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Research Article

Growth, Acid Production, Bile Tolerance and Adherence to Columnar Epithelial Cells of Four Species of Bifidobacteria

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Abstract

Growth, acid, production, bile acids tolerance and adherence to sheep epithelial cells were quite different between the four species of bifidobacteria. The following species were studied, *B. infantis, B. longum, B. angulatum* and *B. breve*. The log phase of *B. infantis* and *B. longum* was observed during the first 10 hrs post-inoculation. Whereas the log phase of *B. angulatum* and *B. breve* were found to be after 14 h of inoculation. Growth rate and acid production for *B. infantis* and *B. longum* were different from *B. angulatum* and *B. breve*. The former reduced the pH faster than the latter. *B. longum* had the highest growth rate and *B. angulatum* had the lowest acid production. All the studied species exhibited some degree of bile tolerance. *B. longum* and *B. breve* were more resistant to bile acids than the other two species. Adhesion of the studied species to the columnar epithelial cells of the small intestine of sheep was studied. All the tested species showed some degree of adhesion; however *B. Infantis* adhered more than the other three species

Keywords: Bifidobacteria; Bile Acids; Epithelial Cells; Adhesion

Abbreviations

ATCC: American Type Culture Collection; MRS: De Man-Rogosa-Sharpe Medium; MRSL: De Man-Rogosa-Sharpe Medium + lactose; B: *Bifidobacterium*

Introduction

Bifidobacteria were found to be predominant bacteria in the intestinal flora of breast fed infants. It has been found that, there is a specific relationship between human milk oligosaccharides grown bifidobacteria and intestinal epithelial cells. The growth of bifidobacteria on human milk oligosaccharides enhances epithelial binding and can induce anti-inflammatory response tin the intestinal epithelial cells [1]. The human intestine is sterile at birth but then rapidly colonized by bacteria. Bifidobacteria are among the first bacterial colonizers of the intestine of neonates [2-4]. Intestinal micro flora is a highly active society of microorganisms, processing a diverse complex of enzymes that perform extremely varied functions. So the balance between beneficial bacteria and the harmful ones plays a crucial role in maintaining not only the intestinal health, but also the overall health of the individual [5]. Probiotics are defined as live microorganisms that when administered in adequate amounts, confer a health benefit in the host [6].

Probiotics improve intestinal microbial balance as a mean of infection control. Probiotics are microorganisms selected mostly from bacteria that form a part of the normal intestinal micro flora of humans. Bifidobacteria have been used as probiotics in humans, they are used in food, supplied as dietary supplement, or as active component of registered medication these bacteria, should not only be capable of surviving passage through the digestive tract by tolerating acid and bile, but should also have the ability to proliferate in the gut [7]. They are increasingly recognized as potential bacteria with advantage properties, they contribute to digestion, immunity promotion, and production of vitamins cholesterol lowering and inhibition of pathogens [8].

A large number of products mostly are dairy origin products containing bifidobacteria are produced worldwide. Different species of bifidobacteria were able to grow in six different types of milk [9]. Strains belonging to the genus *Bifidobacterium* are seldom found in food products. Recently there are incorporated in yoghurt manufacture along with yoghurt starter and production of probiotic cheese, because of their health and therapeutic benefits [10]. Bifidobacteria are nutritionally fastidious microorganisms that require specific growth factors as only a limited number of these bacteria can grow in minimal culture conditions [11].

The ability to the adhere of bifidobacteria to the intestinal epithelium cells play an important role in gut colonization as it prevents the peristaltic elimination of bacteria and providing a com-

petitive advantage in this ecosystem [12]. Adhesion promotes the modulation of the immune system and prevents pathogenic bacteria from attaching to the gut mucosa.

So approaches for the selection of an ideal strain of bifidobacteria are still difficult and require considerable resources. Consequently, the aim of this study was to investigate the effect of environmental conditions on the growth of four species of bifidobacteria. Adhesion of these bacteria to sheep epithelial cells was also studied.

Materials and Methods

Microorganisms

Bifidobacterium longum ATCC 2259, *Bifidobacterium angulatum* ATCC 2238, *Bifidobacterium breve* 2258 NCFB and Bifido bacterium infantis were obtained from the department of dairy science Faculty of Agriculture, Minia University, Minia, Egypt.

