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BENEFICIAL EFFECT OF ISOLATED PROBIOTIC STRAIN

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ABSTRACT

The aim of this research was to isolate Bifidobacterium spp. as probiotic strain from healthy infant stool. After that, study the role of this strain in lowering cholesterol level, enhancement of immunoglobulins (IgG & IgM) and effect of this strain on pathogenic bacteria in intestinal tract. The final aim is to use this strain to produce functional dairy products. Twenty-three presumptive *Bifidobacterium* strains were isolated from healthy infant stools. On the basis of all of the identification tests twelve strains isolated from the stool were identified as *B. breve*, eight strains were identified as *B. infants* and three strains were identified as *B. bifidum*. *B. breve* (9/12 strains), *B. infants* (7/8 stains) and *B.bifidum* (2/3 strains) were tolerable to bile salt. However, *B. breve* (8/12 strains) and *B. infants* (5/8 strains) and *B. bifidum* (1/3 strains) were tolerant in pH 3.0. All strains are not able to grow at pH 1.0 or 2.0. *Bifidobacterium breve* (No. 7) that is characterized by low pH stability, bile salt tolerance and has antimicrobial activity was selected from healthy infant stools to be used as probiotic in mice nutrition

This strain has significant role in lowering cholesterol level, enhancement of IgG and has positive effect in decreasing count of *E. coli* and *Staph. aureus* in intestinal tract. On the other hand, the count of *B .breve* and organoleptic score of probiotic yogurt with this strain reached to high score during the storage period.

Keywords: probiotic, Bifidobacteria, lowering cholesterol, immunoglobulins (Ig)

INTRODUCTION

The intestinal microbiota is a complex ecosystem harboring in the colon up to 1011 bacteria/g belonging to about 400 different bacterial species. This indigenous microbiota performs important metabolic and immunological functions and acts as a biological barrier against pathogens. The gastrointestinal tract of the newborn, sterile during fetal life, becomes rapidly colonized with bacteria derived from the mother and from the environment.

The number of Bifidobacteria in the colon of adults is 108 - 1011 CFU, but this number decreases with age. *B. adolescentis* and *B. longum* are major Bifidobacteria species in the adult intestine and *B. infants* and *B. breve* are predominant species in the intestinal tract of human infants (Gavini et al., 2001).

B. adolescentis, *B. animalis*, *B. bifidum*, *B. breve*, *B. infants*, *B. lactis* and *B. longum* are probiotics (Holzapfel *et al.*, 1998). The health of humans and animals are improved when these viable cells are added to diets (Salminen *et al.*, 1998). They help to synthesize the vitamins in yogurt and improve the absorption of minerals and protein (Ding *et al.*, 2005).

Yamamah et al (2005) found that the use of *B. bifidum* and *L. acidophilus* proved to minimize stool frequency significantly and improve consistency of stools in infant watery diarrhea, both with rotavirus and negative case in comparison to placebo, *B. bifidum* strain seemed to be the most effective.

Probiotics are more precisely defined as mono or mixed cultures of living microorganisms, which beneficially affect the host (human or animal) by improving the properties of indigenous microflora (Havenaar et al., 1992).

All species of *Bifidobacteria* derived from human are non-spore forming, non-motile, anaerobic, Gram positive bacteria.

Bifidobacteria had long been recognized as bacteria with probiotic, nutritive and therapeutic properties (Bezkoravainy, 2001; Holzapfel *et al.*, 2001). In recent years, there has been an increasing interest in the incorporation of the intestinal species *Lactobacillus acidophilus* and *B. species* into fermented milk products. These species are frequently associated with health promoting effects in human and animal intestinal tract. Mehanna et al., (2002) demonstrated that probiotic strains can be introduced into fresh cheese and till two months of storage. At that time numbers were still above the recommended threshold for a probiotic effect. On the other hand add prebiotic substance such as inulin, honey, date and cellulose as a healing agent with probiotic strains play a key role in enhancing the activity of probiotic bacteria (Mehanna et al, 2003a; Mehanna et al., 2003b; Mehanna and Hosney 2003 and Ibrahim et ai., 2003). These probiotic effects are generally related to inhibition of pathogenic species, reducing the risk of colon cancer, increasing the immune response and decreasing (Bezkoravainy, 2001; Holzapfel *et al.*, 1998) concentration of cholesterol in blood plasma (Gilliland, 1990; Gurr, 1987).

