Bifidobacterium spp.: *in vitro* Evaluation to Adhere to Human Enterocyte HT-29 Cells, in Different Colon pH

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Abstract

The adhesion of two different Bifidobacterium strains was studied using HT-29 cell line as in vitro model for intestinal epithelium. All the strains tested have been used as probiotics, and most of them are used in the dairy and food industry. Bifidobacterium longum BB536 was used as positive control. Bacteria adhesion to HT-29 cell culture observed microscopically after Gram staining. Among the test strains, Bifidobacteruim longum BB536 was the most adhesion strain compared with Bifidobacterium pseudocatenulatum G4. For the pH there is significant different in the adhesion in the different part of the colon, whereas observe the best adhesion in pH 5.7 (Transverse) for the both strains.

Keywords: Probiotics; Adhesion; Bifidobacterium; HT-29 cell line.

Introduction

The last 19 century microbiologists describe microflora in the gastrointestinal (GI) tract of healthy individuals that different from those found in diseased individual. These beneficial microfloral found in the GI tract termed probiotic [1]. The probiotic showed the ability to adhere in the surface of digestive system, large intestine colon. The extensive in vitro study was done on the ability of human probiotic to survive from low pH, bile salt, and adhesion properties. Adhesion of probiotic to human epithelium cell has been suggested as an important prerequisite for probiotic action. Adhesions of probiotic are likely to persist longer in the intestinal tract this to showing the ability to metabolic, immunmodulatory, stabilize the intestinal mucosal barrier, and provide competitive exclusion of pathogen bacteria [2], [3],[4],[5]. Adhesion to intestinal cell it's properly for probiotic, since probiotic attach to intestinal cell and colonize in gastrointestinal with other bacteria species[6]. Appropriate for different human intestinal cell culture models simulating the human situation has been used widely to study the specific functions of the human intestinal cell[7]. Bifidobacteria, normal colonize of the human GI tract[8]; can reach a concentration of 1010 CFU/g of intestinal contents[2]. These bacteria are believed to present several healthy, nutritional and therapeutic benefits to human hosts including reduction of blood cholesterol, improvement of lactose utilization in malabsorbers, deconjugation of bile acids and increased immunity in animal hosts [8], [9], [10], [11]. Based on clinical reports, bifidobacteria appear to reduce the incidence of rotavirus infection, traveler's diarrhea and antibiotic associated diarrhea [12]. They are also reportedly antagonistic towards pathogens belonging to the genera *Salmonella, Escherichia, Proteus, Shigella and Candida*[13]. Mukai [14] suggest that the cell surface proteinaceous components are involved in the adhesion of *Bifidobacterium*. The study of cellular as well as the interaction of cells with substrate (in vivo and in vitro adhesion) is particularly important for understanding the mechanisms that regulate bacterial adhesion and therefore colonization. Protein ligands present on the cell surfaces and/or in the culture medium have been identified by Bernet[15]in some strains of bifidobacteria of human origin (*B. breve, B .longum, B. bifidum and B. infantis*). Many studies were done as in vitro model system adhesion of probiotic, such as the human colon carcinoma cell line HT-29, Caco2, and HT29-MTX are important in the assessment of adhesion properties[13]. HT-29 used as model for small intestine and large intestine colon. The location of probiotic adhesion provided with interaction with the intestinal mucosal surface and contact with gut associate lymphoid tissue(GALT)to stimulate immune system. The theoretical benefits of probiotic bifidobacteria in the intestinal, mediated by modulation the functionality of the intestinal microbial, the gut barrier, and immune system of the host, and the both therapeutic and prophylactic roles have been proposed and trailed in animal and human, in recent years, studies of the probiotic effects of bifidobaeria have been focused in these areas: adherence properties, resistance to infection diseases, and prevention of colon cancer [16]. From the identification of a possible probiotic strain, lead to its production and marketing, through its growth in laboratory, summarizing the whole process existing behind its development, microencapsulation technologies, safety tests, and the studies performed to test its resistance to human secretions and stability.

