Bifidobacteria: their significance in human intestinal health

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INTRODUCTION

Discovery and properties of *Bifidobacterium*

Bifidobacterium was first isolated in 1899 from a healthy breast-fed infant by Tissier of the Pasteur Institute in France. It is an anaerobic, Gram-positive, non-sporeforming, pleomorphic rod, and was originally named *Bacillus bifidus communis.* The name signifies the branching morphology of the bacteria; bifidus in Latin meaning cleft in two parts. Later, the bacterium was once placed within the genus *Lactobacillus as L. bifidus.* In 1960s, it was accepted as an independent genus and classified as *Bifidobacterium.* Apart from the properties mentioned above, the main phenotypic characteristics of *Bifidobacterium* is producing lactic acid and acetic acid as the main products of glucose utilization.

Bifidobacterium species and classification

There are currently about 30 known *Bifidobacterium* species. The *Bifidobacterium* species that inhabit the human intestinal tract are rather distinct from those that inhabit the intestines of animals (Mitsuoka, 1984). The representative species of human origin include *B. longum, B. breve, B. infantis, B. bifidum, B. adoltescentis* and *B. pseudocatenulatum.* Representatives of animal-derived species include *B. pseudolongum, B. thermophilus* and *B. animalis.* Species of animal origin are never isolated from the human intestinal tract and human origin species are almost never found in animal intestines. The reason for this host-specificity is unknown, but is suspected to be due to differences among species in the ability to colonize the host intestinal tracts. Among the *Bifidobacterium* species used in various yogurts, *B. animalis* is often identified (Yaeshima et al, 1996). The reason for using this species is that animal-derived species are more acid-resistant and therefore preserve better in the yogurt. Recently, the *B. animalis* strains isolated from these yogurts have been found to have some genetic differences compared to the type strain of *B. animalis,* and these strains have been named *B. lactis* (Meile *et al,* 1997). This species is also not an inhabitant of the human intestinal tract. The use of human-origin species as food supplement seems to be the reasonable and correct choice.

Ecology in the human intestinal tract

Before birth, the human foetus is germ-free and intestinal bacteria do not exist. From the time of birth, bacteria begin to colonize the intestinal tract forming the intestinal microflora. At birth, many bacterial species gain access into the intestinal tract, but bifidobacteria gradually become established as the main bacteria, and predominate in the intestinal microflora during the neonatal period. This tendency is especially marked in breast-fed infants. According to a study,