B. longum and B breve were reported to prevent carcinogens from affecting DNA. ***B. longum*** reduces the creation of tumors, and creates anticancer linoleic acid (Rosberg-Cody *et al.*, 2004). As for other diseases, bifidobacteria was also suggested to reduce serum cholesterol and alleviate constipation (Leahy *et al.*, 2005).

The aim of this research was to isolate *B.* spp. as probiotic strain from healthy infant stool. After that, study the role of this strain in lowering cholesterol level, enhancement of IgG & IgM and effect of this strain on pathogenic bacteria in intestinal tract. The final aim is to use probiotic strain to produce functional dairy products.

MATERIAL AND METHODS

I. Bacterial Strains: *B.* strains were isolated from healthy infant stools, *Lactobacillus delbreuckii* ssp *bulgaricus* and *Streptococcus thermophilus* (to manufacture of yogurt) obtained from Chr. Hansen, Horsholm. Denmark.

Pathogenic strains indicators used to study the antagonistic activity are: *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeroginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633, *Klebsiella pneumonia* ATCC 18833, *Salmonella typhimurium* ATCC 13311 (all strains obtained from Cairo MIRCEN).

II. Animals: Twenty male albino male mice (6 wk old) with an average initial body weight of 20 ± 5. Animals were placed in individual metabolic cages and housed in a room that was maintained at a constant temperature of 22° ±2°C, a relative humidity of 60 ±5%. Mice were housed on a 12-hrs. light: dark schedule, with free access to water and rat and mouse standard diet

containing (g/100 g): 64 starch, 23 protein, 3.5 fat, 5 fiber, 1 vitamin mixture and 3 salt mixture.

Animals were divided into two groups of ten rats each. The first group received basal diet only for 3 weeks. A second group was fed by basal diet containing 10 g yogurt with isolated probiotic strain (final concentration 5 $x10^8$ cfu/g) also for 3 weeks.

The fecal samples were collected before, during, and after treatments. On the last day of the experimental period rats were killed by carbon dioxide and blood was collected from orbital plexus on Na2-EDTA (1 mg/1 ml blood).

III-1. Isolation and phenotypic characterization

A 10 g sample of healthy infant feces was taken aseptically. It was transferred to sterile plastic bags and then homogenized in 90 ml. of sterile buffered peptone water (BPW). Five 10-fold dilutions of the homogenates were then prepared and these were inoculated on plates of MRS agar (Oxoid) *(De Man et al., 1960)* and supplemented plus 0.05% L-cysteine HC1 (Sigma Chemical Co., St. Louis, Mo), acidified with glacial acetic acid to pH 5.7 and incubated anaerobically using Gen Kits in Oxoid jar for 48 hrs at 37°C. Colonies with typical characteristics were randomly selected from plates and tested for Gram stain, cell morphology, and catalase reaction before further sugar fermentation and characterization tests (Harrigan and McCance 1990). During test the cultures were kept in MRS agar plus 0.05% L-cysteine HC1 at refrigeration temperature.

2. Biochemical characterization and presumptive

Identification Growth at 15 and 45°C in tubes containing MRS broth without beef extract and glucose, and fermentation of carbohydrates were determined as described by Bonaparte and Reuter (1996) carbohydrates tested were (arabinose, mannose, salicin, mannitol, sorbitol and melezitose (Difco) xylose (Merck, Darmstadt, Germany) and sterile water were used as positive and negative controls.

IV- Selection of probiotic bacteria.

