

Establishment of Intestinal Bacteriology

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Research on intestinal bacteria began around the end of the 19th century. During the last 5 decades of the 20th century, research on the intestinal microbiota made rapid progress. At first, in my work, I first developed a method of comprehensive analysis of the intestinal microbiota, and then I established classification and identification methods for intestinal anaerobes. Using these methods I discovered a number of ecological rules governing the intestinal microbiota and the role of the intestinal microbiota in health and disease. Moreover, using germfree animals, it was proven that the intestinal microbiota has a role in carcinogenesis and aging in the host. Thus, a new interdisciplinary field, “intestinal bacteriology” was established.

Key words: intestinal microbiota, intestinal bacteria, intestinal bacteriology, functional foods

INTRODUCTION

Research on intestinal bacteria began in 1719, when the first microscopic observations of fecal bacteria were made by Leeuwenhoeck. In 1886, Escherich isolated *Bacillus coli* (*Escherichia coli*) from infants. In 1899, Tissier of the Pasteur Institute isolated *Bacillus bifidus* (*Bifidobacterium*), a type of anaerobic lactic acid bacteria, from the feces of breast-fed infants, and this led to widespread research on infant nutrition and the intestinal microbiota in the pediatric field. In 1900, the Austrian pediatrician Moro discovered *Bacillus acidophilus* (*Lactobacillus acidophilus*). These two important types of enteric lactic acid bacteria appeared in rapid succession, and the debate on the classification of *Lactobacillus*, including the problem of differences from lactic acid bacteria and differentiation from yogurt-derived *Lactobacillus*, started in earnest.

Research on the intestinal microbiota began to determine if individual differences in enteric infections were caused by differences in intestinal microbiota, but the intestinal bacteria studied were mainly limited to aerobes such as *E. coli* and *Enterococcus*, which are easy to culture. In 1935, Eggerth and Gagnon reported that anaerobic bacteria of the genus *Bacteroides* outnumbered *E. coli* in human feces, but the conventional thinking

that *E. coli* was the predominant enteric bacteria was firmly rooted and this new knowledge went unheeded for another 20 years.

In the 5th decade of the 20th century research on the intestinal microbiota began in Europe, Japan and the United States. Dwayne Savage summarized this matter in a review entitled “Microbial Biota of the Human Intestine: A Tribute to Some Pioneering Scientists” [1]. The present review describes the advances in intestinal microbiota research upon which “intestinal bacteriology” was established and functional foods were developed.

DEVELOPMENT OF METHODS FOR COMPREHENSIVE INVESTIGATION OF THE INTESTINAL MICROBIOTA

Development of methods of comprehensive analysis of the intestinal microbiota was carried out by Haenel et al. [2–6], Mitsuoka et al. [7–11] and Drasar [12].

“BL agar”

In 1953, I started graduate work on the intestinal microbiota. First, the fecal material of a human adult diluted with a diluent was prepared, Gram stained, and examined for the number and shape of bacteria under microscope. Then, gram-positive and gram-negative bacteria of different sizes and shapes were observed (Fig. 1). The number of bacteria in one gram was in the range of 3 to 5 × 10¹¹. So, 0.01 ml of the diluted fecal material was smeared on a nutrient agar medium and incubated aerobically. Subsequently, only one or several kinds of coliform bacteria and enterococci colonies appeared on the agar. The number of bacteria per gram feces was

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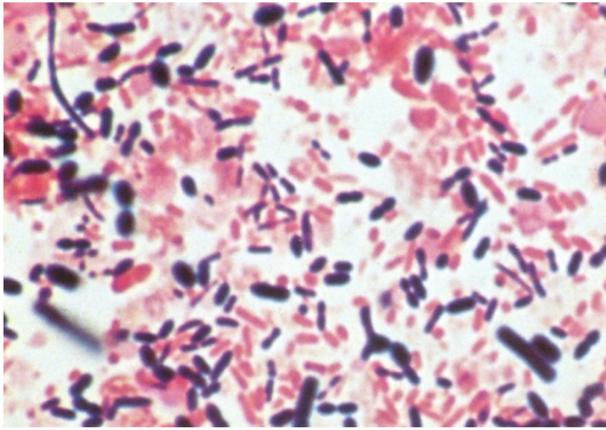


Fig. 1. Photomicrograph of the Gram-stained fecal stool of an adult.

usually between 10^5 and 10^8 . This figure was far less than that estimated from direct smear observation under microscope.

Therefore, I developed BL agar (glucose blood liver agar) for culture, differentiation, and isolation of various intestinal bacteria and cultivated the fecal material of human adults in an anaerobic jar. I discovered that obligately anaerobic bacteria such as *Bifidobacterium* and *Bacteroides* spp. constitute predominant microorganisms in human adult feces (Fig. 2). This discovery formed the basis for the establishment of “intestinal bacteriology” and the development of functional foods.

“Plate-in-bottle method”

In 1950, Hungate [13] developed the “anaerobic roll-tube method” for the isolation of cellulolytic anaerobic bacteria in the rumen. This culture technique was also applied to study the intestinal microbiota and made possible the growth of fastidious anaerobes from the intestinal materials of humans and animals. This method, however, is admittedly cumbersome for routine use. To overcome these difficulties, we developed the “plate-in-bottle method” (Fig. 3) [9], which made possible the growth of fastidious anaerobes as surface colonies on agar media without using special facilities.

This technical improvement enabled the cultivation of over 70% of the microscopic count of bacteria in animal feces, and often more than 90%.

Method for comprehensive investigation of intestinal microbiota

In 1969, we established a comprehensive method using the “plate-in-bottle method”, 4 nonselective media



Fig. 2. Discovery of BL agar and predominant flora of bifidobacteria in an adult fecal specimen.

1, 2, 3, 7: *Bifidobacterium*. 4: *Megasphaera elsdenii*. 5, 6: *Bacteroides*. 8: *Eubacterium*. 9: *Veillonella*



Fig. 3. Plate-in-bottle method.

and 10 selective media [8] for analysis of the intestinal microbiota of humans and animals (Table 1). I described the details of this method in a book entitled “A Color Atlas of Anaerobic Bacteria” [11].

Despite explosive development in modern molecular techniques, it has to be emphasized that classical microbiological techniques are still not obsolete but are complementary to the molecular techniques.

CLASSIFICATION, DIFFERENTIATION AND HABITATS OF LACTIC ACID BACTERIA OF HUMANS AND ANIMALS

During the 1960s and 1980s, significant advances

Table 1. The media and cultural method for comprehensive investigation of intestinal microbiota

Medium	Organisms usually enumerated	Incubation method	Incubation time (days)
Nonselective media			
Medium 10 (modified)	Fastidious anaerobes	Plate-in-bottle method	5
EG agar	Anaerobes	} Steel wool method } Replaced air with CO ₂	} 3
BL agar	Anaerobes		
Trypticase soy blood agar	Aerobes	Air	1-2
Selective media			
BS agar	Bifidobacteria	} Steel wool method } Replaced air with CO ₂	} 3
CS agar	Eubacteria		
NBGT agar	Bacteroidaceae		
Neomycin Nagler agar	Lecithinase-positive clostridia		
VS agar	Veillonellae and Megasphaera		
LBS agar (modified)	Lactobacilli		2
DHL agar	Enterobacteriaceae	} Air	} 1
TATAC agar	Streptococci		
PEES agar	Staphylococci		
Potato dextrose agar	Yeasts and molds		

were made in the bacterial taxonomy of *Lactobacillus*, *Bifidobacterium* and other anaerobes. Moore and his coworkers published the “Anaerobe Laboratory Manual 4th Ed.” [14], and I published a color atlas for the isolation and identification of anaerobic bacteria [11].

