The Journal of Basic & Applied Zoology (2013) **66**, 255–262

The Egyptian German Society for Zoology

The Journal of Basic & Applied Zoology

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Role of some selected *Bifidobacterium* strains in modulating immunosenescence of aged albino rats

Hanan A. El-Bakry [a,*], Wafaa M. Zahran [a], Shaimaa A. Anter [a], Ahmed S. Zahran [b]

[a] *Department of Zoology, Faculty of Science, Minia University, Egypt*
[b] *Department of Food Production, Faculty of Agriculture, Minia University, Egypt*

Received 30 December 2012; revised 8 April 2013; accepted 26 May 2013
Available online 24 June 2013

KEYWORDS

Probiotics;
Bifidobacteria;
Aging;
Immunosenescence;
Rats

Abstract Probiotic administration has been associated with enhanced immune function in elderly subjects. However, approaches for selection of an "ideal" strain of bifidobacteria are still difficult. The aim of the present study is to investigate the possible modulatory effects of three strains of *Bifidobacterium* species (*Bifidobacterium adolescentis* ATCC 15704, *Bifidobacterium breve* ATCC 15700 and *Bifidobacterium longum* ATCC 15707) on haematological and immunological parameters of aged albino rats corresponding to normal adult ones. The animals were divided into six groups; three groups of aged rats were fed yoghurt inoculated with one of the *Bifidobacterium* strains; one group of aged rats was fed yoghurt alone (control aged); two groups of adult and aged rats were provided with normal diet and assigned as normal groups. The total leucocyte count was significantly increased in the three bifidobacteria-treated aged groups as compared with both normal and control aged rats. Serum IgA level was considerably increased in all treated rats. On the contrary, serum IgE level was significantly decreased in rats supplemented with yoghurt inoculated with *B. adolescentis* or *B. breve*. Both *B. adolescentis* and *B. breve* groups showed significant enhanced production of TNF-α. Furthermore, the production of cytokine IL-8 was significantly increased in the *B. adolescentis* group. Interestingly, it was apparent that only *B. adolescentis* had the most pronounced effect on aged rats to regain nearly normal values as measured in normal adult rats. Conclusively, the present work indicates that dietary consumption of selected bifidobacteria strains may have a particular application in the elderly especially in terms of immunomodulation.

Introduction

Intestinal microflora is a highly active society of organisms, possessing a diverse complex of enzymes that perform extremely varied functions, both beneficial and harmful. The delicate, yet critical, balance maintained among this enormous bacterial population plays a crucial role in maintaining not only intestinal health, but also the overall health of the individual

* Corresponding author.
E-mail addresses: elbakry.h@gmail.com, elbakry_h@yahoo.com (H.A. El-Bakry).
Peer review under responsibility of The Egyptian German Society for Zoology.

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http://dx.doi.org/10.1016/j.jobaz.2013.05.002

(Percival, 1997). Therefore, disturbance of the ecological balance in the gastro-intestinal system may be detrimental to health. This has highlighted the importance of probiotics that improve intestinal microbial balance, as a mean of infection control. Probiotics are micro-organisms selected mostly from bacteria that form a part of the normal intestinal microflora of humans. In this respect, bifidobacteria are increasingly recognized as potential probiotics with advantageous properties; they are important species of the intestinal tract and associated with a healthy status in humans (Holzapfel et al., 1998).

Aging or senescence is a post-maturational process that occurs at all levels of biological organizations. It is associated with a progressive decline in a variety of physiological functions including the immune-responsivity (Hakim et. al., 2004). There is strong evidence that a poorly functioning immune system can contribute to decreased disease resistance and reduced life expectancy in the elderly (Wayne et al., 1990). The composition of the gastro-intestinal (GI) microflora also changes with increasing age of the host. These changes involve a decrease in the number and diversity of bifidobacteria and bacteroides and an increase in the number of enterobacteria, clostridia, streptococci, and enterococci in elderly humans and in old animals (Azizpour et al., 2009).

The age-related changes in the GI bacterial community in elderly humans are likely related to changes in nutritional habits (Lesniewska et al., 2006). Therefore, an attractive mean of restoring immune function is dietary intervention. Ouwehand et al., (2008) reported that probiotic administration has been associated with enhanced immune function in elderly subjects, and the changes observed in the immune parameters correlated with changes in the intestinal microbiota composition. Further confirmation of enhanced immunity and increased resistance to infection has been demonstrated in both humans and animals (Orrhage et al., 1994; Yasui et al., 1992).