Growth media

Bifidobacteria were inoculated (1% v/v) and grown in Lactobacilli MRS broth (Oxoid, Basingstoke, UK) supplemented with 5% (w/v) lactose. Solid medium was obtained by adding 1.5% Bactoagar to the supplemented MRS broth. MRSL was supplemented with 0.05% (w/v) L-cysteine HCL (Win Lab, Gemini House, Middlesex, Hab 7ET, UK) as a reducing agent. The cultures were maintained on slopes and subcultured weekly and stored at 5C between transfers. Growth was carried out under anaerobic conditions (Gas Pak System, BBl Cockeysvile, MD, USA).

Growth studies

The four cultures of bifidobacteria were evaluated for growth in MRS broth plus 5% lactose (MRSL medium). Each culture was subcultured at least twice prior to experimental use. Growth was carried out at 37 C and was monitored by recording absorbance at 660 nm (ultrospec. II spectrophotometer, LKB, Biochram, UK). In the case of high growth, 1ml of samples was diluted with 100 mM phosphate buffer (pH 7.0). The pH of the samples was recorded (coming, pH meter 240).

Comparison for bile tolerance

Cultures were tested for growth in MRSL broth medium with or without added bile (oxgall, Sigma Chemial Co., ST.Louos, Mo, USA). The Procedure was that of [13]. Freshly prepared cultures were inoculated (1%) into MRSL broth containing 0.3% oxgall, inoculated at 37 C in a water bath, and monitored for growth every hour by measuring the absorbance at 660 nm. Comparison among cultures was based on the time required for each culture to increase the absorbance at 660 nm by 0.30 absorption units. Growth curves were plotted and a time required for turbidity to reach an optical density of 0.30 was measured.

Bacterial adhesion to intestinal epithelial cells

Adhesion of bifidobacteria to columnar epithelial cells sheep was carried out using the procdure of [14]. Adhesion was tested by examining the slides under the light microscopy of Gram stained samples.

Comparison of adhesion between species was studied by noticing the number of bacterial cells attached to the columnar epithelial cells (concentration of bacteria on epithelial cells).

Results

The relationship between growth of bifidobacteria and certain environmental conditions such as acid production, acid tolerance and reduction in the redox potential was investigated in this study.

Growth and acid production were quite different among the *Bifidobacterium* Species examined in this study (Figure 1,2). The log phase of both *B. infantis* and *B. longum* was found to be after 10 h post-inoculation, whereas the log phase of *B. angulatum* and *B. breve* were found to be after 14hrs of inoculation. The difference of pattern of growth probably due to the difference in β- galactosidase system Adhesion ability of bifidobacteria to epithelial cells is considered one of the most important characteristics for use as probiotic bacteria. Adhesion of bifidobacteria to columnar epithelial cells of the small intestine of sheep was examined. Figure 3 shows the appearance of sheep epithelial cells after the removal of the adherent bacteria. It has been revealed that adhesive bacteria showed a concentration of organisms on the epithelial cells.

Figure 3 shows an example of the adherence of bifidobacterium breve to sheep epithelial cells. Fig 3 shows that the growth rate of B. longum and B. breve enhanced greatly by the addition of L-cysteine to the medium. Thus, these two species might be more obligatory bacteria in comparison with the other two species.

Figure 1: Growth of bifidobacteria (*B. infantis, B. longum, B. angulatum* and *B. breve*) in MRSL broth incubate at 37°C.

Figure 2: Changes in pH of MRSL with 1% bifidobacteria (*B. infantis, B. longumm, B. angulatum* and *B. breve*).

Figure 3: Adhesive of B. breve to sheep epithelial cells (A.) adhesive and (B.) non adhesive cells (x150).

L-Cysteine-HCl was added to the medium to decrease the oxidation reduction potential Table 1 shows that the growth rate of *B. longum* was enhanced by the addition of L-Cysteine to the medium. Thus, the other three species might be more obligatory anaerobic bacteria.