Lactobacillus

Lactobacilli are isolated from the intestine of humans and animals. The predominant *Lactobacillus* flora are especially harbored in the intestines of animals such as pigs, chickens, dogs, mice, rats and hamsters.

Rogosa and Sharpe [15] provided a detailed description of *L. acidophilus*. Lerche and Reuter [16] grouped *L. acidophilus* strains into five biotypes, and I [17] expanded the number of biotypes to ten biotypes.

Johnson et al. [18] divided *L. acidophilus* species into six distinct homology groups, A-1, A-2, A-3, A-4, B-1 and B-2. Until recently, it was not possible to distinguish the genospecies recognized in the *L. acidophilus* group on the basis of phenotypic characteristics. Phenotypic criteria were developed that proved useful for the differentiation of genospecies of the *L. acidophilus* group as shown in Table 2, in which the differential characteristics of lactobacilli recovered from the gastrointestinal tract are also included. Homology groups A-4 and B-2 should be

considered two new species, for which we proposed the names *L. gallinarum* and *L. johnsonii*, respectively [19]. The intestinal strains formerly identified as *L. fermentum* were classified as a new species, *L. reuteri* [20].

Habitats of the genospecies of the *L. acidophilus* group and other *Lactobacillus* species are as shown in Table 3, *L. acidophilus* can probably be isolated from humans, mice and rats. *L. amylovorus* is mainly from pigs and occasionally isolated from cattle, and it can probably be isolated from chickens. *L. crispatus* is most common in the intestine of humans and chickens. *L. gasseri* is the most common isolate from humans, and it is occasionally isolated from cattle. *L. johnsonii* is more commonly isolated from chickens and is occasionally from humans and pigs. *L. murinus* is synonymous with *L. animalis* and is isolated from cattle, dogs, mice, and rats; it can also probably be isolated from pigs and chickens. *L. intestinalis* is common in mice and rats. *L. salivarius* has been commonly isolated from humans, pigs, and chickens. Finally, *L. agilis* is often isolated from pigs and chickens. Anaerobic lactobacilli have been isolated from the gastrointestinal tract of humans and animals, one strain being identified as *L. ruminis* [21] and others being identified as *L. vitulinus* [21], *L. aviarius* [22] and *L. hamsteri* [23]; *L. ruminis* is occasionally isolated

Table 2. Differential characteristics of *Lactobacillus* species recovered from the intestine of humans and animals^a

Species		G+C (mol %)	Gas from glucose	15°C	Aerobic growth	Motility	Isomer of lactic acid	Ribose	Esculin	Cellulose	Trehalose	3.5% NaCl	4.5% NaCl	5.0% NaCl	0.04% SDS	
<i>L. acidophilus</i>	Group															
<i>L. acidophilus</i>	(A-1) ^b	32-37	-	-	+	-	DL	-	+	+	+	-	-	-	-	-
<i>L. amylovorus</i>	(A-3)	40	-	-	+	-	DL	-	+	+	+	-	-	-	-	+
<i>L. crispatus</i>	(A-2)	35-38	-	-	+	-	DL	-	+	+	-	+	-	-	-	+
<i>L. gallinarum</i>	(A-4)	33-36	-	-	+	-	DL	-	+	+	-	+	+	-	-	+
<i>L. gasseri</i>	(B-1)	33-35	-	-	+	-	DL	-	+	+	+	+	+	+	-	-
<i>L. johnsonii</i>	(B-2)	32-38	-	-	+	-	DL	-	+	+	+	+	+	+	+	+
<i>L. murinus</i> ^c		43-44	-	-	+	-	L	+	+	+	v	-	-	-	-	-
<i>L. intestinalis</i>		32-34	-	-	+	-	DL	-	-	v	-	-	-	-	-	-
<i>L. salivarius</i>		34-36	-	-	+	-	L	v	v	-	v	-	-	-	-	-
<i>L. agilis</i>		44	-	-	+	+	L	+	+	+	+	-	-	-	-	-
<i>L. ruminis</i>		44-47	-	-	-	+	L	-	+	+	-	-	-	-	-	-
<i>L. vitulinus</i>		34-37	-	-	-	-	D	-	+	+	-	-	-	-	-	-
<i>L. hamsteri</i>		33-35	-	-	-	-	DL	+	+	+	v	-	-	-	-	-
<i>L. aviarius</i>		39-43	-	-	-	-	DL	-	v	v	+	-	-	-	-	-
<i>L. casei</i>		45-47	-	+	+	-	L	+	+	+	+	-	-	-	-	-
<i>L. plantarum</i>		44-46	-	+	+	-	DL	+	+	+	+	-	-	-	-	-
<i>L. reuteri</i>		40-42	+	-	+	-	DL	+	-	-	v	-	-	-	-	-
<i>L. brevis</i>		44-47	+	+	+	-	DL	+	v	-	-	-	-	-	-	-

^a Symbols: +, 90% or more strains positive; -, 90% or more strains negative; +), 80-90% strains positive; v, 21-79% strains positive.

^b DNA homology group of Johnson et al. (1980).

^c Synonymous with *L. animalis*.

from cattle and Bulgarian people; *L. vitulinus* is isolated from cattle and Papuan highlanders; *L. hamsteri* is the most common isolate from hamsters; and *L. aviarius* is a specific species from birds, including chickens and ducks.

Bifidobacterium

For many years bifidobacteria were included in the genus *Lactobacillus* as *Lactobacillus bifidus*, but they are now classified in a separate genus *Bifidobacterium* as suggested by Orla-Jensen [24] on the basis of their characteristic morphology, biochemical characters, cell wall constituents and DNA base composition.

In the past, several workers recognized only one species in the genus *Bifidobacterium*, but Reuter has proposed eight species on the basis of carbohydrate fermentation and several biotypes for strains isolated from humans [25]. He recognized the following species and biotypes in the genus *Bifidobacterium*: *Bifidobacterium bifidum* a and b; *B. infantis*; *B. parvulorum* a and b; *B. breve* a and b; *B. lactentis*; *B. liberorum*; *B. adolescentis* a, b, c and d; and *B. longum* a and b.