Strains belonging to the genus *Bifidobacterium* are seldom found in food products (Bergonzelli et al., 2005). Recently, they are incorporated in yoghurt manufacture (along with yoghurt organisms) and cheese manufacture because of their health and therapeutic benefits (Clemente, 2012). Nevertheless, approaches for selection of an ''ideal'' strain of bifidobacteria are still difficult and indeed require considerable resources. Consequently, the aim of the present study was to investigate the possible modulatory effects of three strains of bifidobacterial species (*Bifidobacterium adolescentis* ATCC 15704, *Bifidobacterium breve* ATCC 15700 and *Bifidobacterium longum* ATCC 15707) on haematological and immunological parameters of aged albino rats corresponding to normal adult ones. These strains were individually incorporated in yoghurt manufacture along with yoghurt bacteria (starter) in order to assess the relative elder consumer interesting health enhancing properties of such probiotics.

Materials and methods

Animals

Twenty adult male albino (*Rattus norvgicus*) rats of 8–12 months old, weighing from 95 to 120 g, and 140 aged male albino rats (24–30 months), weighing from 350 to 400 g were obtained from the animal house of the National Institute of Cancer (Cairo, Egypt). All animals were weighed, single-housed in isolated polypropylene cages, and provided with diet and water *ad libitum* under good hygienic laboratory conditions. They were maintained for two weeks as an acclimatization period before the beginning of the experiment.

Bacterial strains and yoghurt manufacture

Yoghurt starter culture *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were obtained from Dairy Science Department, Faculty of Agriculture, Minia University. Three bifidobacteria strains (*adolescentis* ATCC 15704, *B. breve* ATCC 15700 and *B. longum* ATCC 15707) were obtained from the Cairo Microbiological Resource Center, Faculty of Agriculture, Ain Shams University. The cultures of both yoghurt starter and *Bifidobacteria* strains were prepared as previously mentioned by Anter et al. (2010). Yoghurt was then stored in the refrigerator to restrict the activity of the starter cultures and prevent the development of access acidity. On the next day, yoghurt was supplied to rats as 95 parts plus five parts of the water together with diet and water for 30 consecutive days. Feeding cups were exchanged daily.

Experimental design

Two groups of 10 adult and 10 aged rats were provided with normal diet and water *ad libitum* for 30 days and assigned as normal groups. Sixty of the aged rats were divided into four groups, each of 15 rats, and treated as follows: the first group was fed yoghurt starter culture (*S. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) and considered as a control group. Each of the remaining three groups was fed yoghurt fermented with starter strains (*S. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus*), in addition to one of the bifidobacteria strains (*B. adolescentis*; *B. breve*; *B. longum*) for 30 consecutive days. Weight of rats, in all groups, was monitored once weekly during the experimental period. The experiment was repeated once more for the confirmation of the results.

Collection of samples

At the end of the experimental feeding period, adult and aged animals of all groups were fasted for 16 h and then sacrificed under deep anaesthesia; Blood samples were collected individually from the jugular vein of each rat in heparinized and non-heparinized tubes for measuring haematological and immunological parameters respectively.

Haematological studies

Haemoglobin concentration (gm/dl) as well as total number of RBCs and WBCs was determined using standard protocols according to Dacie and Lewis (1991). For differential leucocytes count, thin blood films were prepared, allowed to dry rapidly in air, fixed in methanol and stained with Giemsa. The slides were examined microscopically and the percentage of each type of leucocytes in relation to the total number of leucocytes was calculated. Any remarkable morphological features recorded in different leucocyte types were registered and photographed.

Immunological studies

Blood samples were collected in sterile tubes without anticoagulant, left for clotting and centrifuged at 3000 rpm for 15 min for separating sera. The obtained serum samples were analysed for measuring the following immunological parameters:

1- Determination of the immunoglobulins A (IgA) and E (IgE)

Serum Ig A and Ig E concentrations were measured in rats of all experimental groups by Enzyme-Linked Immune Sorbent Assay (ELISA) kits (CellTrend Company, Egypt). The results were expressed as ng/ml.

2- Determination of the cytokine tumour necrosis factor-alpha (TNF-α) and interleukin-8 (IL-8)

Part of the serum samples collected from rats of all tested groups were used for the determination of TNF-α and IL-8 concentrations by ELISA using TNF-α immunoassay (Koma Biotech Inc.) and the Quanti Kin Mouse IL-8 (Cusabio Biotech co., Ltd.) kits according to the manufacture's manual. Results were expressed as pg/ml for both measured cytokines.

Statistical analysis

The data of all groups were represented as means ± standard error (SE) and were statistically analysed using the *T* test and one and two way analysis of variance (ANOVA test using the SPSS program version 10.0). For all statistical tests, differences were considered to be significant if $P < 0.05$.

