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Bifidobacteria and their role in human health

Tomotari Mitsuoka

Department of Biomedical Science, Faculty of Agriculture, The University of Tokyo, Tokyo, Japan

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SUMMARY

There is a growing consensus on the beneficial effects of bifidobacteria in human health. It is now clear that bifidobacteria that exist in the large intestine are helpful for maintenance of human health and are far more important than *Lactobacillus acidophilus* as beneficial intestinal bacteria throughout human life. In other words, the reduction or disappearance of bifidobacteria in the human intestine would indicate an "unhealthy" state. Oral administration of bifidobacteria may be effective for the improvement of intestinal flora and intestinal environment, for the therapy of enteric and hepatic disorders, for stimulation of the immune response, and possibly for the prevention of cancer and slowing the aging process. However, for consistent and positive results further well-controlled studies are urgently needed.

INTRODUCTION

Since the discovery of bifidobacteria by Tissier (1900) much attention has been directed to the habitat, nutritional and immunological role, biochemistry and taxonomy of these bacteria. Although in the past much confusion existed in the taxonomy of bifidobacteria, recent progress of the definitive speciation in this genus has facilitated more detailed microecological research in bifidobacteria. This paper provides a review of the taxonomy and ecological significance of bifidobacteria, and discusses some of the roles of bifidobacteria in human health.

ADVANCES IN TAXONOMY OF BIFIDOBACTERIA

Bifidobacteria were first described in 1900 by Tissier and named by him *Bacillus bifidus* [16]. They are Gram-positive, strictly anaerobic, fermentative rods, often Y-shaped or clubbed at the ends. Since Cruickshank [1] and Weiss and Rettger [18] reported serological, morphological, and cultural similarities between bifidobacteria and *Lactobacillus acidophilus*, *Lactobacillus bifidus* was the generally accepted designation in the United States which, however, was not so widely accepted in Europe. Considerable confusion in the taxonomy of bifidobacteria resulted in misunderstanding the ecological description of the intestinal flora; that is, bifidobacteria have been considered to be the most important organisms for infants, while *L. acidophilus* is the predominant beneficial bacteria for adults.

Since 1958 the taxonomy of bifidobacteria has been the subject of renewed interest, and there is general agreement among taxonomists, that on the basis of morphological, cultural, and biochemical characteristics bifidobacteria should be classified in the genus *Bifidobacterium* as suggested by Orla-Jensen [13]. The genus *Bifidobacterium* is now classified into 25 species on the basis of DNA/DNA homology. On the basis of their phenotypic characteristics, however, *B. adolescentis*, *B. angulatum*, *B. pseudocatenulatum*, and *B. dentium*; *B. pseudolongum* and *B. globosum*; *B. animalis*, *B. suis*, *B. pullorum*, and *B. gallinarum*; *B. coryneforme* and *B. asteroides* are not differentiated from each other. At present time, fermentation patterns are still the principal guidelines used for the differentiation of bacterial species. Therefore, it is especially important to develop additional phenotypic characteristics that correlate best with the recognized genotypes. In this review I use phenotypic traits to speciate *Bifidobacterium*.

ECOLOGICAL SIGNIFICANCE OF BIFIDOBACTERIA IN HUMAN HEALTH

Although bifidobacteria have been considered to be the most important organisms in infants, and lactobacilli and *Escherichia coli* are more numerous bacteria for children and adults than bifidobacteria, it has now become clear that bifidobacteria also constitute one of the major

Correspondence: T. Mitsuoka (Present address), Nippon Veterinary and Zootechnical College, 1–7–1 Kyonan-cho, Musashino-Shi, Tokyo 180, Japan.