1. Bile tolerance

In order to assess bile salt tolerance of bacteria, the isolates of *B. strains*, were incubated in MRS broth (pH 7.0) plus 0.05% L-cysteine at 37°C for 24 hrs under anaerobic conditions. MRS broth was supplemented with 0.3% (w/v) Oxgall (Sigma, USA, pH 7.0). All bacteria were inoculated as 30 µl volume and incubated at 37°C for 3 hrs. Then, bacteria were spread onto BL agar plates (Pacher and Kneifel. 1996) to confirm the survival of bacteria and anaerobically incubated at 37°C for 48 hrs. If colonies were formed on the BL media, they were decided as the bacteria to have bile salt tolerance (De Smet *et al*, 1994 and Kimoto *et al*, 1999).

2. Growth at low pH

To assess low pH tolerance, the first isolates, *Bifidobacterium*, were grown in MRS plus 0.05% L. cysteine media and harvested by centrifugation (5000 rpm for 10 mm at 4°C). The pellet was resuspended in the same volume of the same media adjusted to pH 1, 2 or 3 with 10% (wt/vol.) HC1. Control cultures at pH 7 were included. Resuspended cells were incubated at optimum temperature for 3 hrs. After incubation, viable counts were determined by spread onto BL media to discriminate the survival of bacteria

and anaerobically incubated at 37°C for 48 hrs. If the colonies were formed on the BL media after 48hrs incubation, they were confirmed as the bacteria to have low pH tolerance [Kimoto *et al*, 1999].

3. Antibacterial activities of the strains of isolated B. spp.:

Antimicrobial effects of presumptive strains of *B. spp.* on *E. coli*, *S. aureus*, *P. aeroginosa*, *B. subtilis*, *K. pneumonia* and S.*typhimurium* were determined by the agar diffusion method (Fleming et al., 1985). For the detection of antibacterial activity of the strains of *B. spp.*, MRS broth supplemented with 0.05% L. cysteine was used. Ten milliliters of broth was inoculated with each strain of *B. spp.* and were incubated at 37° C for 48 hrs. After incubation, a cell-free solution was obtained by centrifuging (6000 x g for 15 min) the culture, followed by filtration of the supernatant through a 0.2 µm pore size (Schleicher & Schuell, Germany) cellulose acetate filter. Some supernatants were neutralized with 1 N NaOH to pH 6.5, and the inhibitory effect of the hydrogen peroxide was eliminated by the addition of catalase (5 mg/ml). Un-neutralized (general inhibitory effect) and neutralized (bacteriocin and bacteriocin-like metabolites) supernatants of the strains of *B. spp.* were checked for antibacterial activity against pathogenic bacteria in inoculated nutrient agar (Schillinger & Lucke 1989 and Reinheimer *et al.* 1990). Once solidified, the dishes were stored for 2 hrs in a refrigerator. Then 0.1 ml of cell free supernatants was filled in 8-mm diameter sealed wells cut in the nutrient agar. The inoculated plates were incubated for 24 hrs at 37° C, and the diameter of the inhibition zone was measured with calipers in millimeters (Harris *et al.*, 1989).

V - Preparation of yoghurt:

Cultures of *Str. thermophilus, L. delbreuckii subs. bulgaricus* were maintained by routine propagation in 10% sterile reconstituted skim milk supplemented with 0.5% yeast extract. Subculturing was prepared using 1% with incubation at optimum temperature for 18 hrs. and stored at 5 °C. Subculturing was done at least twice prior to the experimental use. The culture of isolated *B. breve* was routinely grown for 24 hrs. at 37 °C in MRS broth *(De Man et al., 1960)* plus 0.05% cysteine HC1 and incubated under anaerobic conditions.

Fresh cow milk (8.6% solid not fat, 3.3% fat) was heated to 90 °C for 10 min, and then cooled to 40°C. Yoghurt starter was inoculated at 2% level (volume/volume). Inoculated milk was prepared to provide 2 formulae (control and treatment).