Results

Haematological studies

Red blood cells (RBCs) number and haemoglobin (Hb) concentration

No changes were registered in the total number of RBCs or in the concentration of Hb of aged rats after feeding them with yoghurt inoculated with each of the studied *Bifidobacterium* strains (*B. adolescentis* ATCC 15704, *B. breve* ATCC 15700 and *B. longum* ATCC 15707; (Table 1).

Total leucocyte (WBCs) number

The total leucocyte (WBCs) number of normal adult rats was $6 \pm 0.4 \times 10^3$ cell/mm^3. This number was significantly decreased in both normal and control aged rats. On the other hand, it was significantly increased in the three bifidobacteria-treated aged groups as compared to both normal and control aged rats. It was clear that rats fed yoghurt inoculated with *B. adolescentis* showed the best results (Table 1). However, when comparing the recoded values of total leucocyte count in the three treated aged groups with normal adult animals, a significant decrease was observed in WBCs of all rats. Again the *B. adolescentis* group recorded the nearest mean value to those of rats of the adult group (Table 1); Fig. 1).

Differential leucocyte count

Lymphocytes were the prevalent type of leucocytes in blood films of normal adult, normal aged and control aged rats representing mean percentages of 68.50 ± 1.241, 67.70 ± 1.461 and 68.10 ± 1.859, respectively. A significant decrease was recorded in the percentages of lymphocytes in all treated aged rats compared to normal adult as well as normal and control aged animals, exhibiting the lowest value of 48.30 ± 2.716 in rats fed yoghurt inoculated with *B. adolescentis* (Fig. 2).

Neutrophils represent the second predominant leucocytes' type in blood films of normal adult, normal aged and control aged rats showing a mean percentages of 28.10 ± 1.394, 30.80 ± 1.526 and 28.80 ± 1.625, respectively. They exhibited a significant increase in rats of all treated groups compared to normal and control groups; the *B. adolescentis* group, however, showed the highest mean percentage (44.00 ± 2.646) followed by *B. breve* and *B. longum* (Fig. 3).

Similarly, monocytes of treated rats recorded a significant increase when compared with normal and control groups (adult and aged; Fig. 4). Both eosinophils and basophils were not detected in the examined blood films of both adult and aged albino rats.

Moreover, blood films of normal adult rats exhibited normal morphological appearance of erythrocytes, lymphocytes, monocytes and neutrophils (Fig. 5). On the other hand, normal and control aged rats showed the existence of some morphological alterations in some leucocytes, where many hypersegmented neutrophils were seen (Fig. 6). The most noticeable observation was the detection of band neutrophils and leucocytes with ring-shaped nuclei in both normal and control aged rats (Figs. 6 and 7). After feeding aged rats with yoghurt inoculated with one of the bifidobacterium strains, the number of ring cells was reduced (Fig. 8), but some cells with nuclear features between band and segmented neutrophils were observed (Fig. 9 and Fig. 10).

Immunological studies

Table 2 shows immunological effects after supplementation of aged rats with yoghurt fermented with yoghurt starter (*S. sal-*

Table 1 Changes of the total red blood cells counts (cell × 10^6/mm^3), total Hb concentration (gm/dl) and total leucocyte counts (cell × 10^3/mm^3) of normal (adult and aged) rats, control aged rats and treated (administered bifidobacteria for 30 consecutive days) aged rats.

		N. adult	N. aged	C. aged yoghurt	*B. adolescentis*	*B. Breve*	*B. longum*
RBC count	Mean ± SEM	457.4 6.60	446.3 8.15	450 6.82	440.8 8.64	448.8 7.22	444.9 7.35
Hb concentration	Mean ± SEM	13.53 0.167	13.51 0.187	13.43 0.225	13.47 0.129	13.87 0.183	13.58 0.207
WBC count	Mean ± SEM	6.00 0.4006	2.24[a] 0.8718	2.37[a,b] 0.9354	5.56[a,b] 0.5322	4.65[a,b] 0.170	4.88[a,b] 0.7125

Values are mean ± SEM. N., normal; C., control; B., *Bifidobacterium*.
[a] $P < 0.05$ vs normal adult rats.
[b] $P < 0.05$ vs normal aged animals.