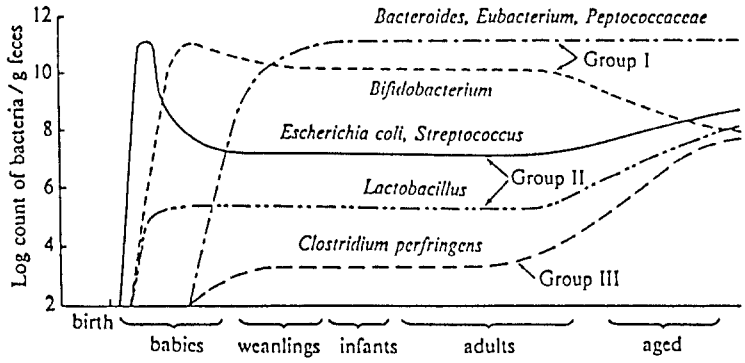

Fig. 1. Changes in the fecal flora with increased age.

organisms in the colonic flora of healthy children and adults [10]. In the feces of children and adults the numbers of bacteroidaceae, eubacteria, and peptococcaceae outnumber bifidobacteria, which constitute 5 to 10% of the total flora. The number of enterobacteriaceae and streptococci decreases to less than 10^8 cfu per gram of feces. Lactobacilli, and veillonellae are often found, but the numbers are usually less than 10^8 cfu per gram of feces.

In elderly persons bifidobacteria decrease or disappear, clostridia including *C. perfringens* significantly increase, and lactobacilli, streptococci, and enterobacteriaceae also increase [8] (Fig. 1).

There are definite differences in the *Bifidobacterium* species isolated from humans of different age groups and from animals [7]. The infant-type *Bifidobacterium* species are never isolated from children and adults, and animal-type *Bifidobacterium* species are never isolated from the intestine of humans. Most common bifidobacteria isolated from infants belong to *B. breve* and *B. infantis*. *Bifidobacterium bifidum*, *B. longum*, and *B. adolescentis* are occasionally isolated from infants. In contrast, *B. adolescentis* and *B. longum* are found in high numbers in the intestines of children, adults and elderly persons.

Monkeys harbor *B. longum* and *B. adolescentis* biovars A and B; dogs *B. adolescentis* biovars C and D, and *B. pseudolongum*. The great majority of the pig and cattle strains are identified as *B. pseudolongum* and *B. thermophilum*. From the intestine of chickens *B. pseudolongum*, *B. gallinarum*, and *B. thermophilum* are commonly isolated. Mice and rats harbor *B. pseudolongum* and *B. animalis*, while guinea pigs and rabbits harbor *B. animalis* and *B. magnum*.

Although the composition of the intestinal flora is rather stable in healthy individuals, it can be altered to an abnormal flora by many endogenous and exogenous factors, such as peristalsis disorders, cancer, surgical operations, liver or kidney diseases, pernicious anaemia, blind loop syndrome, radiation therapy, emotional stress, aging, disorders of immune systems, administration of antibiotics, etc. The abnormal flora are generally characterized by a remarkable increase in bacterial counts in the small intestine, by an increase of aerobes, mostly enterobacteriaceae and streptococci, by the reduction or disappearance of bifidobacteria, and/or often by the incidence of *C. perfringens*.

This ecological evidence would suggest that bifidobacteria should exist in the large intestine for maintenance of health and are far more important than lactobacilli as the beneficial intestinal bacteria throughout human life. In other words, the reduction or disappearance of bifidobacteria in the human intestine would indicate an "unhealthy" state.

ROLE OF BIFIDOBACTERIA IN HUMAN HEALTH

The intestinal flora are composed of 100 trillion bacteria comprising 100 species, and contains variety of enzymes that perform extremely varied types of metabolism in the intestine. Thus, the intestinal flora influence the host's health, including nutrition, physiologic function, drug efficacy, carcinogenesis, aging as well as the host's immunological responses, resistance to infection, and responses to endotoxin and various other stresses. Within the intestine bacteria are implicated in the conversion of various substances, resulting in the production of both beneficial and deleterious products to the host. In addition, bacterial toxins and cell components produced by some bacterial species modify the host's immune responses, enhancing or inhibiting immune function. The beneficial intestinal flora protect the intestinal tract from proliferation or infection of harmful bacteria, whereas the deleterious bacteria manifest pathogenicity when the host's resistance is decreased [6] (Fig. 2).