In 1969, I [26] studied strains of bifidobacteria isolated from human feces, the rumen of cattle and sheep and the intestine of animals such as pigs, chickens, rats, mice, guinea pigs and rabbits and compared the results with those obtained by Reuter [25]. In his study, he clearly differentiated nonhuman strains from human strains by

Table 3. Habitats of *Lactobacillus* species in the intestine of humans and animals^a

Species		Humans	Pigs	Chickens	Cattle	Dogs	Mice	Rats	Hamsters
<i>L. acidophilus</i>	Group								
<i>L. acidophilus</i>	(A-1) ^b	?					?	?	
<i>L. amylovorus</i>	(A-3)	M	M	M	+				
<i>L. crispatus</i>	(A-2)	M		M					
<i>L. gallinarum</i>	(A-4)			M					
<i>L. gasseri</i>	(B-1)	M			+				
<i>L. johnsonii</i>	(B-2)	+	+	M					
<i>L. murinus</i> ^c			?	?	M	M	M	+	
<i>L. intestinalis</i>			M	M			M	M	
<i>L. salivarius</i>		M	M	M					
<i>L. agilis</i>			+	+					
<i>L. ruminis</i>		+			M				
<i>L. vitulinus</i>		+							
<i>L. hamsteri</i>									M
<i>L. aviarius</i>					+				
<i>L. casei</i>		+							
<i>L. plantarum</i>		+							
<i>L. reuteri</i>		M	M	M	M	M	M	M	M
<i>L. brevis</i>		+							

^a Symbols: M, major component of *Lactobacillus* species; +, occasionally recovered; ?, questionable.

^b DNA homology group of Johnson et al. (1980).

^c Synonymous with *L. animalis*.

their carbohydrate fermentation patterns and ability to grow at 46.5 C and proposed the creation of new species, *B. pseudolongum* a, b, c, and d and *B. thermophilum* a, b,

c, and d and a new variant, *Bifidobacterium longum* ss. *animalis*.

Subsequently, Scardovi and his coworkers [27–31] proposed, mainly based on DNA homology, the establishment of eleven additional species for organisms isolated from the intestine of honey bees, pigs, chickens and rabbits, the rumen of cattle and sheep and sewage. The eleven species were *B. indicum*, *B. coryneforme*, *B. asteroides*, *B. ruminale*, *B. globosum*, *B. suis*, *B. pullorum*, *B. magnum*, *B. catenulatum*, *B. dentium*, and *B. angulatum*.

Furthermore, these workers confirmed the separation of species *B. bifidum* and *B. longum* at the genetic level and proposed that *B. lactentis* and *B. liberorum* be merged into a single species, *B. infantis*. Likewise, they noted that *B. parvulorum* was considered a synonym for *B. breve*. They also proposed that *B. ruminale* and *B. thermophilum* be regrouped into a single species because of their similarities. They also found similarities between *B. pseudolongum* and *B. globosum*, but they did not consider that these organisms were enough alike to merge them into a single species. In 1974, Scardovi and Trovatelli [32] recognized the genetic dissimilarity between *B. longum* ss. *longum* and *B. longum* ss. *animalis* and proposed elevation of the subspecies *animalis* to the species level as *B. animalis*.

Actinomyces eriksonii was first described by Georg et al. [33] in 1965. This organism was isolated from human pleural fluid and a lung abscess. In 1972, Holdeman and Moore [14] reclassified *A. eriksonii* and placed it in the genus *Bifidobacterium*, as *Bifidobacterium eriksonii*. Subsequently, Mitsuoka et al. [34] suggested that *A. eriksonii* is synonymous with *B. adolescentis* type b.

The DNA hybridization technique, as used by Scardovi and coworkers, is a significant advance in determinative bacteriology and should help resolve much of the confusion previously encountered. However, on the basis of the fermentation patterns, *B. adolescentis*, *B. angulatum*, *B. pseudocatenulatum*, *B. catenulatum* and *B. dentium*; *B. pseudolongum* and *B. globosum*; *B. animalis*, *B. suis*, *B. pullorum* and *B. gallinarum*; *B. thermophilum*, *B. boum* and *B. choerinum* and *B. coryneforme* and *B. asteroides* cannot be differentiated from each other. At the present time, fermentation patterns are still the principle guidelines used for specific differentiation of bacteria. The differential scheme for a rapid routine identification of phenotypic species of bifidobacteria is shown in Table 4.

In view of the relationship between *Bifidobacterium* species or biotypes and the ecological distribution in humans and animals, it must be stressed that, in general,

each animal species harbors certain species or biotypes of bifidobacteria (Table 5). The great majority of the animal strains, with the exception of the strains isolated from monkeys and dogs, were clearly differentiated from human strains by their growth temperature and carbohydrate fermentation patterns and classified as new species, *B. thermophilum*, *B. pseudolongum* and *B. animalis* [26]. The isolates from chickens and pigs were identified as *B. thermophilum* and *B. pseudolongum*, while the strains from rodents (mice, rats and guinea pigs) and ruminants (cattle and sheep) belonged to *B. pseudolongum* and/or *B. animalis*. On the other hand, isolates from dogs were identified as *B. adolescentis*, *B. longum* and *B. pseudolongum*, and the strains from monkeys were identified as *B. adolescentis* [35].

ECOLOGICAL RULES GOVERNING THE INTESTINAL MICROBIOTA

Major bacterial groups composing the intestinal microbiota

The major bacteria detected in the intestinal microbiota are broadly categorized as follows: lactic acid bacteria, including *Bifidobacterium*, *Lactobacillus* and *Enterococcus*; putrefactive bacteria, including *Clostridium* including *C. perfringens*, *Bacteroidaceae*, *Peptococcaceae*, *Veillonella*, *Escherichia coli*, *Staphylococcus*, *Pseudomonas aeruginosa*; and other bacteria, including *Eubacterium*, *Ruminococcus*, *Megasphaera*, *Mitsuokella*, *C. butyricum*, and *Candida*. These bacteria are also divided into anaerobes and aerobes on the basis of the oxygen requirement or into non-pathogens and pathogens on the basis of the pathogenicity.

Composition of the intestinal microbiota of human adults

With the use of strict anaerobic culturing techniques, it is now accepted that the most prevalent microorganisms in human adult feces are obligately anaerobes, while easily culturable aerobes are expected to account for less than 10^{-3} of anaerobe numbers [14] (Fig. 4). The most prevalent anaerobe is *Bacteroidaceae*, and the average count of this organism is $10^{10.9}$ per gram of wet feces. The second most prevalent microorganisms are those of the genus *Eubacterium*, with average counts of $10^{10.4}$ per gram. The third most prevalent microorganisms are those of the *Peptococcaceae* family, including *Ruminococcus*, *Coprococcus*, *Peptostreptococcus* and *Peptococcus*. The average count for these bacteria is $10^{10.2}$ per gram. The fourth most prevalent microorganisms are those of the genus *Bifidobacterium* with an average count of $10^{10.0}$