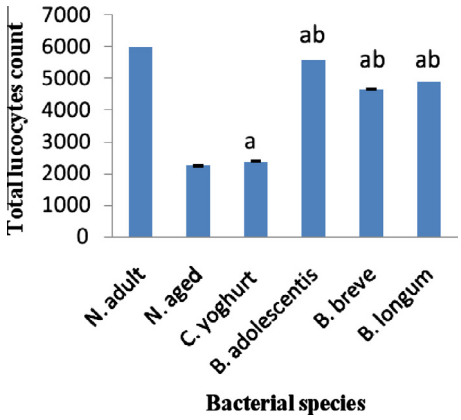

Figure 1 Changes in the total leucocyte number of normal, control, and bifidobacteria-treated rats. Values are mean ± SEM. N., normal; C., control; B., Bifidobacterium. a = $P < 0.05$ vs normal adult rats; b = $P < 0.05$ vs normal aged animlas.

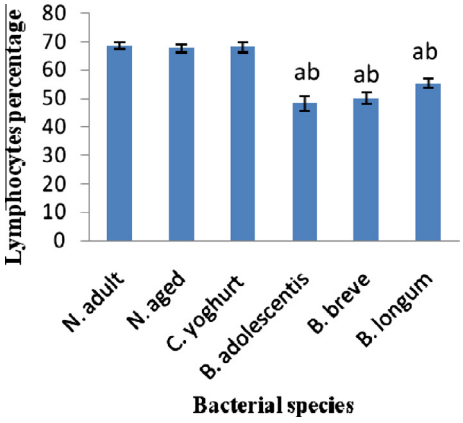

Figure 2 Changes in lymphocyte percentages of normal, control, and bifidobacteria-treated rats. Values are mean ± SEM. N., normal; C., control; B., Bifidobacterium. a = $P < 0.05$ vs normal adult rats; b = $P < 0.05$ vs normal aged animlas.

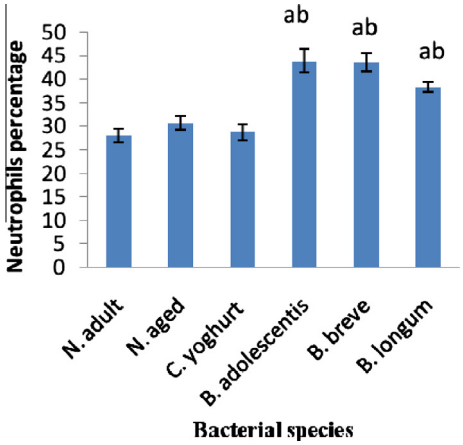

Figure 3 Changes in neutrophil percentages of normal, control, and bifidobacteria-treated rats. Values are mean ± SEM. N., normal; C., control; B., Bifidobacterium. a = $P < 0.05$ vs normal adult rats; b = $P < 0.05$ vs normal aged animlas.

ivarius subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) alone (control group) or inoculated with one of the three

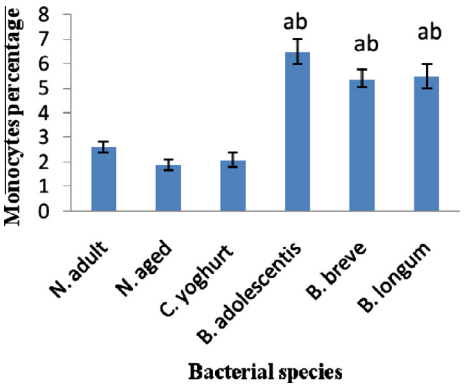

Figure 4 Changes in monocyte percentages of normal, control, and bifidobacteria-treated rats. Values are mean ± SEM. N., normal; C., control; B., Bifidobacterium. a = $P < 0.05$ vs normal adult rats; b = $P < 0.05$ vs normal aged animlas.

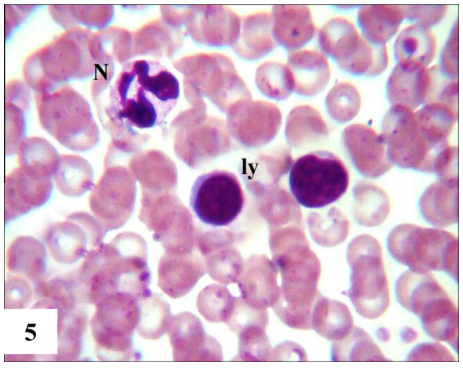

Figure 5 Stained blood film of a normal adult rat, showing normal erythrocytes, lymphocyte (ly) and neutrophil (N) (Giemsa stain, ×1000).