bifidobacteria constitute over 95% of the intestinal flora in breast-fed infants (Yoshioka et al, 1991). The increased resistance to infection in breast-fed infants may partly be attributed to the predominating bifidobacteria in the intestinal microflora. The number of bifidobacteria in bottle-fed infants is lower than that in breast-fed infants. Nevertheless, even in bottle-fed infants, bifidobacteria remain to be the predominant bacteria. Bifidobacteria which are dominant during infancy gradually decrease in number from the time of weaning, and bacteria such as *Bacteroides* and *Eubacterium* become predominant. The changes in bifidobacteria with age are shown in Figure 1 (Mitsuoka, 1978). Even in adults, *Bifidobacterium* is one of the main and important component of the intestinal microflora. The number of bifidobacteria is further reduced during old age, accompanied by increases of *Clostridium* and other species. Figure 2 compares the electron micrographs of the faeces from a breast-fed infant and an adult. The bacteria seen in the faeces of the breast-fed infant are almost exclusively bifidobacteria and no other bacteria are observable. In the faeces of the adult, however, a wide variety of bacterial types are observed such as rods and cocci, large and small, but there are very few bacteria with the morphology of bifidobacteria. This difference in microflora is also observed by culturing faeces. The intestinal microflora changes with age, accompanied by a reduction of bifidobacteria. The effects of changes of the intestinal microflora on the intestinal environment are shown in Table 1. The concentrations of intestinal putrefactive products such as ammonia and indole and the activities of enzymes involved in the production of these substances are extremely low in breast-fed infants compared to adults. Although these changes cannot be attributed entirely to the differences in the intestinal microflora, a strong effect can be expected.
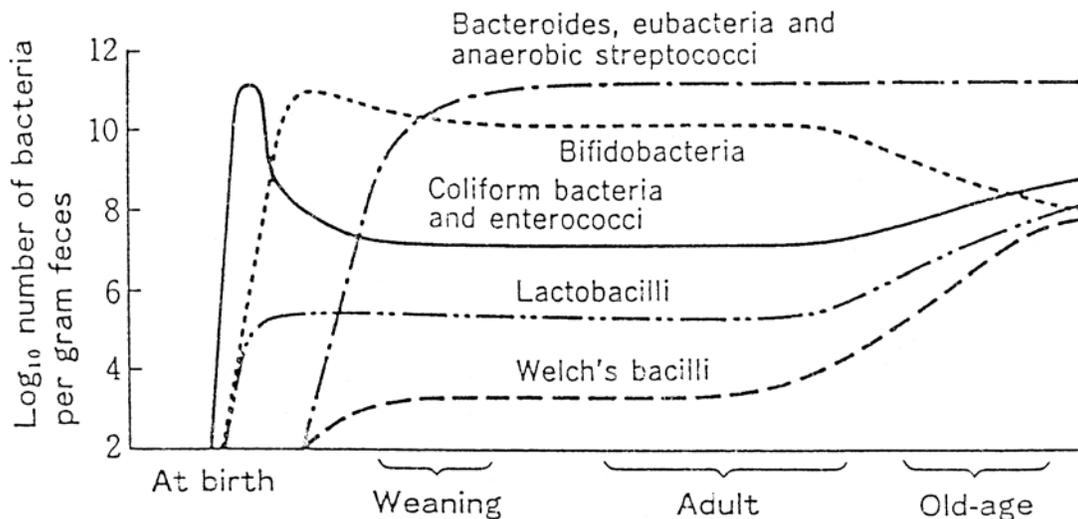
Figure 1. Change of intestinal flora with age (Mitsuoka 1978)