Several benefit health affect have been claimed to be based on the presence of *Bifidobacterium* in the colon [17]. As consequence, bifidobacteria now have a long history of safe use as dietary adjuncts, *B. adolescentis, B. animalis, B. lactis, B. bifidum, B. breve, and B. Longum* have generally regarded as safe status [21]. Fermented dairy products enriched with probiotic bacteria have industrial into one of the most successful categories of functional foods. The aim of this study was to investigate the adhesion of *Bifidobacterim* strains *B. pseudocatenulatum* G4 and *B. longum* BB536 to the model intestinal epithelium consisting of HT-29 cell-line under different pH levels with different times.

Materials and Methods

1. Bacterial and culture conditions

Two probiotics *Bifidobacterium pseudocatenulatum* G4 and *Bifidobacteruim longum* BB536 strains were used, these strains were taking from Dr. Shuhaimi as a kindly gift from Faculty of Biotechnology. Whereas, *B. longum* BB536 was used as positive control. The bacterial were grown in 1 ml De Man, Rogosa and Sharpe (MRS) broth (Merck) from stocks stored at -75 ^{0}C in 40% glycerol, B.

pseudocatenulatum G4 and B. *longum* BB536 was grown under anaerobic condition. After 16 h growth at 37 ^{0}C, the bacteria were harvested by centrifugation (2000 X g for 10 min) and washed twice with phosphate buffer salin (PBS; pH 7.2) and resuspended in PBS. The absorbance (600 nm) was adjusted to 0.25±0.02 in order to standardize the number of bacteria (10^{7} to 10^{8} CFU ml^{-1}) [16].

2. HT-29 cell culture:

The HT-29 cell line from American Type Culture Collection. The cells were cultured in Dulbecco's modified Eagle's minimal essential medium (DMEM, Sigma) supplement with 10 % (v/v) fetal calf serum (Sigma), 100 U ml^{-1} penicillin and 100 mg ml^{-1} streptomycin (Sigma), at 37 0C in at atmospheric of 10% CO_2 / 90% air. For adhesion assays HT-29 monolayers were prepared on glass coverslips placed in 6-well tissue culture plates. The cell culture medium was changed every other day and replaced by fresh non-supplemanted DMEM least 1 h before the adhesion assay.

3. In vitro adhesion assay

In the case of study, the adhesion of bacterial strains to HT-29 cell cultures was examined by adding 1 ml from bacterial supernatant to each well of tissue culture 6-well. After incubation for 1.5 h the HT-29 cell culture were washed four times with 1 ml of PBS and fixed with methanol for 2 min, dried ad Gram staining. The two species of were tested at the same time in triplicate in three independent experiment. Adhesion bacteria were detected microscopically by counting 20 randomised fields per coverslip.

4. Preparation different pHconcentration

The different PBS pH concentration was adjusted by adding HCL 37 % to (5.6, 5.7, 6.6, and 6.8).

5. Study the adhesion in different pH concentrations

To study the adhesion of bacterial in different pH concentration the same procedure was used in adhesion assay, (2000 X g for 10 min), then mix it with PBS adjusted in different pH (5.6, 5.7, 6.6, and 6.8). The strains tested at the same time in triplicate in three independent experiments.

Table 1: Adhesion of *Bifidobacteruim to human intestinalepithelial HT-29 cell culture*

Bacterial strain	Adhesion (%) mean± S.D.
Bifidobacterium pseudocatenulatum G4	55.85±4.40
Bifidobacteruim longum BB536	225.45±7.9