Much recent research has focused on bifidobacteria to establish the importance of these bacteria in influencing

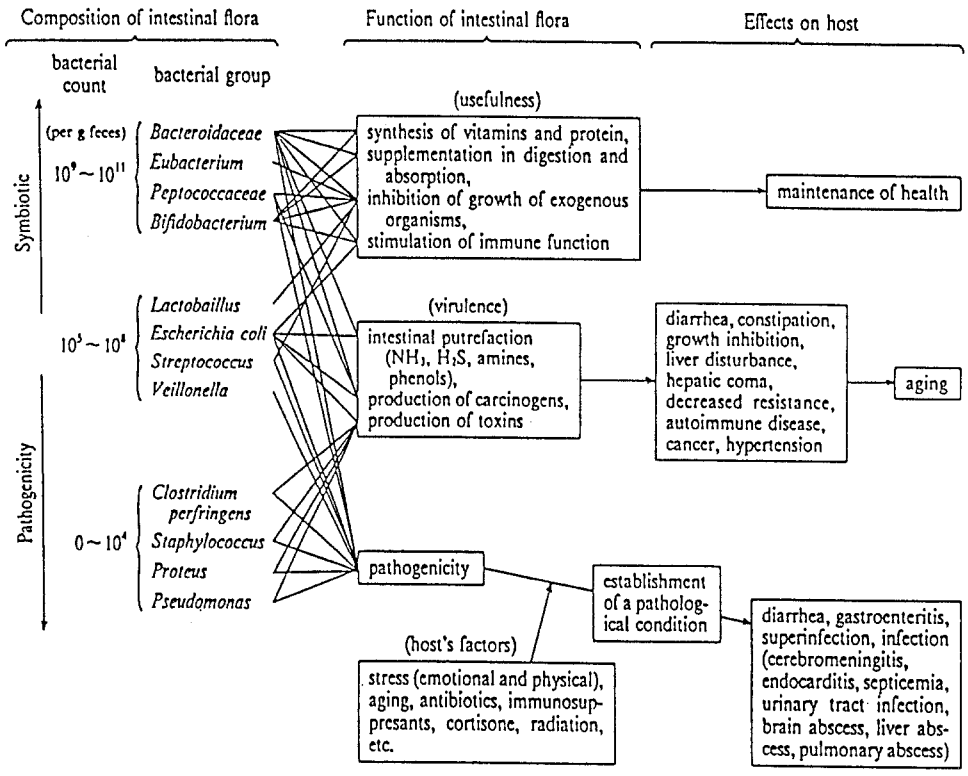

Fig. 2. Relationships between intestinal flora and host.

certain normal functions of the intestinal tract and in exploring their role in human health and diseases. In Japan, bifidobacteria are used as dietary supplements or as starter cultures for yogurt and other cultured milk products with the thought that such products may help the promotion of health. The effects of the daily intake of such products are reported to be as follows: (1) to suppress the putrefactive bacteria as well as intestinal putrefaction, so as to prevent constipation and geriatric diseases, including cancer; (2) to prevent and treat antibiotic-associated diarrhea; and (3) to stimulate the immune response, thus contributing to a greater resistance to infection.

Scavenging function

Most *Bifidobacterium* species metabolize a wide range of indigestible oligosaccharides to acetic and lactic acids and subsequently act as effective scavengers in the large intestine when much indigestible oligosaccharides are ingested, while *E. coli* and *C. perfringens* do not.

In our studies with volunteers, improvement of intestinal flora as well as of the intestinal environment were observed after oral adminstration of various oligosaccharides, including fructo-oligosaccharides, isomalto-oligosaccharides, galacto-oligosaccharides, palatinose condensate, raffinose, and soybean oligosaccharides. Most of the oligosaccharides stimulated the growth of bifidobacteria in vitro [4,9] and in vivo [9] (Fig. 3), and caused reduction of fecal pH, β-glucuronidase, azoreductase, and indole, serum cholesterol and triglyceride levels as well as the blood pressure of elderly patients with hyperlipemia [9]. From the results presented here, it may be concluded that oligosaccharides enhance the intestinal bifidobacteria, to improve the intestinal flora, stool consistency, and lipid metabolism.