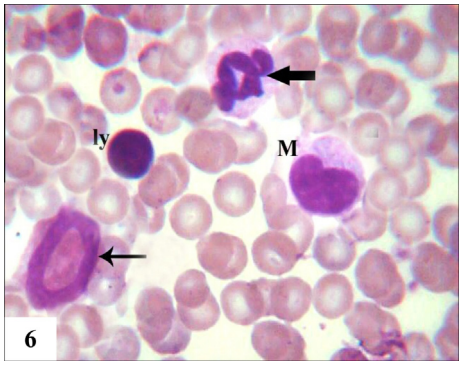

Figure 6 Stained blood film of a normal aged rat showing normal lymphocyte (ly), monocyte (M), hypersegmented neutrophil (thick arrow) and leucocyte with ring-shaped nuclei (thin arrow) (Giemsa stain, ×1000).

studied *Bifidobacterium* strains (*B. adolescentis* ATCC 15704, *B. breve* ATCC 15700 and *B. longum* ATCC 15707) in comparison with normally-fed adult ones.

1- Changes in IgA and IgE concentrations

Data of Table 2 show that serum IgA mean value of normal adult rats was 670.3 ± 34.17 ng/ml. This value was signifi-

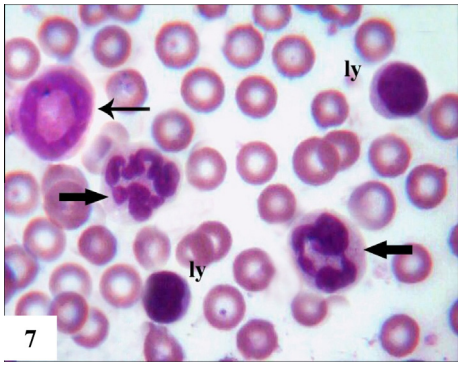

Figure 7 Stained blood film of a control aged rat fed yoghurt showing normal lymphocyte (ly), hypersegmented neutrophil (thick arrows) and ring cell (thin arrow) (Giemsa stain, ×1000).

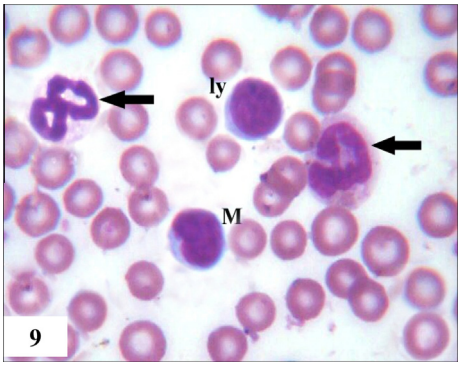

Figure 9 Stained blood film of an aged rat fed yoghurt inoculated with *B. breve* showing large lymphocyte (ly), rnonocyte (M) and cells in different stages between band and segmented neutrophil (thick arrows) (Giemsa stain, ×1000).

cantly decreased in normal aged rats reaching a mean value of 401.7 ± 24.93 ng/ml. On the other hand, aged rats of the control group exhibited slight a non-significant increase in IgA with a mean value of 416 ± 30.54 ng/ml. However, after administration of bifidobacteria the mean values of serum IgA were considerably increased. In this case, rats supplemented with yoghurt inoculated with *B. adolescentis* showed the most significant higher IgA mean value as compared with normal aged rats followed by *B. breve* and *B. longum*, respectively. Interestingly, it was apparent that only *B. adolescentis* had the most pronounced effect on aged rats to regain nearly normal IgA level as measured in normal adult rats.

Serum IgE levels were significantly increased in normal aged rats when compared with those of the corresponding normal adult animals. In contrary, serum IgE levels were decreased in both control and bifidobacteria-treated aged rats compared to normal aged ones. However, this decrease was significant in rats supplemented with yoghurt inoculated with *B. adolescentis* or *B. breve*, where the lowest values of IgE levels were recorded in the *B. adolescentis* group followed by the *B. breve* group. In this respect it is noteworthy to mention that *B. adolescentis* showed the most pronounced effect on decreasing body secreting IgE, approaching a value very close to that estimated in normal adult rats.

2- Changes in serum TNF-α and IL-8 concentrations

Rats consumed yoghurt inoculated with each of the studied bifidobacteria species, showed noticeable enhanced production of TNF-α in comparison to normal aged rats as well as control aged rats (Table 2). Nevertheless, this enhanced production was only significant in both *B. adolescentis* and *B. breve* groups, respectively. However, *B. adolescentis* was the only probiotic that can regain the production of TNF-α to nearly reach the normal level as estimated in adult rats.

In addition, it was obvious that the production of the cytokine IL-8 was increased in all groups of treated aged rats as compared to normal aged ones. Then again, among the tested *Bifidobacterium* species, the only species that can induce significant increased production of IL8 to reach nearly the normal levels registered in normal adult rats was *B. adolescentis*; both *B. breve* and *B. longum* showed non-significant increased values.