The intestinal flora of an adult is composed of approximately 100 species of bacteria, which are present at a level of 10^{10} to 10^{11} per gram of colonic content (Figure 3). These intestinal bacteria can be classified into 3 groups depending on their effect on the intestinal environment; that is, beneficial bacteria, harmful bacteria and bacteria exhibiting an intermediate property. Harmful bacteria are those that possess pathogenicity or transform food components into harmful substances, and they include *Clostridium, Veillonella, Proteus* and the *Enterobacteriaceae* family. Beneficial bacteria represented by *Bifidobacterium* and *Lactobacillus* suppress the harmful bacteria and exert many beneficial physiological effects. They have no harmful effect
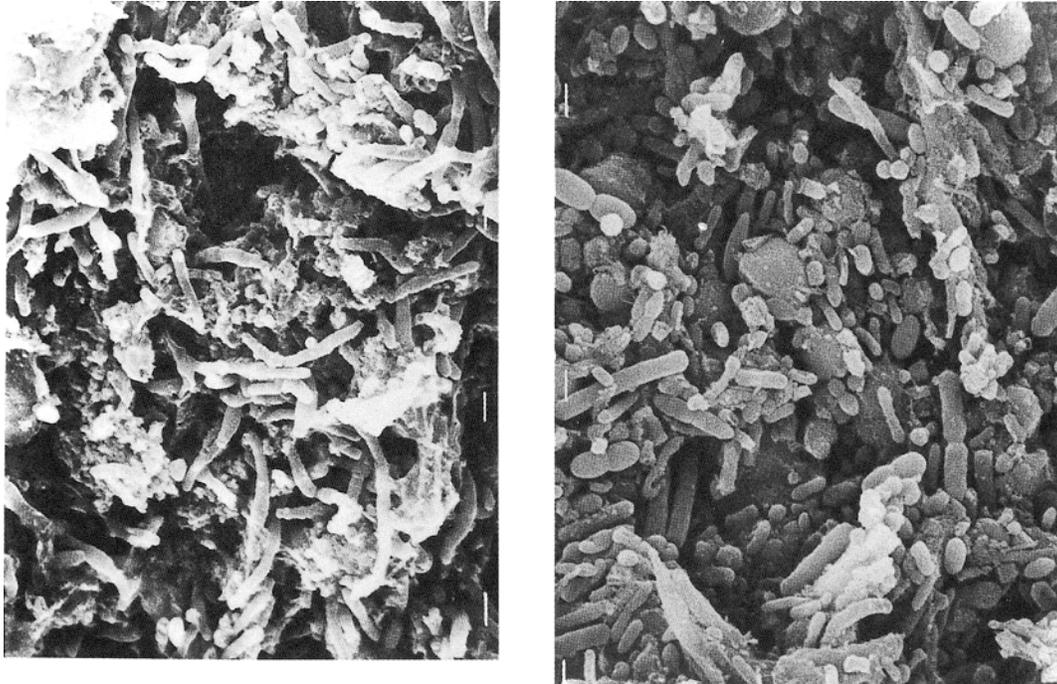
Figure 2. Electron micrographs of infant (A) and adult (B) faeces
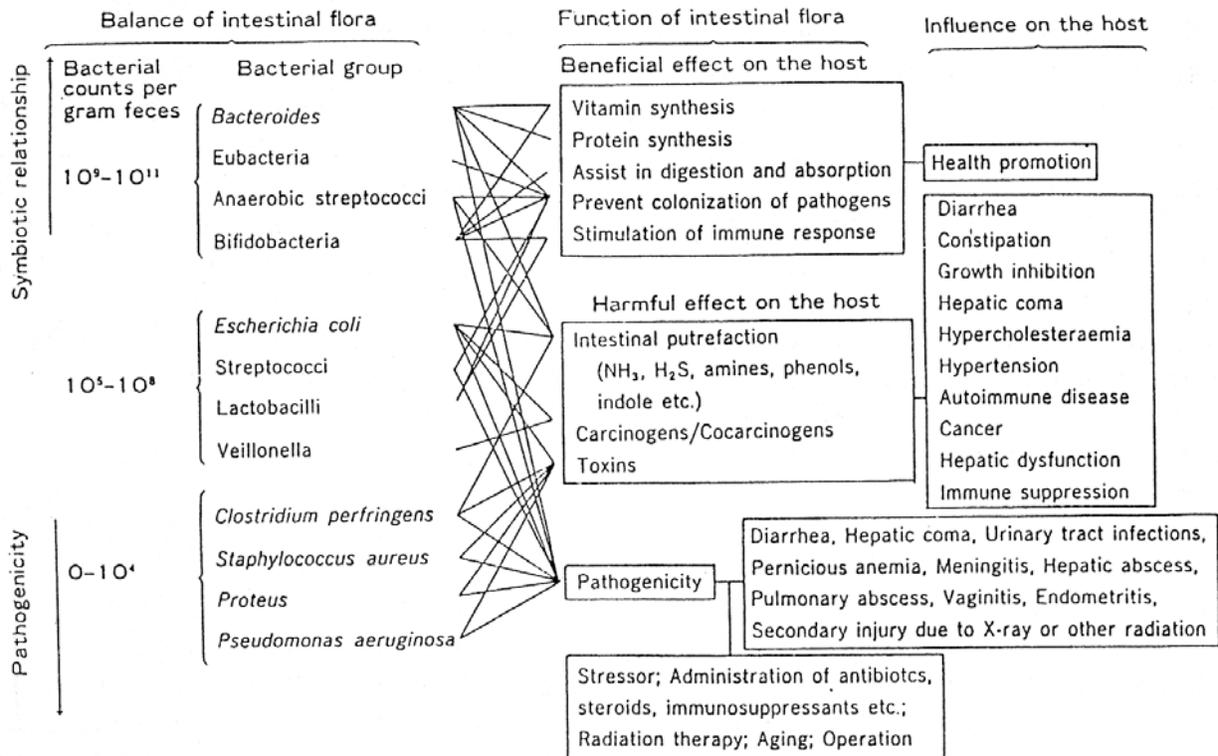
Figure 3. Interrelationships between intestinal flora and the human health (Mitsuoka 1978)