S.D, Standard deviation

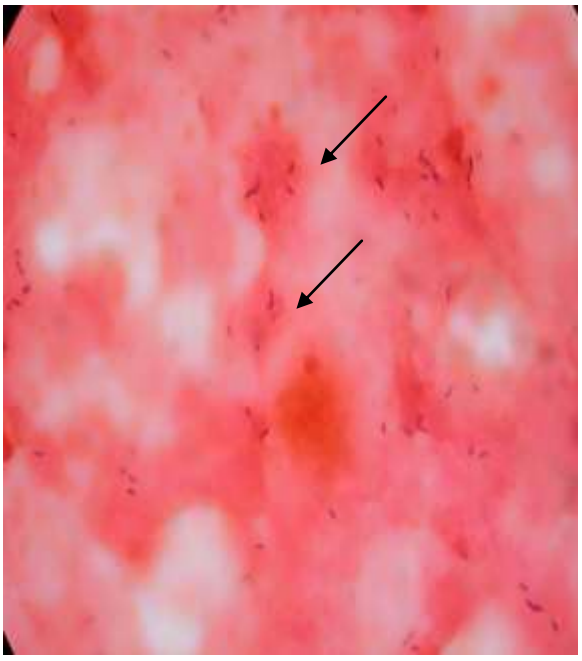

Fig. 1(A)Adhesion of *Bifidobacteruim longum* BB536 to human epithelium cell(HT-29).Adhesion scores in 20 randomized microscopic filed per coverslip were determined.

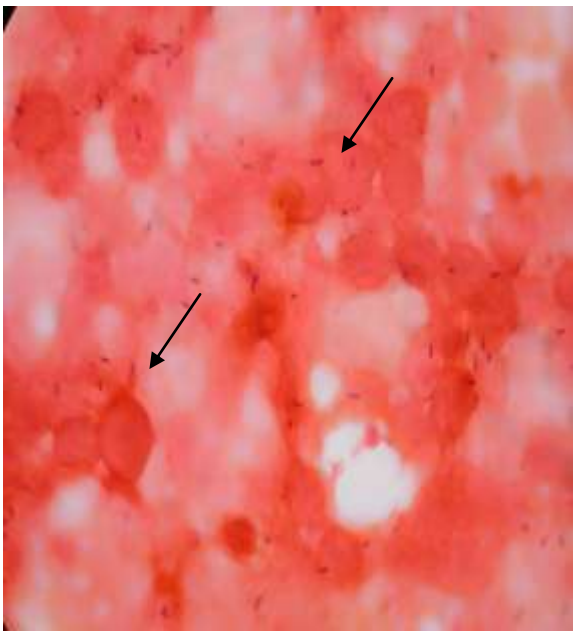

Fig. 1 (B) Adhesion *Bifidobacterium pseudocatenulatim* G4 to HT-29 cell. The cell culture observed using light microscopy Gram-staining (magnification x1000).

Statistical analysis

Student's *t*-test was used to indentify differences among tested strains.

Results

Bifidobacteruim longum BB536 was used as a control to compare with *Bifidobacterium pseudocatenulatum* G4, to HT-29. However, the adhesion of *B. pseudocatenulatum* G4 did significant different from adhesion of *B. longum* BB536 (Table 1). When Gram staining preparation were observed visually, those strains indentified as *B. longum* BB536 and *B. pseudocatenulatum* G4 (Fig. 1 A and B) show the adhesion of both strains to HT-29.

For adhesion in different pH concentration the result show the 5.7 is a pest pH for *Bifidobacteruim* then 5.6, and the result show the low level of adhesion in 6.6 and 6.8 (Fig.2).