Table 4. Characteristics of *Bifidobacterium* species from humans and animals^a

Species and biovars	Growth at 46.5°C Clot of litmus milk	Mono-			Saccharide								Poly-alcohol			Glucoside												
		Arabinose	Xylose	Ribose	Hexose	Di-		Tri-		Poly-				Mannitol	Sorbitol	Inositol	Esculin	Salicin	Amygdalin	α-Methyl-glucoside								
					Mannose	Fructose	Sucrose	Maltose	Cellobiose	Lactose	Trehalose	Melibiose	Raffinose	Melezitose	Dextrin	Starch	Glycogen	Inulin										
<i>bifidum</i> a	- +	-	-	-	-	+	+s	-	-	+	-	(+)/s/-	-	-	-	-	-	-	-	-	-	-	-					
<i>bifidum</i> b	- +	-	-	-	-	+	-	-	-	+	-	+/+s	-	-	-	-	-	-	-	-	-	-	-					
<i>infantis</i>																												
ss. <i>infantis</i> a	- +	-	-	+s	+s	+s	+	+	(+) _s	+	-/(+) _s	+	+	-	(+) _s	-	+	-	-	-	-	-	-	+	-	v		
ss. <i>infantis</i> b	- +	-	-	+	+s	+	+	+	+	+	-/(+) _s	+	+	-	+s	+s	-	+	-	-	+	-	-	-	(+) _s	-	v	
ss. <i>liberorum</i>	- +	-	+	+	+s	+	+	+	-	+	+s	+	+	-	(+) _s	(+) _s	-	-	-	-	-	+	-	+	+s	+s	-	+s
ss. <i>lactentis</i>	- +	-	+	+s	+s	+	+	+	-	+	-	+	+	-	-	+s	-	+	+	-	+	-	(+)	-	-	-	+	+s
<i>breve</i>																												
ss. <i>breve</i> a	- +	-	-	+	+	+	+	+	+	+	-	+	+	-	v	v	v	(+) _s /-	+	+	-	+	+	+	+	+	+	+
ss. <i>breve</i> b	- +	-	-	+	+	+	+	+	+	+	+	+	+	+s	v	v	v	(+) _s /-	+	+	-	+	+	+	+	+	+	+
ss. <i>breve</i> c	- +	-	-	+	+	+	+	+	+	+	-	+	+	+	v	v	v	(+) _s /-	+	+	-	+	+	+	+	+	+	+
<i>parvulorum</i> a	- +	-	-	+	+	+	+	+	+	+	-	+	+	-	+	+	v	v	-	-	-	+	+	+	+	+	+	+
<i>parvulorum</i> b	- +	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	v	v	-	-	-	+	+	+	+	+	+	+
<i>thermophilum</i> a	+ -	-	-	-	-	+	+	+	v	-	v	+	+	-	+	+	+	+	-	-	-	(+) _s						
<i>thermophilum</i> b	+ -	-	-	-	-	+	+	+	+s	-	-	+	+	+	+	+	+	+	-	-	-	(+) _s						
<i>thermophilum</i> c	+ +	-	-	-	-	+	+	+	v	+	+	+	+	-	+	+	+	+	-	-	-	(+) _s						
<i>thermophilum</i> d	+ +	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+s						
<i>adolescentis</i> a	- +	+	+	+	+/+s	+	+	+	+s	+	+	+	+	-/+s	+	+	+	v	+	+	-	+	+	+	+	+	+	+
<i>adolescentis</i> b	- -	+	+	+	+s/+	+	+	+	+s	+	v	+	+	-/+s	+	+	+	v	+	-	-	+	+	+	+	+	+	+
<i>adolescentis</i> c	- v	+/+s	+/+s	+	+s/+	+	+	+	+s	+	v	+	+	-/+s	v	v	v	v	-	+	-	+	+	+	+	+	+	+
<i>adolescentis</i> d	- v	+/+s	+/+s	+	+s/+	+	+	+	+s	+	v	+	+	-/+s	+s	+s	-	-	-	-	-	+	+	+	+	+	+	+
<i>longum</i>																												
ss. <i>longum</i> a	- +	+	+	+	+s/-	+	+	+	-	+	-	+	+	±	±	±	-	-	-	-	-	-	-	-	-	-	-	+s
ss. <i>longum</i> b	- +	+	+	+	+	+	+	+	-	+	-	+	+	±	±	±	-	-	-	-	-	-	-	-	-	-	-	+s
<i>animalis</i> a	- v	+	+	+	-	+/+s	+	+	-	+	-	+	-	±	±	±	-	-	-	-	-	±	±	±	±	±	±	±
<i>animalis</i> b	- +	+	+	+	+	+/+s	+	+	-	+	-	+	-	±	±	±	-	-	-	-	-	±	±	±	±	±	±	±
<i>pseudolongum</i> a	- +	+	+	+	-	+s	+	+	-/+s	+	-	+	-	+	+	+	-	-	-	-	-	-	±	±	±	±	±	+s
<i>pseudolongum</i> b	- +	+	+	+	+s	+s	+	+	+/+s	+	-	+	-	+	+	+	-	-	-	-	-	-/+s	+s	-/+s	+/+s	+/+s	+/+s	+/+s
<i>pseudolongum</i> c	- +	+	+	+	+s	+s	+	+	+s	+	-	+	+	+	+	+	-	-	-	-	-	±	±	±	±	±	±	+s
<i>pseudolongum</i> d	- -	+	+	+	+s	+s	+	+	+s	-	-	+s	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+s

^aSymbols: +, 90% or more of strains are positive; -, 90% or more of strains are negative; v, 11–89% of strains are positive.

per gram. Other anaerobes often found in human feces include *Clostridium*, *Megasphaera* and *Veillonella*. The aerobic microbiota is predominantly represented by *E. coli*, *Streptococcus* including *Enterococcus*, and *Lactobacillus*, but their counts are less than 10^8 per gram feces.

Microbiota in each region of the gastrointestinal tract of human adults

With respect to total bacterial counts in the oral cavity of the adult, there are already 10^7 bacteria per gram of saliva (Fig. 5). However, the number of bacteria transiently decreases in the stomach as a result of gastric acid; only about 10^2 to 10^3 bacteria are detectable in each gram of gastric juice. Although the number of organisms

gradually increases beginning in the small intestine, the bacterial count in the upper small intestine is still (10^3 to 10^4 /g), with *Lactobacillus*, *Streptococcus* and *Veillonella* being the primary bacteria detected in this region.

The lower part of the ileum in the small intestine shows increasing total bacterial counts and exhibits some of the flora seen on transition into the large intestine. A mixed flora is detected here consisting of bacteria that make up the flora of the upper small intestine as well as bacteria seen in the microbiota of the large intestine.

A marked change in the flora then occurs as from the ileocecal valve into the large intestine. The total bacterial count rises abruptly to 10^{11} or greater per gram. Anaerobic bacteria including *Bacteroides*, *Eubacterium*, *Peptostreptococcus*, *Clostridium* and *Bifidobacterium*

Table 5. Habitats of *Bifidobacterium* species in the intestine of humans and animals^a

Species	Human		Monkey	Dog	Pig	Cattle	Chicken	Mouse, rat	Guinea pig, rabbit
	Infant	Adult							
<i>B. bifidum</i>	+	+							
<i>B. longum</i>	+	M	+						
<i>B. infantis</i>	(M)								
<i>B. breve</i>	M								
<i>B. adolescentis</i> a	+	M	+						
<i>B. dentium</i>		M	M						
<i>B. adolescentis</i> c	+	M		M					
<i>B. adolescentis</i> d		M		M					
<i>B. pseudolongum</i>				M	M	M	M	M	
<i>B. animalis</i>								+	M
<i>B. gallinarum</i>									
<i>B. magnum</i>									M
<i>B. thermophilum</i>					M		M		

^aSymbols: M, major component; (M), formerly major component; +, occasionally isolated.