Enumeration of *B. breve* was done according to Blanchette *et al.* (1996) using modified MRS agar with 0.05 L-cysteine HCl. Two groups were prepared. The 1st was control; made of *Str. thermophilus* + *L. bulgaricus*. The 2nd was formula treatment; made of 5% *B. breve* in addition to *Str. thermophilus* + *L. bulgaricus*.

VI – Analysis:

1. Fermented milk: *Bifidobacterium ssp.* was counted on MRS agar with 0.05% L. cysteine. The resultant fermented milk (yogurt or yogurt plus *B. spp.* were evaluated when fresh and during storage period at refrigerator temperature by 20 experienced staff members of Biology Department - Faculty of Applied Science, Umm Al-Qura University according to the

international dairy federation IDF (1997) and British Standard institutes (1986) Part 1, as follow: acceptability flavor, appearance and texture.

2. Blood sampling and serum analysis:

2.1 Triglyceride (TG) and cholesterol determination: TG and total cholesterol levels (mmol/L) in plasma were determined by TG/GB kit and cholesterol/HP kit (Boehringer Mannheim, Indianapolis, IN), respectively.

2.2 Determination of IgG and IgM level

IgG and IgM in serum of mice were determined by IgG ELISA kit (Roche, Diagnostics Penzberg, Germany) and IgM) kit (EXBIO, Prague, Czech Republic)

3. Fecal Microbial Analysis

All fecal samples were collected fresh by gently squeezing the rectal area of the mouse. The fecal pellets were immediately placed in tubes kept in anaerobic jars and the analysis was carried out within 30 to 60 min of collection. Anaerobic conditions were maintained as far as possible during the analysis. Following homogenization, a series of 10-fold dilutions of the specimens was made in a sterile phosphate buffer.

Triplicate plates were made of each sample. Strains of *Staph. aureus* were enumerated on Bird Parker medium (Oxoid), strains of *E. coli* were counted on Brilliant green agar (Oxoid) supplemented with 0.5% glucose (FAO, Reported, 2001) and MRS agar with 0.05 L-cysteine HCl agar for *Bifidobacteria*. Plates of anaerobe and *B.* were incubated anaerobically in an anaerobic chamber (Gen Kits in Oxoid jar) for 3 days at 37° C. Plates for the enumeration of aerobic organisms and coliforms were incubated at 37° C for 2 days.

4. Statistical Analyses

Results obtained were subjected to Student's t-test using SPSS (1993) version 6.0. Standard error and level of significance were calculated and compared to control animals or with the values of before administration (0 day) of the respective group.

RESULTS AND DISSCSION

Isolation and Identification

Twenty-three presumptive *Bifidobacterium* strains were isolated from healthy infant stools. All isolates were catalase-negative, Gram positive, bacilli in a typical arrangement and were also unable to grow aerobically in solid media. On the basis of all of the identification tests twelve strains isolated from the stool were identified as *B. breve*, eight strains were identified as *B. infants* and three strains were identified as *B. bifidum*.

Metabolic characteristics and presumptive identification of *B. breve, B. infants* and *B. bifidum*, isolated from stool are shown in table (1) (according to Bonaparte and Reuter, 1996).

Table 1: Biochemical identification of *Bifidobacterium* (According to Bonaparte and Reuter, 1996)

Isolated No.	Gram stain	catalase	Carbohydrates						Isolated strains
			Ara	Man	Sal	Mant	Sor	Xyl	
3	+	-	-	+	+	+	+	-	*B. breve*
4	+	-	-	+	+	+	+	-	*B. breve*
7	+	-	-	+	+	+	+	-	*B. breve*
8	+	-	-	+	+	+	+	-	*B. breve*
9	+	-	-	+	+	+	+	-	*B. breve*
13	+	-	-	+	+	+	+	-	*B. breve*
14	+	-	-	+	+	+	+	-	*B. breve*
15	+	-	-	+	+	+	+	-	*B. breve*
16	+	-	-	+	+	+	+	-	*B. breve*
17	+	-	-	+	+	+	+	-	*B. breve*
18	+	-	-	+	+	+	+	-	*B. breve*
21	+	-	-	+	+	+	+	-	*B. breve*
1	+	-	-	+	+	-	-	-	*B.infants*
2	+	-	-	+	+	-	-	-	*B.infants*
5	+	-	-	+	+	-	-	-	*B.infants*
10	+	-	-	+	+	-	-	-	*B.infants*
11	+	-	-	+	+	-	-	-	*B.infants*
19	+	-	-	+	+	-	-	-	*B.infants*
20	+	-	-	+	+	-	-	-	*B.infants*
23	+	-	-	+	+	-	-	-	*B.infants*
6	+	-	-	-	-	-	-	-	*B. bifidum*
12	+	-	-	-	-	-	-	-	*B. bifidum*
22	+	-	-	-	-	-	-	-	*B. bifidum*