Discussion

The data presented herein demonstrate that both normal and control aged rats exhibited a significantly decreased number of WBCs in comparison with adult animals. According to Ogawa et al. (2000) and Hakim et al. (2004), cells of immune

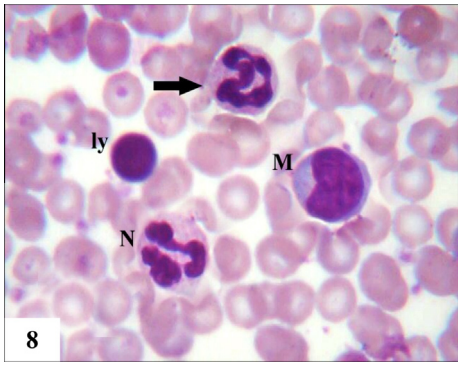

Figure 8 Stained blood film of an aged rat fed yoghurt inoculated with *B. adolescentis* showing normal lymphocyte (ly), neutrophil (N), monocyte (M) and hypersegmented neutrophil (thick arrow) (Giemsa stain, ×1000).

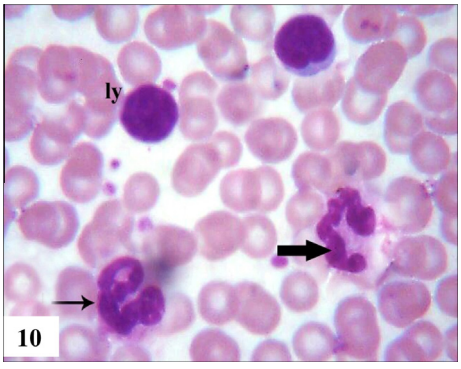

Figure 10 Stained blood film of an aged rat fed yoghurt inoculated with *B. longum* showing lymphocyte (ly), nearly hypersegmented neutrophil (thick arrow) and nearly band neutrophil (thin arrow) (Giemsa stain, ×1000).

Table 2 Measurements of serum immunoglobulins (IgA and IgE), as well as TNF-Alpha and IL-8 of normal (adult and aged) rats, control (fed yoghurt) aged rats and treated (fed yoghurt fermented with bifidobacteria for 30 consecutive days) aged rats.

Groups		IgA ng/ml	IgE ng/ml	TNF-alpha pg/ml	IL8 pg/ml
N. adult	Mean ± SEM	670.3 34.17	58.8 5.30	1551.9 84.22	964 49.38
N. aged	Mean ± SEM	401.7[a] 24.93	91.1[a] 3.85	870.38[a] 32.14	729.6[a] 22.96
C. aged yoghurt	Mean ± SEM	416.8[a] 30.54	83.7[a] 4.26	904.5[a] 26.97	748.8[a] 43.86
B. adolescentis	Mean ± SEM	689.3[b] 35.29	50[b] 3.29	1669.5[b] 73.78	972.9[b] 65.34
B. breve	Mean ± SEM	557[a b] 7.77	69.1[b] 1.48	1220.8[b] 65.42	816.9[a] 47.07
B. longum	Mean ± SEM	465.9[a] 43.61	78.8[a] 7.00	1048.5[a] 58.33	754.9[a] 51.06

Values are mean ± SEM. N., normal; C., control; B., *Bifidobacterium*.
[a] $P < 0.05$ vs normal adult rats;
[b] $P < 0.05$ vs normal aged animals. See text for more details.

system are constantly renewed from haematopoietic stem cells. With age, a reduction in the overall capacity for renewal of these cells has observed. This may explain the recorded leucocyte depletion in aged rats compared with adult ones. Nilsson-Ehle et al. (1995) and Ogawa et al. (2000) stated that the proliferative activity of bone marrow peaks in the middle age (adult) and then gradually decreases, but reduction in marrow cellularity is found only with extreme ages. They added that such reduction, perhaps associated with increased apoptosis. Insufficient haematopoiesis in bone marrow stroma to support the circulating blood with renewed cells can led to an increase in the proportion of less mature leucocytes such as band neutrophil (Cornbleet, 2002). Thus, some of the deficits of immunosenescence begin with stem cells, which in turn reflect on leucocyte number and abnormalities in circulating blood as has been observed in aged rats in the present study.

Immunosenescence can be expressed by changes in the function or proportions of leucocytes that contribute to innate immunity, such as phagocytes and natural killer (NK) cells (Butcher et al., 2000). In *in vitro* studies, activities of both polymorphonuclear and mononuclear phagocytes were diminished in elderly subjects (McLachlan et al., 1995; Wenisch et al., 2000), mostly through a decline in opsonin-mediated phagocytosis (Butcher et al., 2000). Nevertheless, a decline in innate immune cell function is generally considered to be a contributing factor to decreased immunity in the elderly (Butcher et al., 2000). Moreover, because both NK cells and phagocytes (particularly monocytes) secrete many immunoregulatory cytokines, their potentially diminished function in elderly individuals may have important downstream effects on immune events in the integrated immune system, such as lymphocyte activation and differentiation. Accordingly, interventions that can combat immunosenescence by restoring cellular immune function are highly desirable (Gill et al., 2001).

