenulatum* NB48 and *B. bifidum* NB42 increased TER significantly (p<0.05).

Discussion and Conclusions

In the present study, only strain NB48 from 5 *B. psedocatenulatum* isolates and strain NB42 from 2 *B. bifidum* isolates increased TER. This implied that the enhancement effect of *Bifidobacterium* spp. on TJs integrity is strain-specific. It has been recognized that commensal bacteria and probiotics are beneficial to host by their ability such as immunomodulation, protection against pathogens and enhancement of the integrity of intestinal barrier function (Ohland & Macnaughton, 2010). At present, there are a limited number of the investigations for *Bifidobacterium* with the ability to enhance the integrity of TJs. Recently, it has been reported that conditioned medium of *Bifidobacterium infantis* can enhance integrity of TJs by altering tight junction protein expression (Ewaschuk et al., 2008) and *B. lactis* 420 cell-free supernatant increases TER more than *B.lactis* HN019, *L.acidophilus* NCFM and *L. salivarius* Ls-33 (Putaala et al., 2008). Furthermore, probiotics protect the disruption of TJs induced by pathogens such as *B. lactis* 420 cell-free supernatant prevents TJs damage induced by EHEC (*Escherichia coli* O157:H7) (Putaala et al., 2008). *L. plantarum* prevents the changing of TJs induced by enteroinvasive *Escherichia coli* (EIEC) and enteropathogenic *Escherichia coli* (EPEC) (Qin et al., 2009; Liu et al., 2010).

In conclusion, two *Bifidobacterium* spp. including *B. psedocatenulatum* NB48 and *B. bifidum* NB42 increased TER significantly as compared with control. Their ability in the prevention or improvement of the intestinal integrity destroyed by important bacterial pathogens is being investigated. These bifidobacteria are potential probiotics for the enhancement of intestinal barrier function.

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Table 1 *Bifidobacterium* isolates used in this study (Jittaprasatsin, 2008; Chaodong, 2011)

Isolates from infant feces	Isolates from breast milk
B. adolescentis B14	*B. bifidum* NB13
B. adolescentis B24	*B. bifidum* NB42
B. catenulatum B38	*B. breve* Bif29
B. longum B9	*B. catenulatum* NB38
B. longum B36	*B. dentium* NB11
B. longum B103	*B. dentium* NB14
B. pseudocatenulatum B11	*B. pseudocatenulatum* NB2
B. psuedocatenulatum B57	*B. pseudocatenulatum* NB45
	B. psedocatenulatum NB48

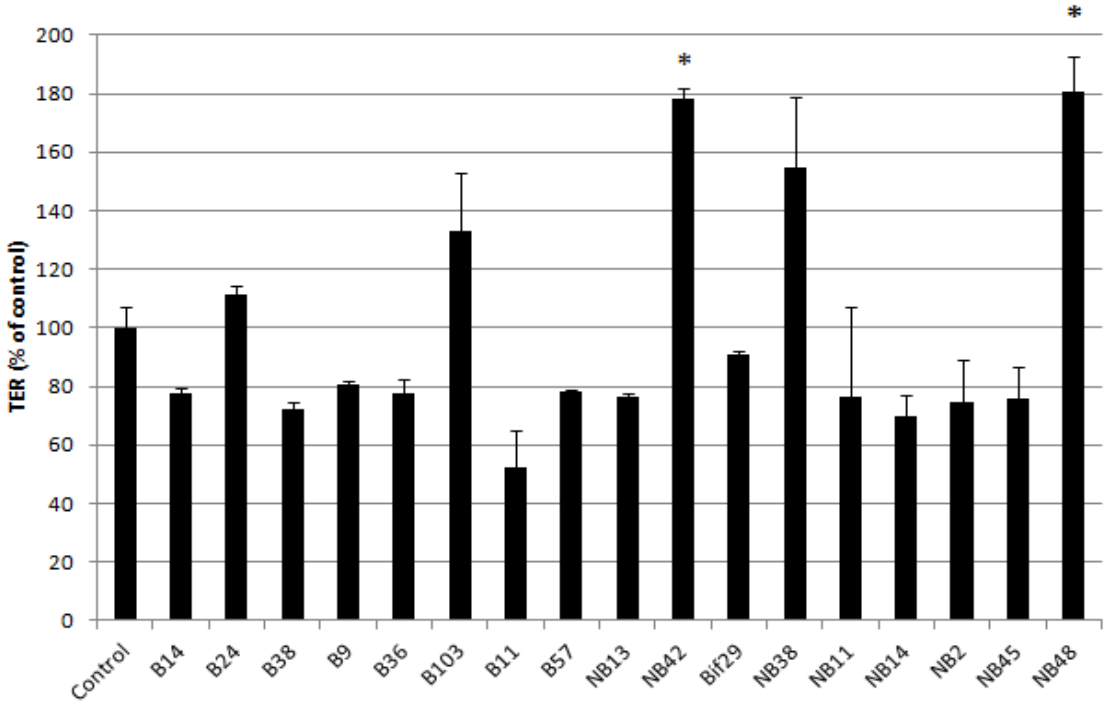

Figure 1 Change in the TER by *Bifidobacterium* spp. isolated from infant feces and breast milk across Caco-2 human colorectal adenocarcinoma cells (*p<0.05)

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