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ORIGINAL ARTICLE

Enhancement of *Bifidobacterium pseudocatenulatum* G4 Adhesion Properties by Calcium Ions

Sulaiman Qahtan Ali[1], Sara Qahtan[2], Shuhaimi Mustafa[3], Abd Manap Yazid[1], Razieh Amini[4], Zamberi Sekawi [5], Farid Azizi Jalilian*[6]

[1]Department of Food Microbiology,Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[2]Department of biology, Faculty of Medicine, Takrit University, Iraq

[3]Halal Products Research Institute, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

4Department of Molecular Medicine, Faculty of medicine, Hamadan University of Medical Sciences, HUMS, Hamadan, Iran

[5]Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

[6]Department of Medical microbiology, Faculty of Medicine, Ilam University of Medical sciences, Iran

*Corresponding author E-mail: azizijalilian@yahoo.com

ABSTRACT

Adhesion to human intestinal cell is considered as one of the main selection criteria of a probiotic strain. The adhesion of Bifidobacteriun pseudocatenulatum G4 was evaluated on HT-29 human epithelium cell–line, at four different pH and three different duration times with and without the present of calcium ions. The strain exhibited a high calcium dependent adhesive property and the unique properties of calcium ions promote both specific and non-specific interactions. A specific change of bacterial adhesion on HT-29 cell line was exhibited with the shift of pH value from 6.8 to 5.6 which simulated the rectum and ascending colon region respectively. B. pseudocatenulatum G4 is capable to adhere on human intestinal cell, promoting the strain as effective probiotic strain for in vitro and in vivo with the present of calcium.

***Key words**: adhesion, bifidobacteria, probiotic, calcium ions, pH*

INTRODUCTION

Bifidobacteria, normally colonize human GI tract [1] can reach a concentration of 10^{10} CFU/g of intestinal contents [2]. These bacteria are believed to promote several healthy and therapeutic benefits to human hosts including reduction of blood cholesterol, deconjugation of bile acids, and increased immunity in animal hosts [1,3,4,5]. Bifidobacteria, the most predominant bacteria in the intestinal flora of infants, are established shortly after birth. Change in profile may be linked to the intake of dietary bifidogenic factors. Basically, *Bifidobacterium* present in a bacillary form as Gram positive staining, and are non-immobile and non-sporulate [6,7].

The theoretical benefits of probiotic bifidobacteria in the intestinal, mediated by modulation the functionality of the intestinal microbial, gut barrier, and immune system of the host, and both therapeutic and prophylactic roles have been proposed and trailed in animal and human. In recent years, studies on probiotic effects of bifidobacteria have been focused in adherence properties, resistance to infection diseases, and prevention of colon cancer [8].

Many studies were done *in vitro* model system to evaluate the adhesion properties of probiotic. Human colon carcinoma cell line HT-29, Caco2, and HT29-MTX are important in the assessment of adhesion properties [9]. The adherence and colonization capability were related to the surface properties of the bacteria. These properties precisely determine the ability of the microorganisms to adhere both to the intestinal mucus and enterocyte cells (epithelium cell) [10].

Furthermore, the experimental evidence shows that adhesion properties may be favored by several factors such as a particular medium, temperature and pH [13]. It has also been recommended that divalent ions, for e.g., calcium, have an influence on probiotic adhesion [11]. Therefore, addition of calcium may be useful to enhance the adhesion properties of probiotic. However, the effect of calcium on the adhesion ability of probiotic bacteria has not been thoroughly investigated. A few reported experiments have shown that the adherence of lactobacilli to Caco-2 and intestine mucus might be due to calcium ions or was depending on the strain [12].

Bacteria adhere only to complementary substrata either by ions or hydrogen bonding by the hydrophobic interaction, and coordination complexes involving multivalent metal ions [13, 3, 5]. Probiotic bacteria display various surface determinants that are involved in their interaction with intestinal epithelial cells. According to Servin and Coconnier *et al*., 142004 the adhesion mechanisms of probiotic is due to passive forces, electrostatic interactions, hydrophobic interaction, lipoteichoic acids and specific structures such as external appendages covered by lectins. The aim of the present study was to investigate the adhesion ability of *B. pseudocatenulatum* G4 and *B. longum* BB536 on HT-29 cell line with the presence of calcium ions at different levels of pH and exposure times.