Ara: arabinose, Man: mannose, Sal: salicin, Mant: mannitol, Sor: sorbitol and Xyl: Xylose. NT: not tested.

Bile salt tolerance: Results of bile salt tolerance were shown in (Table 2). *B.* breve (9/12 strains), *B.* infants (7/8 stains) and *B.* bifidum (2/3 strains) were tolerable to bile salt.

Table 2: No. of strains which tolerate to grow on bile salt

Strain	No. of Strains
B. breve	9/12
B.infants	7/8
B. bifidum	2/3

Gilliland *et al.*, (1984) decided that bile tolerance has been identified as one of the important characteristics that enable probiotic to survive and grow in the intestinal tract.

Low pH tolerance: Low pH tolerance of isolated *B.* was assessed in pH 1.0, 2.0 and 3.0. As shown in Table (3) *B.* breve (8/12 strains) and *B.* infants (5/8 strains) and *B. bifidum* (1/3 strains) were tolerant in pH 3.0. All strains are not able to grow at pH 1.0 or 2.0 (the colonies were formed on the BL media after 48hrs incubation; they were confirmed as the bacteria to have low pH tolerance [Kimoto *et al*, 1999]).

Antibacterial activities of the isolated *B. spp* strains:

Results in table (4) show antimicrobial activity of culture supernatants obtained from eight isolated *B. breve* strains (mm) (which had grown at pH 3 and were tolerable to bile salt) against six pathogenic bacteria strains. The antimicrobial activity produced by *B. breve* isolated has varying inhibition

effect on the growth of *E. coli*, *Staph. aureus*, *P. aeroginosa*, *B. subtilis*, *K. pneumonia* and *S. typhimurium*, as shown in table (4). We noticed that the highest effect was shown on *E.coli* and *P aeruginosa* respectively and the lowest effect was noticed on *B. subtilis*, also we observed that the strain No. 7 had the highest effect. These results agree with those obtained by (Gibson and Wang, 1994 and Fujiwara *et al.*, 1997).

Table 3: No. of strains which had grown at different pH level

Strain	pH		
	1	2	3
B. breve	-	-	8/12
B.infants	-	-	5/8
B. bifidum	-	-	1/3

Table (4) antimicrobial activity of culture supernatants obtained from eight isolated *B. breve* strains (mm)

Isolated No.	Pathogenic bacteria (mm)					
	E.coli	Staph. aureus	P. aeroginosa	B. subtilis	K. pneumonia	S. typhimurium
3	7	6	6	4	6	5
7	9	7	8	5	6	7
8	8	5	7	3	7	5
13	7	5	6	3	6	7
14	7	6	6	4	6	3
16	8	7	7	5	7	4
17	6	5	6	3	5	6
21	7	5	5	4	4	5

V Liévin *et al.*, (2000) recited that the viability of all microorganisms was verified after one and three hours of incubation with *bifidobacteria*-SCS. The viability of *Streptococcus spp* group D, *S. flexneri*, and *C. difficile* was not affected at any time points. The viability of *L. monocytogenes* was not affected after one hour of contact and was affected to a less degree after three hours of contact (3 log decrease). *E. coli, K. pneumoniae, Y. pseudotuberculosis, or S. aureus* viability was not affected or affected to a less degree after one hour of contact, but in contrast was greatly decreased after three hours of contact (5 to 6 log decrease). The viability of *P. aeruginosa* was greatly decreased at both times (6 log decrease).