whatsoever on the host. In the large intestine, the number of *Lactobacillus* is approximately 1/100 that of *Bifidobacterium,* and the influence on the intestinal environment is less than that of *Bifidobacterium.* Finally, *Bacteroides, Eubacterium* and anaerobic streptococci belong to the intermediate group. These bacteria do not show any virulence under normal conditions, but they may cause opportunistic infections when the host immunity or resistance is lowered. Figure 3 shows the interrelationship between intestinal bacteria and human health. Many kinds of bacteria produce harmful substances such as ammonia, amines, hydrogen sulfide and indole from proteins. Consumption of fats increases bile acid secretion, and bile acids are converted by many intestinal bacteria into secondary bile acids which are supposed to have a carcinogenic potential. These show that intestinal bacteria have great influences on the intestinal environment, and the intestinal environment in turn influences the health of the host. The levels of bacteria in the intestinal tract change due to factors such as food and living environment and they are competing with each other, resulting in change of the intestinal microflora. A favorable intestinal microflora can create a healthy intestinal environment. A favorable intestinal microflora consists of a low level of harmful bacteria and a high level of beneficial bacteria represented by bifidobacteria.

Table 1. Faecal characteristics of Infant and Adult

	Infant (3 months)	Adult (23 ages)
Bifidobacteria (%)*	96%	19%
pH	5.30	6.4
Ammonia (μ mol/g)	13.20	54.0
Indole (μ g/g)	0.76	56.2
p-cresol (μ g/g)	ND	68.7
Phenol (μ g/g)	0.72	7.6
Urease**	22.50	911.0
Tryptophanase**	0.33	3.7
ß-glucronidase**	2.09	33.4

*   Bifidobacteria percentage in fecal microflora
**   Enzyme activity: μ mol/h/g

Physiological effect of bifidobacteria

This section will describe the actual physiological effects of bifidobacteria. First, the most important physiological effect is the action of intestinal conditioning. The intestinal conditioning action is an overall effect of improving the intestinal environment, which include improvement of the intestinal flora, inhibition of intestinal putrefactive substances, and improvement of the faecal properties and defecation state (alleviation of constipation or diarrhea). As mentioned earlier, in addition to lactic acid, bifidobacteria produce acetic acid with a strong bactericidal action, which has been shown to suppress harmful bacteria. This effect has also been demonstrated in vitro (Araya-Kojima *et al,* 1995). Table 2 shows the changes of the relative share (percentage) of *Bifidobacterium* and other main bacteria in the intestinal flora after consuming a bifidobacteria-containing yogurt (*B. longum* BB536) compared to a standard yogurt containing no bifidobacteria (Yaeshima, 1997). The proportion of *Bifidobacterium* in the intestinal microflora is increased significantly by consumption of the *B. longum* BB536 yogurt. A simultaneous decrease in ammonia, which is a representative putrefactive product, is observed (Figure 4). Figure 5 shows the effect of consuming this yogurt on the frequency of defecation in

subjects with a constipation tendency. A significant increase in frequency of defecation can be observed after ingestion of the bifidobacteria yogurt. Administration of bifidobacteria [*B. breve* M-16V (Akiyama *et al,* 1994), B. longum BB536 (Akiyama *et al,* 1994)] to extremely small premature infants of 1000g or less enhances early colonization of bifidobacteria and formation of a bifidobacteria flora, accompanied by reduction of necrotizing enterocolitis and other intestinal tract infections.

Table 2. Influence of *Bifidobacterium longum* BB536 yogurt administration on the relative percentages of fecal bacterial groups (Yaeshima 1997)

Bacteria	Control period	Interval period	Test period
Enterobacteriaceae	1.6 ± 1.7[a]	1.4 ± 1.9	1.4 ± 2.7
Bifidobacteria	23.7 ± 14.0	23.1 ± 12.0	34.4 ± 19.2[b,c]
Eubacteria	16.4 ± 9.9	17.2 ± 11.7	12.8 ± 9.1
Bacteroidaceae	51.1± 20.3	54.4 ± 18.1	48.4 ± 19.5
Total aerobic bacteria	4.0 ± 5.9	1.9 ± 1.7	1.7 ± 2.8
Total anaerobic bacteria	96.0 ± 5.9	98.1 ± 1.7	98.3 ± 2.8