MATERIALS AND METHODS

Bacterial strains and growth condition

Bifidobacterium pseudocatanulatum G4 was obtained from the culture collection of Probiotic Laboratory at University Putra Malaysia. *Bifidobacterium longum* BB536 was purchased from ATCC (American Type Culture Collection). The bacteria were cultured in de Man, Rogosa and Sharpe (MRS; Merck, Germany) at 37^0C for 12–16 h under anaerobic conditions. 10mM of $CaCl_2$ was dissolved in Dulbecco's modified Eagle's minimal essential medium (DMEM), and the pH was adjusted to 5.6, 5.7, 6.6, and 6.8. Bacterial strain were grown for 24 h at 37^0C in MRS agar plate, colonies were collected in MRS broth, concentration and responded in DMEM with 10 Mm $CaCl_2$ at a concentration of 1.5×10^8 CFU/ml.

HT-29 cell line culture

The human colon adenocarcinoma cell line (ATCC HTB-38) was purchased from American Type Culture Collection. The cells were grown in Dulbecco's modified Eagle's minimal essential medium (DMEM; Merck, Germany) supplemented with 10% (v/v) fetal calf serum (FCS), contain 100 U ml^{-1} penicillin and 100 mg ml^{-1} streptomycin. Cells were cultured in tissue culture flask in an incubator with 5% CO_2 at 37^0C. For adhesion assays HT-29 monolayers were prepared on glass cover slip and placed in 6-well tissue culture plates. The tissue culture plates maintained for 4 days to confluence to use in adhesion assays. The cell culture media was change everyday and replaced with fresh non-supplemented DMEM 3 h before the adhesion assays.

In vitro adhesion assays

The adherence of *Bifidobacterium* strains to HT-29 cell culture was examined by adding 1ml/ well of *Bifidobacterium* suspension into tissue culture plate, containing HT-29 cell line culture and incubated at 37^0C. Sampling was done at 30, 60 and 120 min. After incubation the cell culture were washed 5 times with PBS (pH 7.2), fixed with methanol, gram stained and counted using 20 randomized microscopy field per well.

Statistical analysis

Statistical analysis was made using the SPSS 14.0 software (SPSS Inc, Chicago, IL, USA). Data were subjected to T-*test* to compare the effect of calcium ion before and after added to DMEM, A probability of $P < 0.05$ was used as the criterion for statistical significant.

RESULTS AND DISCUSSION

Capability of *Bifidobacterium longum* BB536 and *B. pseudocatenulatum* G4 to adhere on HT-29 human epithelium cell line was carried out. The concentration of calcium in adhesion assays was 10 mM equivalent to calcium content in milk. This denotes the importance of calcium as an ingredient of milk-based probiotic foods. Four different pH levels 5.6, 5.7, 6.6, and 6.8 used in this study simulated the ascending, transverse, and descending region of colon and rectum respectively. The bacterial strains were exposed to the cell line in the above-mentioned condition for 30, 60, and 120 min.

Figure 1-4 present the cell number of *B. longum* BB536 and *B. pseudocatenulatum* G4 that successfully adhered on HT-29 cell line in the media with and without calcium at different pH and after exposed at different time. The adhesion of both strains was increased with the increment of exposure time and the present of calcium ions increased the adhesion ability of both strains at all pH levels tested in this study. Both strains show the best adhesion ability in DMEM media supplemented with calcium ions and adjusted to pH 5.7 after 120 min of exposure time. The lowest adhesion was in the DMEM without calcium at pH 6.8. This indicates that calcium enhanced the

adhesion properties of *B. longum* BB536 and *B. pseudocatenulatum* G4. Calcium is known as the element that promote non-specific interactions such as neutralization of the electrical double layer between the cells as well as specific adhesive interactions with protein and polysaccharide adhesion molecules at the cell surface [13]. Bacteria adhere to the epithelium cell by ionic interaction, hydrogen bonding or coordination complexes involving multivalent metal ions. Some bacteria that bearing carbohydrate-binding adhesion will adhere only to substrate possessing the request carbohydrate structure; a much less specific adhesion in the case of a hydrophobic bacterium that will bind to any hydrophobic surface [13].