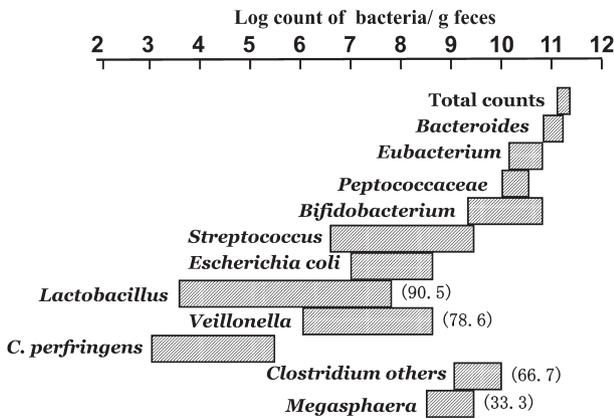


Fig. 4. Composition of fecal microbiota of adults. Figures in parentheses indicate the frequency (%) of detection. Bacterial groups without parentheses: the frequency of detection is 100%.

predominate here. *Enterobacteriaceae*, *Enterococcus*, *Lactobacillus*, *Veillonella* and *Staphylococcus* are only detected at levels of about 10^5 to 10^7 /g.

The composition of the microbiota in the lower large intestine is the same as that of the fecal flora.

Succession of human intestinal microbiota associated with age

The fetus lives in a completely germfree environment in utero in the mother.

After birth, it rapidly becomes colonized by bacteria.

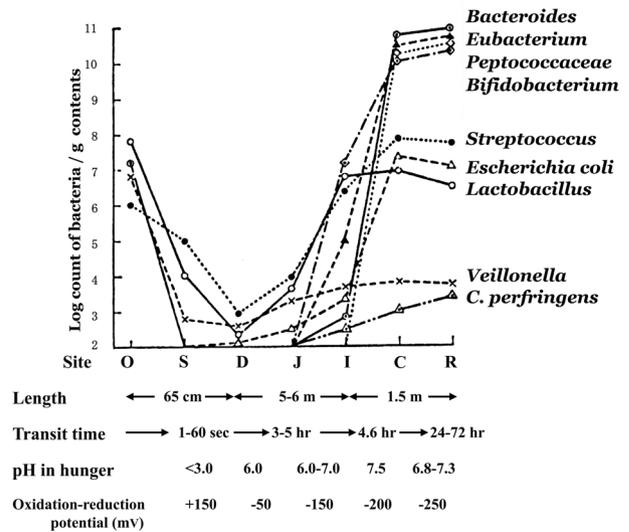


Fig. 5. Microbiota of various regions of the GI tract of human adults. O, oral cavity; S, stomach; D, duodenum; J, jejunum, I, ileum; C, cecum; R, rectum.

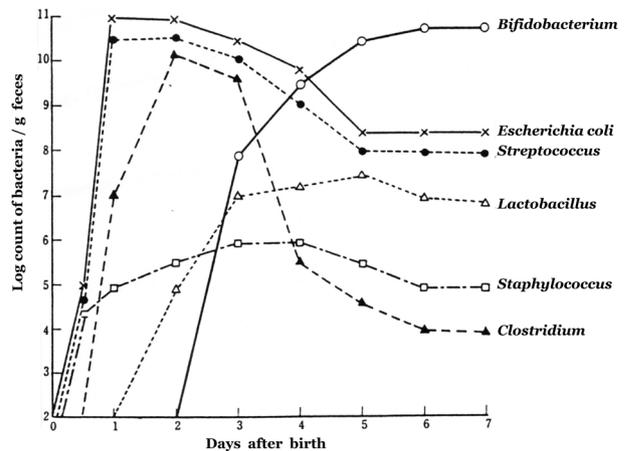


Fig. 6. Changes in the fecal microbiota during the first 7 days after birth.

On the first day after birth, *E. coli*, *Enterococcus*, *Lactobacillus*, *Clostridium* and *Staphylococcus* are found in the stools of almost all newborns, with total bacterial counts reaching 10^{11} per gram.

In breast-fed infants, *Bifidobacterium* generally begins to appear about 3 days after birth, and the previously appearing bacterial groups begin to decrease. On the fourth to seventh day, *Bifidobacterium* becomes predominant, with counts of 10^{10} to 10^{11} per gram. In contrast, the bacterial counts of *E. coli*, *Streptococcus*, *Staphylococcus*, *Bacteroides* and *Clostridium* are

Table 6. Fecal microbiota of different age groups of humans

Bacterial groups	17 infants aged 1-4 days	60 infants aged 5-90 days	29 children aged 4-6 years	29 adults aged 20-64 years	72 senile men aged 65-86 years
Total count	10.1±0.5 ^a	10.5±0.6	10.8±0.3	10.8±0.4	10.5±0.5
Bacteroidaceae	8.6±1.7 (41) ^b	8.2±2.3 (57)	10.4±0.6 (100)	10.3±0.6 (100)	10.0±0.8 (100)
Eubacteria and anaerobic lactobacilli	0 (0)	9.7±0.5 (7)	9.9±0.4 (24)	9.9±0.8 (93)	9.5±0.9 (76)
Anaerobic Gram-positive cocci	0 (0)	9.0±0.6 (5)	8.1±0.9 (14)	8.9±1.8 (52)	7.7±2.2 (35)
Bifidobacteria	9.3±2.5 (47)	9.9±1.5 (90)	10.1±0.6 (97)	9.8±0.7 (100)	9.4±0.8 (85)
Streptococci	8.5±1.7 (100)	8.1±1.9 (100)	7.8±1.3 (100)	7.7±1.3 (100)	8.2±1.3 (100)
Enterobacteriaceae	9.3±1.3 (100)	8.8±2.0 (100)	8.0±1.4 (100)	8.2±1.3 (100)	7.8±1.3 (100)
Lactobacilli	6.4±1.9 (53)	7.3±2.4 (75)	7.0±1.8 (93)	6.7±1.8 (100)	8.0±1.5 (99)
Veillonellae	5.6±2.2 (29)	6.3±2.7 (55)	5.2±1.9 (86)	4.8±2.3 (55)	6.1±2.0 (64)
<i>Clostridium perfringens</i>	5.9±3.0 (35)	6.9±2.0 (32)	5.7±2.0 (79)	4.8±1.7 (52)	6.6±1.9 (92)
Staphylococci	6.2±1.7 (100)	6.8±1.7 (100)	4.0±1.3 (86)	4.4±1.8 (90)	4.3±2.1 (60)
Yeasts	3.5±1.3 (41)	4.0±1.6 (28)	4.2±1.3 (86)	3.7±1.2 (69)	4.6±1.5 (71)

^a Mean ± SD of log bacterial counts (when present). ^b Frequency of occurrence (%).

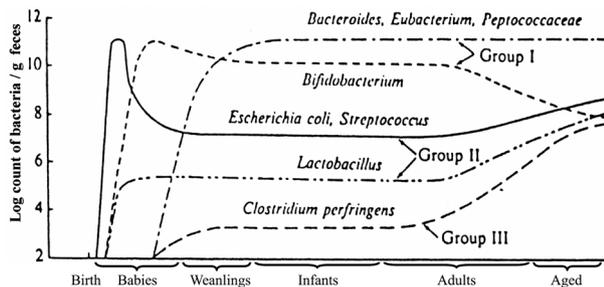


Fig. 7. Changes in the fecal microbiota with increasing age.

reduced to about 1 percent of those of *Bifidobacterium*, with *Bifidobacterium* accounting for close to 100 percent of the overall microbiota. By about day 7, the "balance" of the intestinal microbiota is nearly stable [36] (Fig. 6).