Bifidobacterium breve (No. 7) that is characterized by low pH stability, bile salt tolerance and has antimicrobial activity was selected from healthy infant stools to be used as probiotic in mice nutrition

Analysis of Yogurt

Fig (1) showed the viable count of *B. breve* during yogurt storage at room temperature (10 days). We noticed that the counts of *B. breve* slightly increase (insignificant) during the first five days after that slightly decreased till the end of this period, these results were confirmed by Mehanna (2003a,b). Reducing the viability of *Bifidobacterium* may be due to the presence of lactic acid and acetic acid which inhibit the growth of it (Gomes *et al.*, 1995).

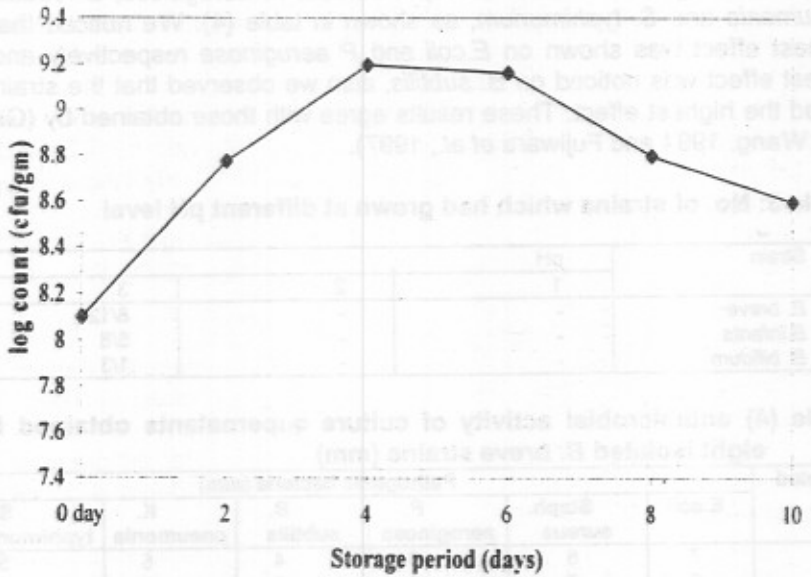

Fig 1: Viability of *B breve* in probiotic yogurt during storage

The results in fig (2) demonstrated that both traditional yogurt and fermented milk with *B. breve* has high organoleptic score during all storage period with slightly decrees after five days (insignificant)

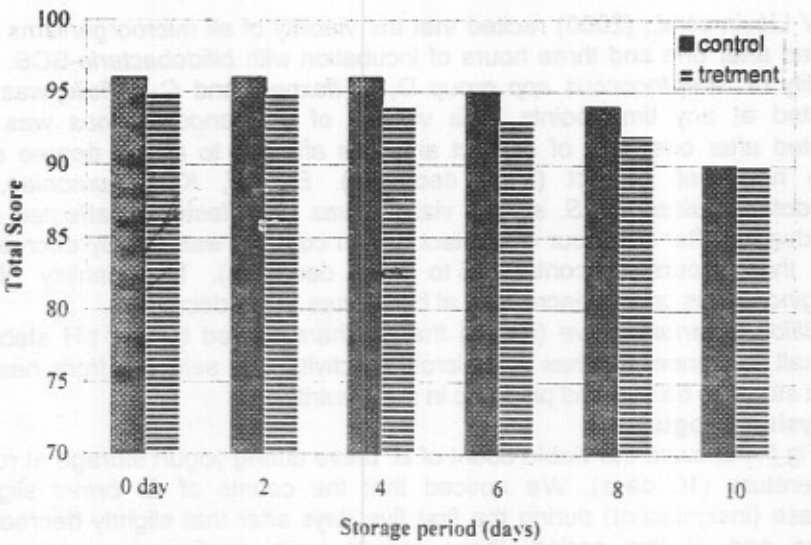

Fig 2: Score of organoleptic properties of yogurt during storage period.