Table 1 present the percentage increase of adhesion on HT-29 cell line for *B. longum* BB536 and *B. pesudocatenulatum* G4 associated to the presence of calcium ion. The study revealed that calcium ions improve the adhesion of both strains on the entire simulated zone. The pH levels were found to play important factor on the effect of calcium in enhancing the adherence ability of bifidobacteria on simulated human colon. *B. pseudocatenulatum* G4 and *B. longum* BB536 have a compatible adherence properties and the highest enhancement was at the simulated rectum zone.

Table 1. Percentage (%) increase of adhesion associated to calcium ion

Strain	***B. longum* BB536**			***B. pseudocatanulatum* G4**		
Exposure Time	**30 min**	**60 min**	**120 min**	**30 min**	**60 min**	**120 min**
pH of Media	**Percentage (%)**					
pH 5.6	72.2	62	57	71.4	64.6	59
pH 5.7	83.3	51.3	60.7	86.6	67	67
pH 6.6	46	55.3	64.1	76	69.2	35
pH 6.8	84	87.9	75.2	76.7	78.5	71.9

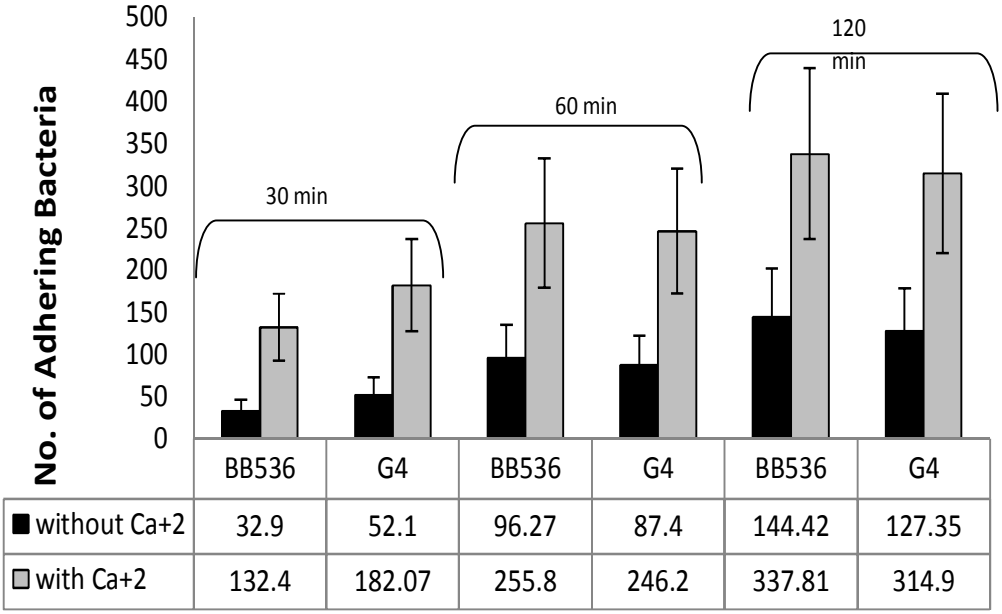
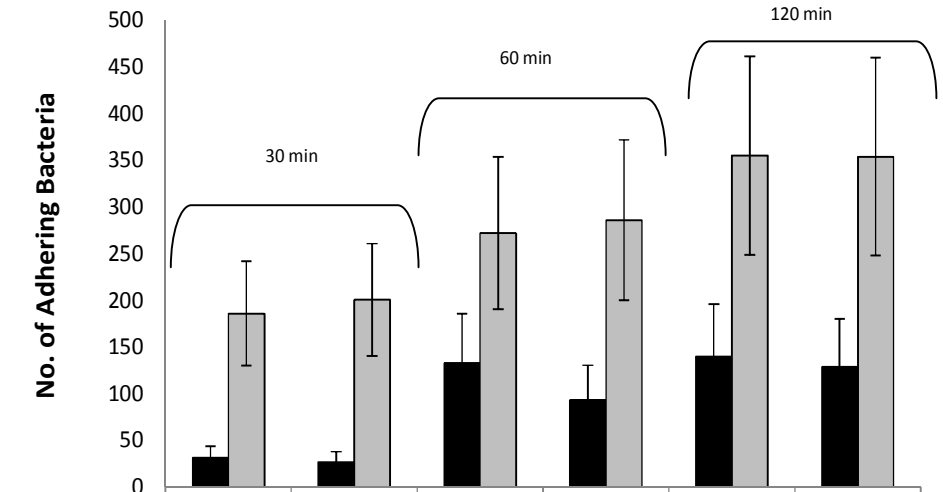
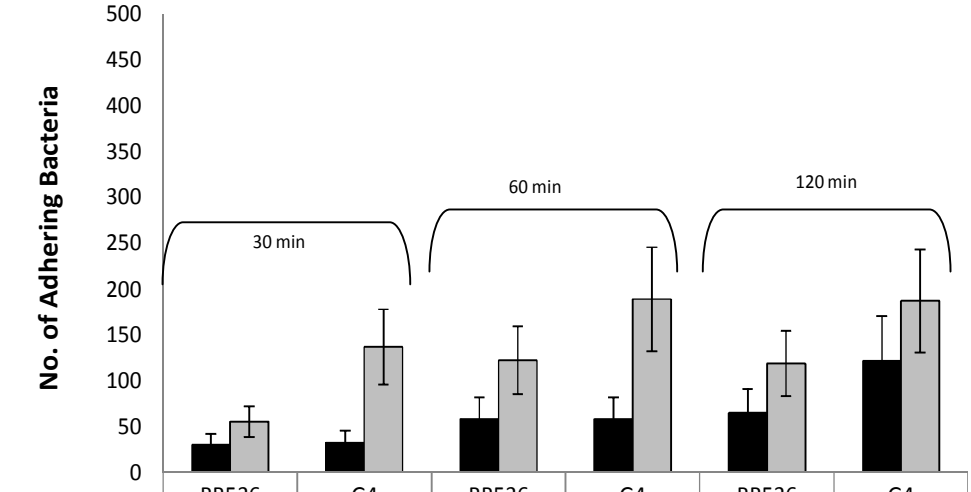