Volunteers (n = 11) consumed 100g of standard yogurt (control period) or *B. longum* BB536 yogurt (test period) per day
a: Data shown are the mean of two values and SD (n = 11)
b: $p<0.05$ (compared with control period)
c: $p<0.05$ (compared with interval period)

The next important effect of bifidobacteria is the immunological activation effect. Bifidobacterial cell or cell component have been reported to induce specific and nonspecific antibody production, nonspecific resistance to toxins, and antitumor effects. In mice mono-associated with *B. longum* BB536, increased production of anti-*B. longum* antibodies and total IgA can be observed with the acquisition of resistance to pathogenic *E. coli* (Yamazaki *et al* 1991). The cell wall of *B. infantis* has been shown to suppress Meth-A fibrosarcoma in mice (Sekine *et al,* 1985). Bifidobacteria also demonstrate antitumor activities. Administration of lyophilized *B. longum* BB536 has been reported to inhibit cancers or aberrant crypt foci (ACF) formation induced by imidazoquinoline derivative (IQ) (Reddy & Rivenson 1993) or azoxymethane (Kulkarni & Reddy 1994). Figure 6 shows the results of administration of lyophilized *B. longum* BB536 on IQ-induced carcinogenesis in various organs (Reddy & Rivenson 1993). Administration of IQ to untreated mice induces cancers in the liver, intestines, and mammary gland. However administration of *B. longum* BB536 inhibits cancers in all these organs, especially, a 100% inhibition is achieved in the large intestine. Administration of *B. longum* BB536 concomitant with lactulose has been reported to achieve an additive effect of ACF inhibition (Challa *et al*, 1997). *B. longum* BB536 has also been shown to possess an action of increasing bone strength (Igarashi *et al,* 1994) which is supposed to be a result of enhanced calcium absorption.

Growth factor of bifidobacteria

Early studies on growth factors of bifidobacteria were research on in vitro growth promoting factors. The growth factors found in these studies include human milk components of N-

acetylglucosamine-containing oligosaccharides, enzymatic cleavage products of proteins, glycoproteins and pantethine compounds (Rasic & Kurmann 1983). Studies on in vivo growth
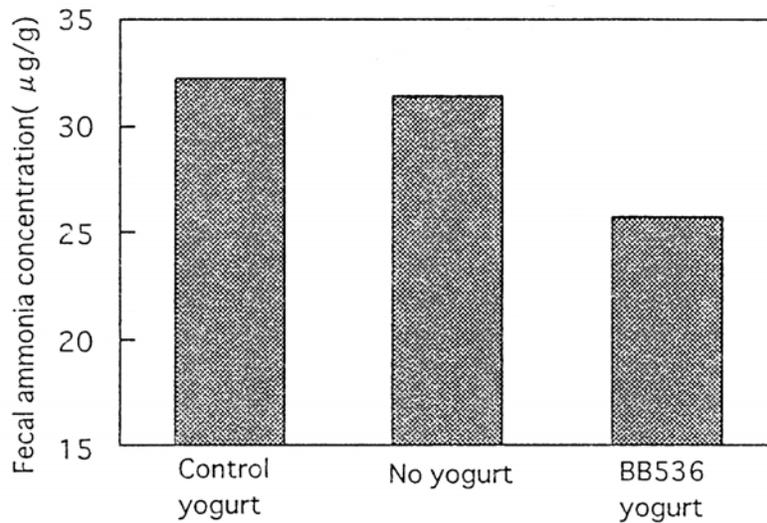
Figure 4. Effect of BB536 yogurt administration on the fecal ammonia concentration (n = 11) (Yaeshima 1997)
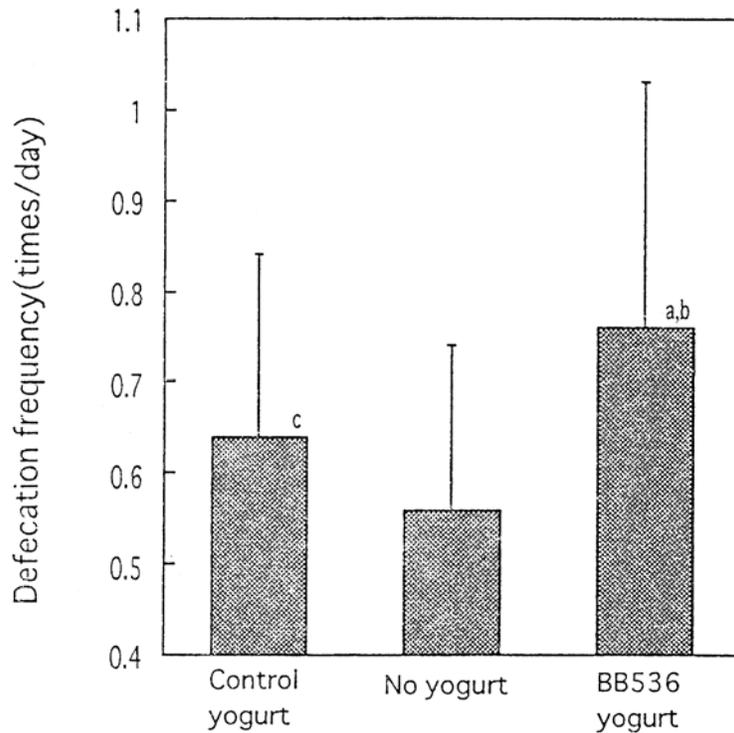
Figure 5. Effect of BB536 yogurt administration on the defecation frequency of women volunteers (n = 39) (Yaeshima 1997)
   a: p<0.001 (compared with control yogurt period)
   b: p<0.001 (compared with no yogurt period)
   c: p<0.01 (compared with no yogurt period)