Blood sampling and serum analysis:

At the end of the experiment, blood cholesterol and TG was higher in control mice than in treated animals which fed probiotic yogurt as shown by the results in fig (3) (significant difference at $P < 0.05$), This results may be due to the effect of *B. breve* strain in lowering cholesterol and TG. These results confirmed by (Hyeong *et al* 2004)

Some natural microorganisms in human intestine are beneficial in terms of lowering serum cholesterol (Fernandes *et al.*, 1987; Fukushima *et al.*, 1999 and Mann & Spoerry, 1974)

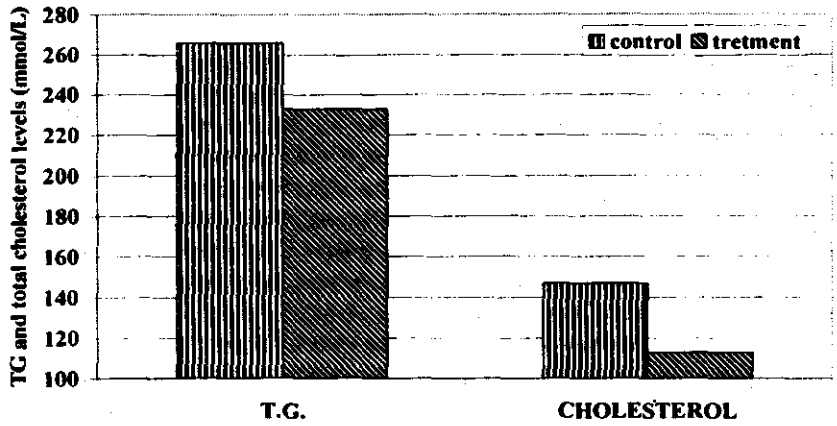

Fig 3: The level of cholesterol and TG in animal blood at the end of experimental feeding.

The lactic acid bacteria (LAB), Lactobacillus and *B.* spp. in particular, have the ability to metabolize cholesterol [De Smet *et al.*, 1995]. Gilliland *et al.*, (1985) reported that *Lactobacillus acidophilus* reduces blood cholesterol by direct breakdown of cholesterol and de-conjugation of bile salt.

IgG and IgM:

The level of IgG (0.455±0.003) was significantly increased ($p<0.05$) in probiotic-feeding mice as compared to the control (0.253±0.001). In the same table, the level of IgM (0.331±0.013) was slightly increased in probiotic-feeding mice as compared to the control (0.301±0.002) (table 5).

Table (5): Levels of IgG and IgM in serum control and treated animals:

	IgG	IgM
Control (yogurt)	0.253±0.001	0.301±0.002
Probiotic yogurt)	0.455±0.003	0.321±0.013

*** Significant at p<0.05 in the same row**

Probiotic consumption has been recommended for immune modulation and general health promotion. The precise mechanisms of immune modulation by probiotics have not been elucidated but they are known to influence both non-specific and specific immune responses in animal models and in humans [Cross, 2002, and Guarner Malagelada, 2003].

Oral introduction of *B.* bifidum was shown to enhance antibody response to ovalbumin (Moreau, *et al.*, 1990) and *B.* breve was shown to stimulate IgA response to cholera toxin in mice (Yasui, *et al.*, 1992). Lactic acid bacteria enhance immune system function at the intestinal and systemic levels. In humans, lactic acid bacteria have been shown to increase IgA-, IgG- and IgM-secreting cells and serum IgA levels, which would increase antibody activity (Kaila, *et al.*, 1992).