Figure 1 - Adhesion of *B. longum* BB536 and *B. pseudocatenulatum* G4 at three different times 30, 60, and 120 min in pH 5.6 with and without calcium ion. Error bar show means ± standard deviation.



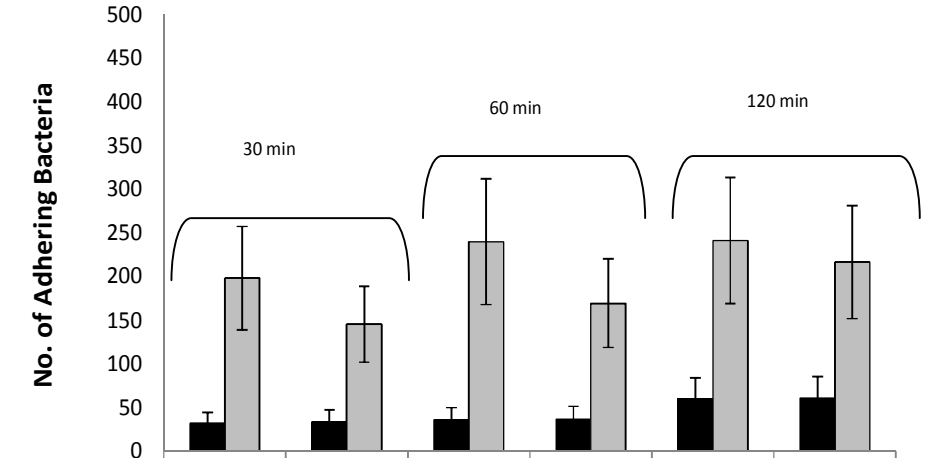
	BB536	G4	BB536	G4	BB536	G4
without Ca+2	31.15	26.87	132.57	93.02	139.87	128.62
with Ca+2	185.92	200.52	272.02	285.92	355.02	353.92

Figure 2 - Adhesion of *B. longum* BB536 and *B. pseudocatenulatum* G4 at three different times 30, 60, and 120 min at pH 5.7 with and without calcium ion. Error bar show means ± standard deviation.