Administration of specially selected lactic acid bacterial (LAB) strains could potentially correct immune defects seen in the elderly and neonates (Gill et al., 2000; Miller, 1979), whose lower numbers of polymorphonuclear cells have decreased phagocytic activity. Furthermore, several strains of LAB were reported to display stimulatory properties on cells of the innate immune system *in vitro*, including macrophages and NK cells (Mishra et al., 2008).

In the present work, feeding aged rats with selected *Bifidobacterium* strains increased the number of WBCs, especially in rats fed *B. adolescentis*. Such increase appeared to be mainly due to the increase in the number of neutrophils and monocytes. Similar results were obtained by Pollmann et al. (1980) who observed that the number of leucocytes in the blood was increased by administration of *Lactobacillus acidophilus* to piglets. In addition, Ebaid and Hassanein (2007) demonstrated that WBC number was increased in adult rats that administered yoghurt fermented with *B. infantis, B. longum, B. adolescentis, B. bifidum* and *B. breve*. They also stated that the proportion of monocytes was significantly elevated in the *B.* adolescentis group, but the change in lymphocytes percentage was not significant. Previous investigations can be extended to confirm the present results in view of the observation that oral administration of *L.acidophilus* or *B. bifidum* to healthy volunteers enhanced the non-specific phagocytic activity of peripheral blood cells. Although both granulocytes and monocytes are the targets for this effect, the global enhancement in phagocytic activity was considered to be mainly due to the granulocyte population, which constitutes a greater proportion of the blood cells and exhibited a greater increment in phagocytic activity (Schiffrin et al., 1995). Moreover, Gill et al. (2001) reported that two 3-week intervention trials have shown that *Bifidobacterium lactis* HN019 supplementation (10^9–10^{10} cfu/d) increases the phagocytic capacity of monocytes and polymorphonuclear cells and the NK cell tumouricidal activity in elderly subjects.

Ouwehand et al. (2002) reported that the intestine is the largest immune organ of the body, where it produces more antibodies than any other part of the body and contain 80% of all antibody-producing cells. They added that the immune system regulates the colonization of the intestinal microflora by interfering with its ability to bind to the mucosa, while parts of bacterial cells and metabolites modulate the activity of the immune system. The principle antibody in the intestine is the immunoglobulin A (IgA). Moreover, Helgeland and Brandtzaeg (1999) postulated that, in contrast to IgG, IgA does not elicit an inflammatory reaction. Thus IgA can bind antigens and exclude them from the intestinal mucosa without causing inflammation. Furthermore, the predominant sites of antigen sampling in the intestine are Peyer's patches (PPs) which constitute parts of the gut-associated lymphoid tissues. PPs are covered with specialized membrane (M) cells. These cells specifically sample the contents of the gut and transfer antigens to antigen presenting cells, which present the antigen to B and T cells. Naïve T cells develop into T helper 1 (Th1) or T helper 2 (Th2) cells. Th1 cells will direct the differentiation of B cells to IgA producing cells, while Th2 cells direct B cell differentiation towards IgE producing cells. Interestingly, M

cells have a preference for the uptake of IgA-complexed antigens, thus stimulating the production of IgA (Ouwehand et al., 2002; Zahran et al., 2007).

In this study, serum IgA level decreased significantly in normal and control aged rats compared with that of adult animals. After feeding the aged rats with *Bifidobacterium* species, serum IgA level increased significantly especially in *B. adolescentis*-fed rats. In contrary, serum IgE level decreased in treated groups compared to normal aged rats. This decline was significant in both *B. adolescentis* and *B. breve* groups only. It was established that aging is accompanied by a reduction in the functional capacity of all the organs in the body and accordingly the activity of the immune system also declines with age. The senescence of the immune system, especially, affects cell-mediated immunity with a decrease in lymphocyte proliferation capacity and IL-2 production (Chandra, 2002).

These results provide additional support to those previously-mentioned reports, demonstrating that IgA plays a significant role in the local immune system which has to deal with food antigens as well as harmful bacteria and viruses (Underdown, 1986). Exposure to food antigens can sometimes cause the development of food allergies (Sampson, 1992). Interestingly, IgA secreted in the intestine is the first defense against the exposure to orally fed food antigens, and IgA secreted in breast milk protects infants from allergenic sensitization by food antigens (Machinger and Moss, 1986). Therefore, this would suggest that bifidobacteria may have a potential to contribute to the prevention of allergy by stimulating the production of IgA to food antigens both in milk and the intestine.