Fecal Microbial Analysis:

Fig (4) described the count of *B. breve* and count of both *E. coli* and *Staph. aureus* in the feces of animals which were analyzed during 21 days. These results showed that the count of *B. breve* was significantly increased at $p < 0.01$ from the beginning of the experimental to the end, while the count of *E. coli* and *Staph. aureus* was dramatically decreased (significantly at $p < 0.01$). These results agree with that recorded by (Mullie, *et al.*, 2002) which mentioned that the cell-free whey obtained from milk fermented with *B. breve* C50 (Bb C50) has been shown to modify the intestinal flora in humans and mice. Also, (Romond *et al.*, 1997) established that modification to the intestinal flora was an activity specific to *B. breve* C50 strain. These results may be due to the bifidobacteria strains which are known to produce antimicrobial substances (Gibson and Wang, 1994).

A number of digestive health benefits have been reported for the polyunsaturated fatty acids. Due to their anti-inflammatory and antibacterial properties, some polyunsaturated fatty acids have the ability to kill harmful bacteria that are likely to be present in the gastrointestinal tract (Das UN, 2002). Bomba *et al* (2003) proposed that dietary fatty acids affect attachment sites of intestinal flora by modifying the fatty acid composition of the intestinal wall.

CONCLUSION

From all these results, we could select *Bif. breve* as a probiotic strain from healthy infant stool. This strain has significant role in lowering cholesterol level, enhancement of IgG and has positive effect in decreasing count of *E. coli* and *Staph. aureus* in intestinal tract. On the other hand, the count of *B.breve* and organoleptic score of probiotic yogurt with this strain reached to high score during the storage period.

In the future, we should make more study on this strain genetically and physiologically before recommendation to use it in industry.

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الفوائد الصحية لسلالة بكتريا البروبيوتيك المعزولة

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****قسم الالبان – شعبة الصناعات الغذائية والتغذية – المركز القومى للبحوث – مصر**

يهدف البحث الى عزل سلالة تنتمى الى مجموعة بكتريا البروبيوتيك من براز الرضع الاصحاء ودراسة دور هذه البكتريا على خفض مستوى كوليسترول دم فئران التجارب و زيادة اجسام المناعة IgG, IgM وتأثير تلك البكتريا على اعداد البكتريا المرضية المتواجدة بالقناة الهضمية وذلك بهدف انتاج منتجات لبنية صحية.

وتم عزل ٢٣ سلالة من بكتريا Bifidobacterium من براز الرضع الاصحاء وصنفت الى ١٢ سلالة *Bifidobacterium breve* و٨ سلالات *Bifidobacterium infants* وثلاث سلالات *Bifidobacterium bifidum* ووجد ان ٧٥% (١٢/٩) من سلالة *Bifidobacterium breve* و ٨٧٫٥ % (٨/٧) من سلالة *Bifidobacterium infants* و٦٦٫٧ % (٣/٢) *Bifidobacterium bifidum* يتحمل النمو فى وجود املاح الصفراء

ووجد ان ٦٦٫٧% (١٢/٨) من سلالة *B. breve* و ٦٢٫٥% (٨/٥) من سلالة *B. infants* و ٣٣٫٣% (٣/١) من سلالة *B. bifidum* لهم القدرة على النمو عند مستوى حموضة pH ٣ وجميع السلالات لا تستطيع النمو عند مستوى حموضة اقل من ذلك

وتم اختبار سلالات *B. breve* التى استطاعت النمو فى وجود املاح الصفراء وpH ٣ فى تأثيرها على البكتريا المرضية واظهرت النتائج ان السلالة رقم ٧ افضل تأثير على البكتريا المرضية. تم تصنيع زبادى باستخدام السلالة رقم (٧) مع البادئ التقليدى وتم تغذية ٢٠ فأر تجارب على الزبادى لمدة ٢١ يوم واظهرت التجارب النهائية ان الزبادى تميز بجودة حسية مرتفعة خلال طول فترة التخزين وان التغذية على الزبادى ادى الى خفض كوليسترول الدم معنويا وكذلك الزيادة المعنوية فى اجسام المناعة والانخفاض المعنوى لاعداد كل من بكتريا *E. coli* and *Staph. aureus* المتواجدة بالقناة الهضمية.