	BB536	G4	BB536	G4	BB536	G4
without Ca+2	29.97	32.4	58.45	58.32	65.22	121.74
with Ca+2	55.5	137	122.5	189.05	118.67	187.02

Figure 3 - Adhesion of *B. longum* BB536 and *B. pseudocatenulatum* G4 at three different times 30, 60, and 120 min at pH 6.6 with and without calcium ion. Error bar show means ± standard deviation.



	BB536	G4	BB536	G4	BB536	G4
without Ca+2	31.72	33.9	35.67	36.52	60.05	60.92
with Ca+2	198.05	145.37	239.85	169.35	241.175	216.52

Figure 4 - Adhesion of *B. longum* BB536 and *B. pseudocatenulatum* G4 at three different times 30, 60, and 120 min at pH 6.8 with and without calcium ion. Error bar show means ± standard deviation.

In conclusion calcium ions enhance the adhesion quality of *B. longum* BB536 and *B. pseudocatenulatum* G4 in human intestinal cells. This observation was similar as reported by Larsen *et al.* (11) where *Lactobacillus* strains high adhesion with in presence of calcium ions.

REFERENCES

1. Jiang T., Mustapha, A., Savaiano D. A. (1996). Improvement of lactose digestion in humans by ingestion of unfermented milk containing *Bifidobacterium longum*. Journal Dairy Science. **79**, 750– 757.
2. Salminen S., Wright A., Ouwehand, A. (2004). Lactic Acid Bacteria. Marcel Dekker, New York.
3. Gill H.S.: Stimulation of the immune system by lactic cultures. (1998). International Dairy Journal. **8**, 535– 544.
4. McNaught C. E., MacFie J. (2001). Probiotics in clinical practice: a critical review of the evidence. Nutrition Research. **21**, 343–353.
5. Gill H.: Probiotics to enhance anti-infective defenses in the gastrointestinal tract. (2003). Best Prac. Res. Clin. Gastroenterol. **17**, 755–773.
6. Rasic J. L., Kurmann J. A. (1983). Bifidobacteria and their role. Basel, Boston, Stuttgart, Birkhauser Verlag.
7. Tuomola E., Crittenden R., Playne M., Isolauri E., Salminen S. (2001). Quality assurance criteria for probiotic bacteria. American Clinic Nutrition. **73**, 393–398.
8. Tuomola E., M., Ouwehand A. C., Salminen S. (1999). Human ileostomy glycoproteins as a model for small intestinal mucus to investigate adhesion of probiotics. Letter in Applied Microbiology. **28**, 159–163.
9. Saarela M., Mogensen G., Fonden R., Matto J., Mattila-Sandholm T. (2000). Probiotic bacteria: safety, functional and technological properties. Journal of Biotechnology. **84**, 197-215.
10. Riedel C., Foata F., Goldstein D., Blum S., Eikmanns B. (2005). Interaction of bifidobacteria with Caco-2 cell─ adhesion and impact on expression profiles. International Journal of Food Microbiology. **110**, 62-68.
11. Larsen, N., Nissen P., Willats W. (2007). The effect of calcium ions on adhesion and competitive exclusion of *Lactobacillus* ssp. and *E. coli* O138. International Journal of Food Microbiology. **114**, 113–119.
12. Bernet M. F., Brassart D. Neeser, J., R. Servin A. L. (1994). *Lactobacillus acidophilus* LA 1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. Gut. **35**, 483–489.
13. Ofek I., Doyle R.: Bacteria Adhesion to Cells and Tissues. Chapman & Hall, New York (1994).
14. Servin A., Coconnier M.: Adhesion of probiotic strains to the intestinal mucosa and interaction with pathogens. (2003). Best Research Clinic Gastroenterology. **5**, 741–754.
1. th edn.Keneth,Arilington,USA.