As the weaning period approaches, the intestinal microbiota begins to resemble that of the predominant Gram-negative rod flora seen in adults. The median total bacterial counts per gram of stool are about 10^{11} /g. There are increased counts of *Bacteroides*, *Eubacterium*, anaerobic *Streptococcus* and often *Clostridium*. *Bifidobacterium* then decreases to about 10 percent of the total flora. In addition, the pattern of *Bifidobacterium* (species and biovars) changes from an infantile pattern, consisting mainly of *B. infantis* and *B. breve*, to an adult pattern, consisting of *B. longum* and *B. adolescentis* [37].

The intestinal flora then begins to again show changes during the transition from middle age to old age. Along with slight reductions in total bacterial counts, there are reductions of *Bifidobacterium*. There are some individuals in whom *Bifidobacterium* becomes completely undetectable. However, the detection rate and

number of *Clostridium perfringens* markedly increases. *Lactobacillus*, *Enterobacteriaceae*, and *Enterococcus* also increase. This phenomenon is thought to result from the effect that aging of physiologic function in the host has on the intestinal bacterial microbiota. This itself may also further accelerate the aging process (Table 6, Fig. 7).

Composition of the intestinal microbiota of various animal species

The compositions of the fecal microbiota of healthy adult animals of 12 different species have been analyzed [38] (Table 7). In general, animals of the same species had a common pattern of fecal microbiota, but patterns different from those of other species. In almost all animal species, the most predominant fecal microbiota were anaerobes, including *Bacteroidaceae*, *Bifidobacterium*, *Eubacterium*, *Lactobacillus*, *Peptococcaceae* and anaerobic curved rods. The numbers of lactobacilli and bifidobacteria varied with the species of animal. In the feces of humans, monkeys and guinea pigs, bifidobacteria outnumbered lactobacilli. Regarding the feces of chickens, pigs, dogs, mice, rats and hamsters, bifidobacteria were present in almost all individuals but in smaller quantities than lactobacilli. In the feces of rabbits and horses, bifidobacteria were occasionally demonstrated as being present but not consistently. They were never demonstrated as being present in the feces of cats and minks.

FACTORS AFFECTING THE COMPOSITION OF THE HUMAN INTESTINAL MICROBIOTA

Individual and day-to-day differences

Individual differences in the intestinal microbiota have

Table 7. Fecal microbiota of twelve adult animals of different species

Bacterial groups	Monkeys	Chickens	Pigs	Dogs	Cats	Minks
Total counts	10.7 ± 0.4 ^a (5) ^b	10.9 ± 0.2 (5)	10.8 ± 0.4 (5)	10.8 ± 0.2 (5)	10.2 ± 0.2 (5)	9.8 ± 0.2 (5)
Bacteroidaceae	10.1 ± 0.4 (5)	10.6 ± 0.2 (5)	10.3 ± 0.8 (5)	10.3 ± 0.3 (5)	9.7 ± 0.4 (5)	7.6 ± 1.5 (5)
Eubacteria	10.0 ± 0.6 (5)	10.2 ± 0.3 (5)	9.2 ± 1.0 (5)	9.9 ± 0.4 (5)	9.4 ± 0.5 (5)	8.4 ± 0.1 (5)
Peptococcaceae	9.8 ± 0.4 (5)	9.9 ± 0.1 (5)	9.8 ± 0.3 (5)	9.6 ± 0.5 (5)	9.6 ± 0.1 (5)	0 (0)
Anaerobic curved rods	9.4 ± 0.2 (2)	0 (0)	9.4 ± 0.3 (5)	8.8 ± 0.7 (2)	9.0 (1)	0 (0)
Bifidobacteria	9.8 ± 0.5 (5)	9.1 ± 0.9 (5)	9.0 ± 0.5 (5)	9.2 ± 0.8 (5)	0 (0)	0 (0)
Lactobacilli	8.9 ± 0.7 (5)	9.5 ± 0.5 (5)	9.9 ± 0.4 (5)	9.6 ± 0.6 (5)	5.2 ± 1.5 (4)	6.1 ± 0.1 (5)
Veillonellae	5.5 ± 1.9 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Clostridia	0 (0)	0 (0)	6.9 ± 1.0 (4)	5.6 ± 1.5 (3)	9.2 ± 0.4 (5)	7.4 ± 1.1 (5)
Fusiform bacteria	0 (0)	0 (0)	0 (0)	9.0 ± 0.7 (2)	0 (0)	0 (0)
Spirochaetes	10.2 (1)	0 (0)	9.5 ± 0.8 (3)	0 (0)	7.3 (1)	0 (0)
Enterobacteriaceae	7.2 ± 1.0 (5)	7.0 ± 0.4 (5)	8.1 ± 0.1 (5)	8.1 ± 1.0 (5)	7.9 ± 0.4 (5)	9.6 ± 0.1 (5)
Streptococci	7.3 ± 1.4 (5)	7.1 ± 0.4 (5)	7.9 ± 1.0 (5)	9.9 ± 0.4 (5)	8.5 ± 0.4 (5)	9.2 ± 0.3 (5)
Staphylococci	4.2 ± 0.5 (5)	6.8 ± 0.7 (5)	3.5 ± 1.1 (3)	3.4 ± 0.8 (4)	6.8 (1)	5.7 ± 0.8 (4)
Corynebacteria	0 (0)	8.6 (2)	6.5 ± 0.5 (2)	0 (0)	0 (0)	0 (0)
Bacilli	6.6 (1)	6.4 ± 1.2 (5)	6.4 ± 0.9 (5)	0 (0)	0 (0)	0 (0)
Yeasts	4.5 ± 1.4 (5)	4.2 ± 1.1 (5)	4.2 ± 0.1 (2)	3.4 ± 0.7 (4)	3.4 ± 1.6 (2)	5.7 ± 0.4 (5)