factors then became popular, and oligosaccharides such as lactulose (Petury 1957, Terada *et al,* 1992) as well as dietary fibers have been found to be effective in promoting intestinal growth of bifidobacteria. These oligosaccharides have several important properties. They are basically indigestible in the human digestive tract and reach the large intestine intact, and also that they are preferentially utilized by bifidobacteria.

Figure 7 shows the changes in proportion of bifidobacteria in the intestinal flora after the consumption of lactulose (Terada *et al,* 1992). The proportion of bifidobacteria increases markedly when lactulose is consumed at 3g/day, and decreases when lactulose consumption is discontinued. Also, faecal ammonia and other putrefactive substances are suppressed by taking lactulose. Therefore, as a bifidobacterial growth factor, lactulose at a relatively small dose is effective in promoting intestinal growth of bifidobacteria. Utilizing this property, lactulose has long been used as a treatment for portal-systemic encephalopathy and also for alleviatig constipation. The mechanisms of action of lactulose in the management of these diseases are that lactulose promotes the growth of bifidobacteria which suppress ammonia production in the intestine, and the organic acids produced by the bifidobacteria promote peristalsis of the intestine. Lactulose has been used in a wide variety of foods, especially as a supplement in infant formulas, contributing greatly to the establishment of a bifidobacteria flora in infants (Tamura *et al,* 1993).
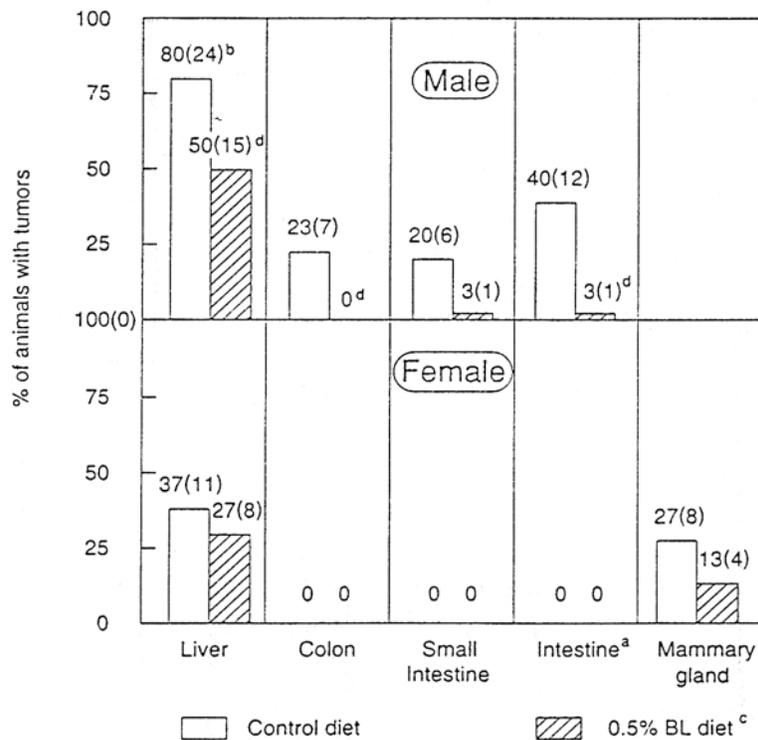
Figure 6. Effect of dietary B. longum BB536 on IQ-induced carcinogenesis. (Reddy 1993)
a. Intestines represents colon and small intestine.
b. Numbers in parenthesis are number of animals.
c. BL diet, control diet containing 0.5% lyophilized B. longum BB536.
d. Significantly different from its respective control diet group.
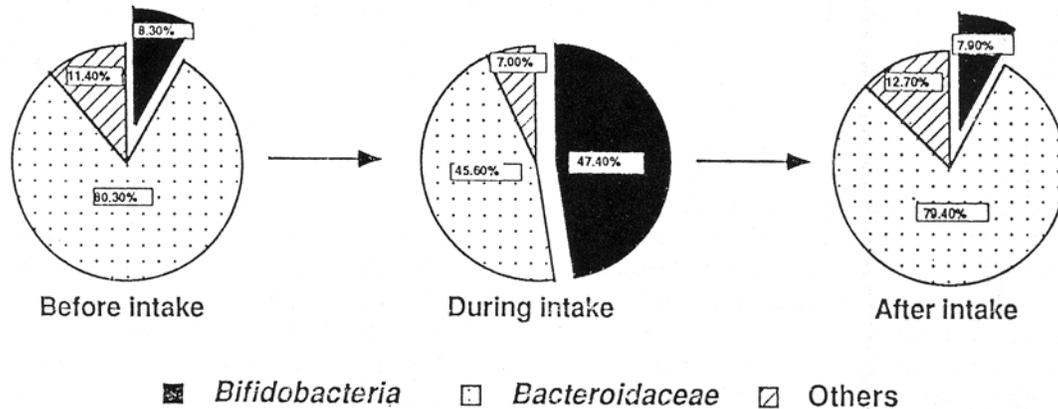