Incubation time (hrs.)	*B. infantis*		*B. longum*		*B. angulatum*		*B. breve*	
	-	+	-	+	-	+	-	+
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.06	0.06	0.06	0.68	0.04	0.06	0.04	0.06
4	0.07	0.79	0.06	0.09	0.06	0.08	0.05	0.08
6	0.22	0.23	0.17	0.39	0.29	0.33	0.30	0.40
8	0.40	0.35	0.30	0.45	0.39	0.41	0.35	0.49
10	0.70	0.75	0.70	0.89	0.60	0.65	0.65	0.79
12	0.65	0.70	0.68	0.85	0.69	0.71	0.70	0.79
14	0.65	0.70	0.60	0.75	0.82	0.80	0.72	0.85
16	0.6	0.65	0.60	0.73	0.75	0.75	0.71	0.75

Table 1: Growth of bifidobacteria (*B. infantis, B. longumm, B. angulatum* and *B. breve)* in MRSL growth with and without reducing agent (cultures were grown at 37°C.).

Table 2 show that there is a difference between the studied species in terms of bile tolerance. Although growth was reduced due to the presence of bile. However, all species showed some degree of bile tolerance. *B. longum* and *B. infantis* were found to be more resistant to bile comparing with the other two species.

Species	Hours to reach A66o nm=0.3	
	MRSL broth	MRSL + 0.30 Oxgall
B. infantis	7	9
B. longum	8	10
B. angulatum	8	14
B. breve	10	17

Table 2: Comparison of bile tolerance of four species of bifidobacteria (*B. infantis, B. longumm, B. angulatum* and *B. breve)*.

Discussion

Growth of probiotics is often thought to be depending on the growth factors such as amino acids, bovine casein digest and yeast extract [15]. However, environmental conditions are also important [16].

[17] found that the log phase of *B. breve* and *B. angulatum* was during the first 12hrs post-inoculation. They also reported that some species of bifidobacteria have two log phases and this pattern of growth could be due tow *B- galactosidase* systems. The reduction of pH was found to be different among studied species. *B. longum* and *B. infantis* reduced the pH faster than the other two species. [17] reported that species of bifidobacteria which produce acid early during growth are less tolerant to acidity than those which produce it later during growth.

Growth of bifidobacteria is also affected by the redox potential. It has been reported that the differences in growth rates among species of bifidobacteria is due to different levels of tolerance to anaerobic conditions [18]. Therefore, L-cysteine-HCl was added to the medium to decrease the oxidation-reduction potential. [17] reported that *B. breve* and *B. angulatum* were found to be enhanced by the presence of reducing agent more than *B. bifidum* and *b. longum* [19] pointed out that bifidobacteria are classified as anaerobic bacteria, although some species are able to tolerate oxygen. It has been reported that oxygen prevents growth by establishing a high oxidation reduction potential [18].

Bile tolerance is a desirable characteristic for bifidobacteria to be used as probiotics. It has been found that bile tolerance was different even between species [20]. They observed that *B. longum* B6 was more tolerant to bile than *B. longu*m ATCC 15078. In [9] pointed out that *Bifidobacterium bifidum* 2203 was the most resistant studied species to bile salts.

The ability of bifidiobacteria to adhere to epithelial cells has been suggested to be an important property of probiotics. Cell adhesion is a multistep process involving contact of the bacterial cell membrane and interacting surfaces [21]. In [22] pointed out that binding of the lipoteichoic acid of B. bifidum to human colonic epithelial cells appeared to be specific, reversible and depends in length of contact time and cell concentration. [23] pointed out that the higher the level of the fatty acid component of the lipoteichoic acids, the better the adhesion. Cell surface proteins had an effect on the adhesion and aggregation ability of probiotic strain *lactobacillus acidophilus* M92 [24]. Further work has to be done regarding the attachment of bifidobacteria to human epithelial cells, the effect of different natural media on the attachment to epithelial cells has to be investigated as well.

Studying growth factors of bifidobacteria will have a great benefits on the microbial balance of the gastrointestinal tract. Such benefits are, improvement of lactose intolerance, decreasing cholesterol levels, treatments of Crohns disease, constipation, ulcreative colitis, colorectal cancer, prevents antibiotic induced diarrhea and prevents pathogen from attaching to the gut mucosa.

Bibliography

1. Chichlowski M., *et al*. "Bifidobacteria isolated from infants and cultured on human milk oligosaccharides affect intestinal epithelial function". *Journal of Pediatric Gastroenterology and Nutrition*55.3 (2012): 321-327.