Bacterial groups	Mice	Rats	Hamsters	Guinea pigs	Rabbits	Horses
Total counts	10.7 ± 0.3 (5)	10.4 ± 0.2 (5)	10.3 ± 0.2 (5)	9.5 ± 0.2 (5)	9.7 ± 0.2 (5)	9.0 ± 0.4 (5)
Bacteroidaceae	10.5 ± 0.3 (5)	9.9 ± 0.2 (5)	9.9 ± 0.4 (5)	8.5 ± 0.7 (5)	9.6 ± 0.2 (5)	7.2 ± 1.6 (5)
Eubacteria	9.1 ± 0.7 (5)	9.5 ± 0.3 (5)	0 (0)	8.1 ± 0.4 (5)	5.6 ± 1.0 (2)	7.7 ± 0.3 (3)
Peptococcaceae	8.9 ± 0.2 (2)	9.3 ± 0.3 (5)	9.7 ± 0.2 (5)	9.1 ± 0.3 (5)	8.3 ± 1.0 (5)	6.8 ± 2.4 (5)
Anaerobic curved rods	8.9 ± 0.5 (3)	9.5 ± 0.4 (5)	9.2 ± 0.5 (5)	8.7 (1)	8.6 ± 0.3 (5)	8.3 ± 0.4 (5)
Bifidobacteria	7.1 ± 1.2 (4)	8.2 ± 0.8 (5)	9.0 ± 0.3 (5)	8.8 ± 0.3 (5)	7.8 (1)	8.5 ± 0.8 (5)
Lactobacilli	9.5 ± 0.4 (5)	9.6 ± 0.3 (5)	9.7 ± 1.2 (5)	8.2 ± 0.7 (5)	0 (0)	7.7 ± 0.5 (5)
Veillonellae	0	4.5 ± 0.3 (5)	4.5 ± 0.5 (5)	2.6 ± 0.3 (3)	0 (0)	4.6 ± 0.4 (5)
Clostridia	8.6 ± 0.5 (4)	2.1 (1)	0	0	2.3 (2)	7.5 ± 0.4 (5)
Fusiform bacteria	9.8 ± 0.5 (5)	9.2 ± 0.5 (5)	9.6 ± 0.7 (5)	0 (0)	0 (0)	0 (0)
Spirochaetes	7.5 ± 0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	7.6 ± 0.5 (5)
Enterobacteriaceae	4.7 ± 1.2 (5)	5.3 ± 1.4 (5)	6.3 ± 0.7 (5)	6.4 ± 1.6 (5)	3.5 ± 1.3 (4)	5.5 ± 1.0 (5)
Streptococci	5.6 ± 0.9 (5)	8.2 ± 0.6 (5)	5.1 ± 1.5 (5)	6.9 ± 1.8 (5)	3.6 ± 0.6 (3)	8.5 ± 0.8 (5)
Staphylococci	4.8 ± 0.7 (5)	5.8 ± 1.3 (5)	4.8 ± 0.6 (5)	7.3 ± 0.7 (2)	3.4 (1)	3.8 ± 0.6 (5)
Corynebacteria	0 (0)	0 (0)	0 (0)	8.3 ± 0.2 (4)	4.6 ± 0.4 (2)	3.9 ± 0.4 (2)
Bacilli	3.5 ± 0.4 (4)	0 (0)	4.4 (1)	7.9 ± 0.4 (5)	0 (0)	6.1 ± 1.0 (5)
Yeasts	0 (0)	0 (0)	0 (0)	2.4 (1)	4.3 (1)	2.8 ± 0.2 (4)

^a Mean ± SD of log bacterial counts (when present). ^b Figures in parentheses refer to the number of subjects that harbor the organism.

also been observed in adults. The intestinal microbiota of six adults were investigated in seven samples collected from each individual during a two-month period Table 8 [39]. As a result, it was revealed that the balances of the intestinal microbiota were nearly stable; day-to-day variations in the predominating bacterial populations were markedly small, but in the minor bacterial populations, they were unstable. The possible causes of the difference in balance of the intestinal flora are physical conditions of the digestive tract, such as peristalsis and the excretion of gastric juice or bile acids, and dietary habits.

Age-related differences in Bifidobacterium species or biotypes

Differences in the predominant *Bifidobacterium* species or biotypes in different age groups of humans were observed [37]. The results are shown in Table 9.

The bifidobacteria isolated from infants belonged to the species *B. bifidum* type b, *B. infantis* ss. *infantis*, *B. breve* ss. *breve*, *B. breve* ss. *parvulorum*, and *B. longum* ss. *longum* type b. These species and biotypes, except *B. longum* biotype b, occurred almost exclusively in infants. In contrast, *B. adolescentis* types a-b and *B. longum* type a were found in high numbers in the intestine of children, adults and senile men, although in the senile men, the occurrence of *B. adolescentis* type b was significantly higher than in the other age groups. These species and biotypes were only occasionally isolated from infants.

Age-related differences in Lactobacillus species

Lactobacilli isolated from infants belonged mainly to the *L. acidophilus* group, *L. salivarius* and *L. fermentum* (*L. reuteri*). After weanings, *L. plantarum*, *L. casei*, *L. brevis*, and *L. buchneri*, which are thought to be of diet

Table 8. Individual and daily differences in the fecal microbiota of 6 healthy adults

Bacterial group	VPA♂	VPB♂	VPC♂	VPD♂	VPE♂	VPF♂	Significance ^b	
	Age 24	Age 24	Age 32	Age 32	Age 32	Age 42	Indv/indiv	Day/day
Total bacteria	11.0±0.2* (100)	11.1±0.1 (100)	11.3±0.2 (100)	11.2±0.2 (100)	11.2±0.2 (100)	11.3±0.2 (100)	•	•
Bacteroidaceae	10.8±0.2 (100)	10.7±0.1 (100)	10.9±0.2 (100)	11.0±0.3 (100)	10.9±0.2 (100)	10.8±0.2 (100)	•	•
Eubacterium	9.9±0.3 (100)	10.4±0.3 (100)	10.6±0.4 (100)	10.3±0.2 (100)	10.3±0.2 (100)	10.7±0.4 (100)	**	•
Peptococcaceae	10.1±0.4 (100)	10.2±0.4 (100)	10.2±0.3 (100)	10.4±0.2 (100)	9.9±0.4 (100)	10.3±0.3 (100)	•	•
Bifidobacterium	9.8±0.4 (100)	9.8±0.6 (100)	10.2±0.3 (100)	8.9±0.8 (100)	10.3±0.4 (100)	10.8±0.2 (100)	**	•
Streptococcus	7.9±1.0 (100)	7.9±0.7 (100)	7.9±0.6 (100)	9.6±0.5 (100)	8.2±0.6 (100)	5.7±1.3 (100)	**	•
Enterobacteriaceae	7.9±0.3 (100)	7.9±0.9 (100)	7.3±0.6 (100)	8.1±0.7 (100)	8.1±0.6 (100)	8.4±0.4 (100)	**	•
Lactobacillus	4.0±1.7 (100)	4.5±1.2 (100)	6.0±1.2 (100)	7.9±0.3 (100)	7.8±0.8 (100)	2.8±0.7 (43)	**	•
Veillonella	8.9±0.9 (100)	7.0±1.1 (71)	0 (0)	8.9±0.3 (100)	6.7±1.0 (100)	6.4±0.3 (100)	**	•
<i>C. perfringens</i>	4.6±1.1 (71)	3.8±1.3 (43)	3.4±0 (14)	5.1±0.9 (57)	4.1±1.7 (57)	4.7±0.6 (29)	•	•
Clostridium—other	9.7±0.3 (100)	9.0±0 (14)	10.1±0.4 (14)	9.3±0.7 (100)	9.5±0.5 (100)	9.5±0.3 (71)	**	•
Curved rods	9.3±0 (14)	9.1±0.7 (29)	9.9±0.5 (100)	0 (0)	0 (0)	0 (0)	**	•
Megasphaera	0 (0)	0 (0)	8.8±0.5 (100)	0 (0)	0 (0)	9.2±0.3 (43)	**	•
Staphylococcus	2.8±0.5 (100)	2.6±0 (14)	3.0±0 (100)	3.7±0.6 (100)	3.3±1.1 (86)	2.8±0.6 (71)	**	•
Yeasts	2.6±0.6 (43)	4.4±2.0 (57)	3.8±0 (14)	2.3±0 (14)	5.6±0.3 (71)	2.7±0.3 (57)	**	•

^a Mean ± SD of log bacterial counts (% the rate of occurrence).

^b Significant difference: *p<0.05; **p<0.01; ***p<0.001.