Figure 7. Effect of lactulose on the fecal Bifidobacterium (Terada 1992)

Application of bifidobacteria

With the intent to actively utilize the physiological effects of bifidobacteria mentioned above, bifidobacteria have been widely applied in foods, medicine and animal feeds. In this type of products, viable bifidobacteria are ingested to improve the intestinal microflora, which in turn improves the intestinal environment and contributes to the health of the intestine, with the ultimate aim to prevent or improve intestinal tract diseases or infection. The application of bifidobacteria in foodstuff had been practiced in Germany since the old days (Mayer, 1948), and was actively adopted in Japan in the early 1980s (Ishibashi, 1993). Later it was spread to Europe and is now used worldwide (Tamime *et al,* 1995). The most representative application of bifidobacteria in foods is bifidobacteria yogurt. Another popular product is bifidobacteria milk which is non-fermented milk with the addition of bifidobacteria. The concept of applying bifidobacteria in these foods is to supply viable bifidobacteria. However, to maintain a desirable level of viable bacteria in the bifidobacteria yogurt or milk until the expiry date requires high level technological know-how. For example, bifidobacteria do not grow in milk under normal conditions, and require special growth promoting substances. In addition, bifidobacteria are strict anaerobes and sensitive to oxygen. Special techniques are required throughout the whole manufacturing process to exclude the effect of oxygen. The technology of producing lyophilized powder has seen great advances, and powder preparation containing viable cells of 10^{10} to 10^{11} per gram is being marketed as food supplement. High quality lyophilized powder can be preserved long-term at room temperature for more than one year. It can be applied in many products in a wide variety of forms such as powdered health food and medicine, capsules and tablets.

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