2. Favier CF., *et al*. "Molecular monitoring of succession of bacterial communities in human neonates". *Applied and Environmental Microbiology* 68.1 (2002): 219-226.

3. Turruni FA., *et al*. "Human gut microbiota and bifidobacteria from compostion so functionality". *Antonie van Leeuwenhoek* 94.1 (2008): 35-50.

4. El-Bakry H., *et al*. "Role of some selected Bifidobacterium strains in modulating immuosenescence of aged slbino rats". *The Journal of Basic and applied Zoology* 66 (2013): 255-262.

5. Percival M. "Choosing a probiotic supplement. CNI601 12/97 Clinical Nutrition Insights Advanced Nutrition Publications, Inc (1997).

6. Food and Agriculture Organization (FAO). World Health Organization (WHO). Report of a joint FAO/WHO expect consultation on evaluation of health and nutrition properties of probiotics in food including powder milk with live lactic acid bacteria" (2014).

7. Vandenplas Y., *et al*. (2014). "Probiotics : an update". *Jornal de Pediatria* 91.1 (2015): 6-21.

8. Moslemi M., *et al*. "Incorporation of propionibacteria in fermented milks as a probiotic". *Critical Reviews in Food Science and Nutrition* 56.8 (2016): 1290-1312.

9. Moawad R., *et al*. "Some physiological properties and antibiotic resistance of four strains of bifidbacteria. Food Safety". 1st International Cinference of Egyptian Society of food safety (2) (2018): 15-18.

10. Clemente A. "Probiotics and prebiotics: an update from the world gastrointestinal organization (WGO)". *European Journal of Food Research and Review* 2.1 (2012): 24-28.

11. Poch M and Bezkorovamty A. "Growth enhancing supplement for varies species of genus Bifiobacterium". *Journal of Dairy Science*71.12 (1988): 3214-3221.

12. Cownway PL and Kjelleberg SC. "Protein mediated adhesion of Lactobacillus fermentium strain 737 to mouse stomach squamous epithelium". *Journal of General Microbiology* 135.5 (1989): 1175-1186.

13. Gilliland S., *et al*. "Assimilation of cholesterol by lactobacillus acidophlus". *Applied and Environmental Microbiology* 49.2 (1985): 377-381.

14. Fuller R. "Ecological flora on the lactobacillus associated with Grope epithelium of the fowl". *The Journal of applied bacteriology y*36.1 (1993): 131-134.

15. Rasic JL and Kurmanm JA. (1993). "Culture media in Bifidobacteria and their sale. Experients supplement vol.39. Birkhouse. Verlag. Basel Appendix 2.P 175.

16. Klaver FA M., *et al*. "Growth and survival of bifidobacteria in milk". *Netherlands Milk and Dairy Journal* 47 (1993): 151-164.

17. Al-Saleh AA., *et al*. "Growth of bifidobacteria: environmental conditions and adherence to epithelial cells". *Milchwissenschaft* 53 .4 (1998).

18. De vries W and Stouthamer AH. (1969). "Factors determining the degree of anaerobic of bifiobactreium strain". *Arch Mikrob* 65 (1969): 275-287.

19. Tamime AY., *et al*. "Microbiological and technological aspects of milk fermented by bifidobacterial". *Journal of Dairy Science* 62.1 (1995): 151:187.

20. Jiang T., *et al*. "Improvement of lactose digestion in humans by ingestion of unfermented milk containing bifidobacterium longum". *Journal of Dairy Science* 79.5 (1996): 750-757.

21. Del Re B., *et al*. "Adhesion ,auto aggregation and hydrophobicity of 13 strains of Bifidbacterium longum". *Letters in Applied Microbiology* 31.6 (2000): 438-442.

22. Del Re B., *et al*. "Adhesion ,auto aggregation and hydrophobicity of 13 strains of Bifidbacterium longum". *Letters in Applied Microbiology* 31.6 (2000): 438-442.

23. Op Den Kamp HJM Peeters., *et al*. "Phospholipid asymmetry in the plasma membrane of malaria infected erythrocyte". *The Journal of General and Applied Microbiology* 131 (1985): 661-668.

24. Kos b., *et al*. "Adhesion and aggregation ability of probiotic strain Lactobacillus acidophilus M92". *Journal of Applied Microbiology* 94.6 (2003): 981-987.

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