Close contact of the studied three *Bifidobacterium* species with the intestinal mucosa may lead to enhance interaction of the probiotics and the intestinal immune system. According to Ouwehand et al. (2002), this interaction stimulates naïve T cells to differentiate to Th1 cells under the influence of IFN-γ, IL-2 and IL-12, while the development of Th2 cells is down regulated under the influence of IL-4. The result of this shift in T- cell differentiation from Th2 cell to Th1 cell is a reduced production of IgE and an increased secretion of IgA, which leads to a reduced allergic response. Such suggestion is coincident with the results of the current study. Several lines of evidence indicate that feeding probiotics appears to be a factor in enhancing IgA. For example, Delcenserie et al. (2008) demonstrated that probiotics can enhance humoral immune responses by increasing IgA producing cells. In addition, Fukushima et al. (1998) suggested that ingestion of probiotic (bifidobacteria; *B. lactis* Bb12) formula stimulate the production of IgA in the gastrointestinal tract of healthy children.

These results are reasonable because Schiffrin et al. (1995) and Gill et al. (2000) showed that yoghurt containing Lactobacillus (acidophilus, casei, plantarum, delbrueckii, gasseri) and Bifidobacterium (longum, bifidum, adolescentis, infantis) produce certain bioactive peptides, which stimulate the proliferation and maturation of T lymphocytes and improve immunity by increasing the number of IgA through producing plasma cells.

There is accumulating evidence that interactions of LAB and their products with immunocompetent cells such as macrophages and T-cells can lead to the production of cytokines which have a manifold effect on immune and non-immune cells. The intestinal epithelium cells are able to produce, *in vitro,* a wide range of pro-inflammatory cytokines such as IL-8 and TNF-α if they are influenced by invasive pathogenic bacteria (Jung et al., 1995). IL-8 is chemokine that attracts and activates neutrophils and monocytes. TNF-α activates immune and inflammatory cells (Galli et al., 1991). Interestingly, IL-8 appears to be a major cytokine produced by enterocytes following an encounter with a probiotic organism. The IL-8 cytokine primarily functions as a neutrophil chemoattractant.

In the current study, aged rats fed bifidobacteria showed noticeable enhanced production of TNF-α in comparison to both normal and control aged rats, but it was only significant in *B. adolescentis* and *B. breve* groups. However, *B. adolescentis* was the only probiotic that can regain the production of TNF-α to nearly reach the normal levels as estimated in adult rats. These results are consistent with those obtained by Ebaid and Hassanein (2007) who reported a significant increase in pro-inflammatory cytokine secretion (TNF-α) in adult rats fed *B. adolescentis* compared to those fed either *B. breve* or *B. infantis*. Furthermore, this study revealed that the production of the cytokine IL-8 increased in bifidobacteria-treated aged rats, especially in the *B. adolescentis* group. These results are in agreement with data reported in healthy adults, where a variety of experimental approaches *in vivo* and *in vitro* have been followed in order to test the effects of LAB on cytokine production by immune cells. For instance, *L. sakei* induces the expression of IL-1β, IL-8 and TNF-α, and this process appears to require cross-talk between the epithelial cells and the underlying leucocytes (Haller et al., 2000). In addition, the incubation of PBMCs from healthy young adults with *L. casei, L. acidophilus* or *Bifidobacterium*, *in vitro*, induced an enhancement of TNF-α production (Solis-Pereyra et al., 1997). However, such results are somewhat different from what have been reported in the study of Ouwehand et al. (2008) where intervention with *B. longum* 2C (DSM 14579) and 46 (DSM 14583) did not influence the levels of TNF-α and IL-10 in elderly subjects. On the other hand, Zhang et al. (2005) stated that quality and dose of probiotic preparations may impact the IL-8 production by enterocytes. Besides, they demonstrated that probiotic strains differ in their capacity to augment IL-8 expression, however, and some strains seem to rather decrease epithelial-cell production of IL-8.

Taken together, the results of the present work suggest that dietary consumption of selected bifidobacteria strains, especially *B. adolescentis*, in a yoghurt-based diet may offer benefit to elderly consumers to prevent some of the harmful effects of immunosenescence. They add to the accumulating evidence that functional foods such as probiotic products may have a particular application in the elderly who are a high-risk group, especially in terms of immunomodulation, protection against pathogens, and perhaps also in the prevention of several age-related diseases.

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