In our laboratory the relationship between liver tumorigenesis and bifidobacteria was studied in gnotobiotic (Gb) C3H/He male mice monoassociated and polyassociated with intestinal bacteria [11]. The incidence of liver tumors was higher in most of the gnotobiotes and conventional mice than in the germ-free mice. Liver tumors were observed in 100% of mice associated with a bacterial combination of *E. coli*, *S. faecalis*, and *C. paraputrificum*, in contrast to 30% of germ-free mice and 75% of conventional mice. However, this tumor-promoting effect by intestinal bacteria was suppressed 46% by the addition of *B. lungum* to the promoting combination, and 65% by *L. acidophilus* [13]. These results suggest that intestinal bacteria are related to both promotion and prevention of cancer. The mechanism of the suppressive effect of bifidobacteria on liver tumors might be related to their ability to detoxify carcinogens.

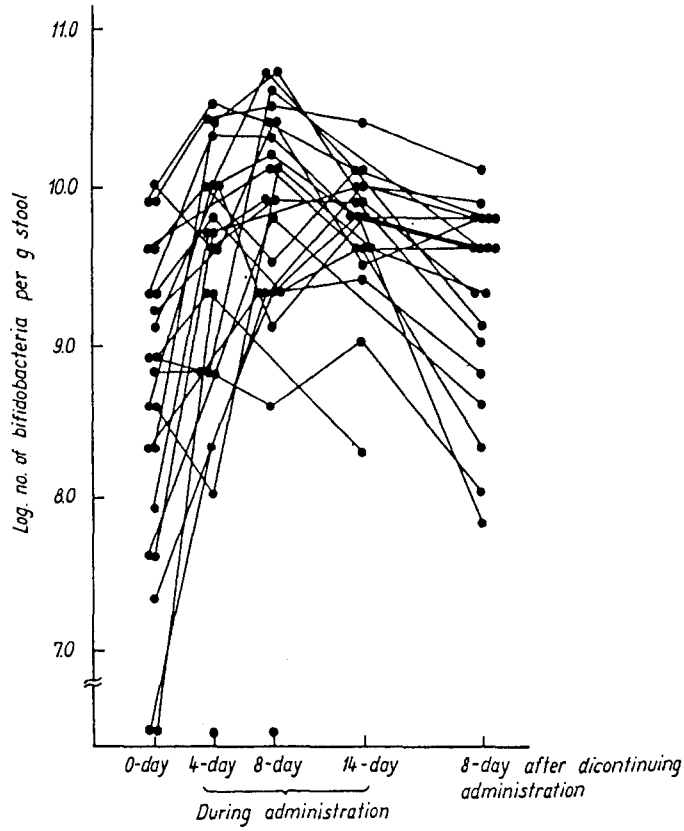

Fig. 3. Effect of fructo-oligosaccharide administration on the counts of bifidobacteria in elderly persons.

Therapy for intractable diarrhea

Orally administered bifidobacteria preparations have been used in the treatment of various intestinal disorders. Hotta et al. [2] investigated the effects of administration of *Bifidobacterium* preparations on infantile intractable diarrhea. Fifteen patients (11 boys and 4 girls), ranging in age from 1 month to 15 years (mean 2.5 years), were receiving antibiotic therapy for the treatment of such diseases as septicemia and respiratory tract infections. In most cases, disturbed intestinal flora were observed; *Candida* or enterococci often dominated, with a marked decrease of anaerobes, especially bifidobacteria. During the disease neither pathogens such as *Clostridium difficile* nor toxins responsible for diarrhea were detected. In all patients the stool frequency and appearance were remarkably improved within 3 to 7 days after adminstration of a bifidobacteria preparation. The fecal flora of all subjects studied also became normal with predominance of resident bifidobacteria or administered *B. breve* and the balances of intestinal flora improved to the normal level.