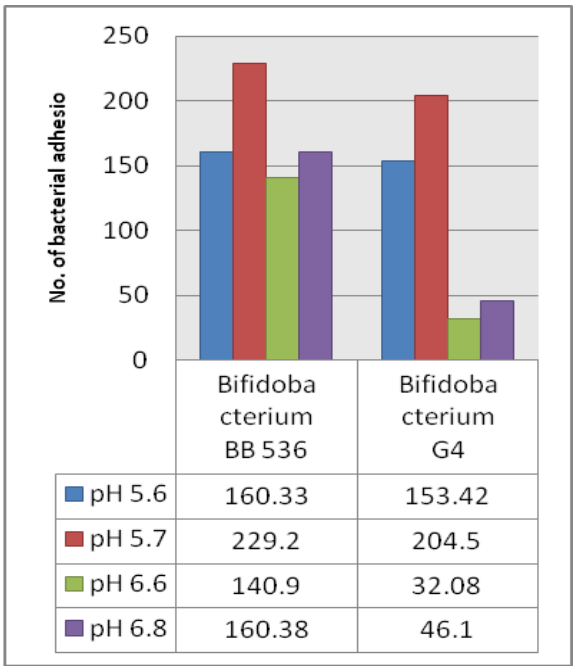

Fig.2. Adhesion of *B. Psedudocatenulatum* G4 and *B. longum BB536* to HT-29 culture in different pH.

Discussion

Because bacterial adhesion to epithelium cells has been considered as one of the most selection criteria for probiotic strains, the HT-29 cell line has been used as an vitro model for intestinal epithelium and the cell line has been used to screen for adhesive strains[18,19,20,21] Bernet *et al*., 1993, 1994). In this study, the adhesion of *B. psedudocatenulatum* G4 and *B. longum BB536* to HT-29 culture was investigated by examination of adhesion between bacteria and HT-29 cell culture to support the adhesion (Fig.1 A and B). The result shown the BB536 has ability to adhesion better than G4 to human intestinal

cell. The mechanisms of adhesion were not studied. Thus, it was not possible to define which strains bound due to specific interaction mediated by adhesions and which strains bound by non-specific interaction.

The capacity of adhesion for these strains is different, BB536 show the ability to adhesion to HT-29 for 1 h better than G4 (Table1). The mean and standardization show in (Table 1). The bacteria adhesion to HT-29 for BB536 bigger than G4.

For colon pH, from the result the best adhesion was observing at 5.7 pH (Transverse), and 5.6 (Ascending) compared with 6.6 and 6.8 (Descanting, and Rectum) this for G4, last two part show low level of adhesion. BB536 also show better adhesion in Ascending, Transverse, and Rectum than Descanting.

Although the conclusions draw from the results of in vitro studies cannot be directly related to in vivo situation, there is evidence relating adhesion to the temporary colonization of human intestinal tract.

Conclusion

From the result we can conclude the optimum pH was 5.7for Bifidobacterial strains. Thus, by demonstrating here the environment factors of human colon play important role effect on probiotic adhesion to epithelium cells like colon pH levels.*B. pseudocatenulatum* G4 express adhesive properties to HT-29 compared with *B. longum* BB536 in our study and with other *Bifidobacterium* strains, *B. longum* NCC 490, *B. breve*, *B. bifidum*, and *B. adolescentis* in other studies [17].

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تقييم قابلية التصاق ا لـ .*Bifidobacterium* spp باستعمال الخلايا الطلائية نوع HT-29 وفي تراكيز مختلفه من الـ pH خارج جسم الانسان

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الملخص

أجري البحث لاختبار قابلية الاتصاق لسلالتين مختلفة من ال(Bifidobacterium B. longum BB536 و.B pseudocatenulatum باستعمال نسيج من الخلايا الطلائية HT-29 في دراسة خارج جسم الانسان. كلا السلالتين المستخدمة في هذه الدراسة قد استعملت في دراسات سابقة كبروبايوتيك بكتريا حيث تم استعمالها في منتجات الالبان. B. longum BB536 استعملت كعامل سيطره موجب. وقد لوحظت قابلية الاتصاق لكلا السلالتين باستعمال المجهر بعد التصبيغ بصبغة كرام. أظهرت النتائج ان السلالة البكتيريه من نوع B. longum BB536 قلبليه على الاتصاق اكثر بالمقارنة مع B. pseudocatenulatum G4. وقد لوحظ فرق بالالتصاق في كلا السلالتين باختلاف ال pH حيث سجل اعلى التصاق في ال pH 5.7 الذي يمثل منطقة المستعرض من قولون الانسان.