Hepatic maintenance function

Müting et al. [12] have shown that bifidobacteria are beneficial for protein metabolism in liver cirrhosis. Thirty-three patients with various degrees of liver cirrhosis were given a reconstituted bifidus milk preparation during an average period of 100 days. The authors observed that this bifidus milk preparation improved symptoms and decreased ammonia, free phenols, and indican in the blood; and also it reduced fecal pH, and increased the count of bifidobacteria.

Koizumi et al. [5] also confirmed the beneficial effects of bifidobacteria in the treatment of liver cirrhosis with portal encephalopathy. Seven patients were given 3 doses of *B. breve* and *B. bifidum* at 10^9 cells of each per day for 1 month; improvement of symptoms and decreased blood ammonia were observed.

Immunoregulatory function

Administration of viable or non-viable intestinal bacteria to germ-free mice enhanced intestinal production of immunoglobulin A(IgA) plasmacytes. Experiments on laboratory animals strengthen the supposition that regular ingestion of bifidobacteria may induce an immunogenic response in humans.

Kohwi et al. [3] found that intralesional repeated injection of viable or killed *B. infantis* inhibited the growth of Meth-A tumor cells transplanted subcutaneously into

syngenic BALB/c mice. Water-soluble cell wall fractions of this organism have an adjuvant effect on the induction of transplantation immunity.

In 1985 Sekine et al. [14] isolated cell wall skeleton (CWS), whole peptidoglycan (WPG), and sonicated WPG from heat-killed *B. infantis* and studied relative antitumor efficacy with syngenic Meth-A fibrosarcoma in BALB/c mice. Among these, WPG was completely retained in the intact cell wall structure. A single subcutaneous injection of CWS, WPG, or sonicated WPG in a mixture with tumor cells resulted in a significant suppression of the tumor growth. However, when these cell wall preparations were injected intralesionally 5 times into mice bearing 5-day-old tumors, WPG resulted in the highest suppression.

The effect of ingested bifidobacteria cultures on the immunosystems of their hosts is now under investigation.

EFFECT OF ORAL ADMINISTRATION OF BIFIDOBACTERIA ON THE INTESTINAL FLORA

Bifidobacteria cultures have been added to the diet of children and adults to modify their intestinal flora. Tanaka et al. [15] observed that feeding 200 ml of Bifidus milk supplemented with 10^7 to 10^8 of *Bifidobacterium breve* per ml per day to healthy subjects (11 children and 5 adults) with an otherwise normal diet resulted in an increase of indigenous bifidobacteria, reduction of the counts of bacteroidaceae, clostridia and enterobacteraceae in the stools, and a decrease of fecal ammonia and urinary indican.

Tohyama et al. [17] also studied the effects of the administration of *B. breve* on bacterial metabolic activities in the intestine. The authors observed that the activities of bacterial enzymes such as β-glucuronidase, tryptophanase and lysine decarboxylase in feces were significantly reduced, and bacterial metabolites such as indican, phenols, ammonia and cadaverin in urine were also reduced.

In our laboratory, the effects of oral administration of 10^9 freeze-dried *Bifidobacterium longum* per day for 5 weeks on the fecal flora and some fecal putrefactive parameters were examined with 5 healthy 25- to 35-year-old volunteers. During the administration the counts of bifidobacteria increased, while the counts and frequencies of clostridia in stools significantly decreased. There was also a decrease of ammonia concentration and β-glucuronidase activity in both feces and serum.

Serum cholesterol in Hartley male rabbits fed with a 0.25% cholesterol diet supplemented with 10^{10} cells of *B. longum* per day for 13 weeks were compared with the control group. In 2 of 3 rabbits, a diet supplement with *B. longum* remarkable suppressed an increase in cholesterol level.

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