Table 9. Frequency of occurrence of species and biotypes of bifidobacteria in feces of various age groups of humans^a

Species and biotype	Age groups			
	Infants	Childrens	Adults	Senile men
No. of total specimens	52	28	42	18
<i>B. bifidum</i> type a	2	11	7	3
b	10		1	1
<i>B. infantis</i> ss. <i>infantis</i> type a	16			
b	13			
ss. <i>liberorum</i>	2	1		
ss. <i>lactentis</i>	1			
<i>B. breve</i> ss. <i>breve</i> type a	1			1
b	1			
c	3			
ss. <i>parvulorum</i> type a	6			
b	2	1		
<i>B. adolescentis</i> type a	5	12	17	4
b	1	8	22	15
c	8	14	23	7
d	1	1	5	1
<i>B. longum</i> type a	5	11	20	5
b	14	9	7	1

^a Figures indicate the numbers of specimens.

Table 10. Differences in *Lactobacillus* species in different age groups

Species	Infants (66)*	Children (29)	Adults (42)	Elderly (21)
<i>L. lactis</i>				2
<i>L. acidophilus</i> group	33	21	22	11
<i>L. salivarius</i>	15	10	19	10
<i>L. plantarum</i>	1	4	9	6
<i>L. casei</i>		6	5	6
<i>L. brevis</i>			4	8
<i>L. buchneri</i>		17	1	1
<i>L. fermentum (reuteri)</i>	20		15	11

*Number of subjects.

origin, were also detected (Table 10).

Differences of the intestinal microbiota in breast-fed and bottle-fed infants

Since the work of Tissier, it has been believed that bifidobacteria are found exclusively in feces of breast-fed infants, whereas in bottle-fed infants, *Lactobacillus acidophilus* is the most commonly found organism. According to our study [40], however, the differences in the occurrences and numbers of bifidobacteria between breast-fed and bottle-fed infants were not significant. Bifidobacteria were present in all 30 breast-fed infants and in 29 bottle-fed infants, with mean counts (when present) of $10^{10.7}/g$ and $10^{10.0}/g$, respectively (Table 11). The principal difference between breast-fed and bottle-fed infants was that in bottle-fed infants, the numbers of enterobacteriaceae, streptococci and anaerobes other than bifidobacteria were significantly greater than in breast-fed infants.

Clinic-dependent Bifidobacterium species or biotypes in the infant intestine

Variations were observed in the occurrences of *Bifidobacterium* species or types in infants in different clinics [37]. In general, all infants in the same clinic harbored a similar predominating *Bifidobacterium* strain(s). *B. longum* type b was a relatively common species isolated from the infants of Clinic A but was only occasionally isolated from the infants of Clinics B and C. *B. infantis* ss. *infantis* type b was a predominant species among infants of Clinic B and was less frequently isolated from infants of Clinic A and C. *B. infantis* ss. *infantis* type a was the most common bifidobacteria isolated from infants in Clinic C but was not isolated in other clinics. This finding suggested that one *Bifidobacterium* strain may be transmitted from infant to infant through the hands of nurses.

Table 11. Comparison of fecal microbiota between bottle-fed infants and breast-fed infants

Organisms	Bottle-fed infants	Breast-fed infants
Enterobacteriaceae	9.5±0.5 ^a (100) ^b	8.6±1.3** (100)
Streptococci	9.8±0.6 (100)	7.9±1.5** (100)
Staphylococci	5.5±1.7 (100)	5.8±1.9 (97)
Lactobacilli	7.2±1.5 (78)	6.8±1.7 (80)
Bifidobacteria	10.0±2.2 (97)	10.7±0.9 (100)
Eubacteria	7.3±4.1 (78)	3.1±4.6** (33)**
Bacteroidaceae	9.9±1.1 (100)	6.1±4.3** (67)**
Peptococcaceae	7.9±4.0 (80)	2.4±4.1** (27)**
<i>Clostridium perfringens</i>	6.4±2.5 (50)	1.0±2.3** (17)*
Clostridia-other	0.9±2.6 (10)	1.3±3.2 (13)
Veillonellae	5.9±4.6 (63)	5.8±3.4 (83)

^a Mean ± SD of log bacterial counts.

^b Frequency of occurrence (%).

Significant difference: **p<0.01; *p<0.05.

Succession of the species and biovars of bifidobacteria found in infants

In 1900, Tissier discovered *B. bifidum* in the feces of infants. In 1950, Norris et al. [41] isolated *Lactobacillus parabifidus* strain Timberlain (ATCC 17930) from infant feces. This strain is considered synonymous to *B. infantis* ss. *liberatorum* György [42] originally designated the organism *L. bifidus* var. *penn* (subsequently changed to *L. bifidus* var. *pennsylvanicus*) isolated from infants. This strain belongs to *B. bifidum*. Dehnert [43] reported his group IV (*B. infantis* ss. *lactentis*) as the predominant bifidobacterial group in the stools of breast-fed infants. Reuter [44] and I [17] reported that *B. infantis* ss. *infantis* predominated in the feces of breast-fed infants. Seeliger and Werner [45] reported that Dehnert's group III (*B. infantis*) was predominant in Bonn (West Germany), although group IV (*B. infantis* ss. *lactentis*) was also present. Petuely and Lindner [46] noted the predominance of Dehnert's group IV (*B. infantis* ss. *lactentis*) in the stools of breast-fed infants. Werner [47] also reported the prevalence of Dehnert's group IV in breast-fed infants and stated that group IV had been found only in the stools of breast-fed infants or of infants fed maternal milk together with cow's milk. In the year after 1980, all of the subspecies of *B. infantis* became very difficult to isolate from infant feces, and *B. breve* ss. *breve* predominates in the feces of both breast-fed and bottle-fed infants. The factors affecting such succession of the predominant bifidobacterial species and biovars are still unknown (Table 12).

Influence of diet

It is often reported that the compositions of intestinal microbiota are influenced by diet. The fecal microbiota of

Table 12. Succession of species and biovars of bifidobacteria occurring in the feces of infants

Species and biovars	Dehnert (1957) Br ^a	Reuter (1963-64) Br+Bo ^b	Mitsuoka et al. (1969) Br+Bo (59) ^c	Mitsuoka et al. (1978)		Mitsuoka et al. (1980)
				Br (20)	Bo (48)	Bo (65)
<i>B. bifidum</i> a		+ ^d	2 ^e	2	4	22
<i>B. bifidum</i> b		+	10	12	3	23
<i>B. infantis</i> ss. <i>infantis</i> a		++	16	1	1	9
<i>B. infantis</i> ss. <i>infantis</i> b		++	13	0	0	3
<i>B. infantis</i> ss. <i>infantis</i> c		++	0	0	1	3
<i>B. infantis</i> ss. <i>liberorum</i>		—	2	0	1	4
<i>B. infantis</i> ss. <i>lactentis</i>	++?	—	1	0	0	0
<i>B. breve</i> ss. <i>breve</i> a		+	1	9	15	65
<i>B. breve</i> ss. <i>breve</i> b		+	1	16	34	141
<i>B. breve</i> ss. <i>breve</i> c		+	3	9	9	35
<i>B. breve</i> ss. <i>breve</i> d		+	0	2	2	0
<i>B. breve</i> ss. <i>parvulorum</i> a		+	6	4	6	19
<i>B. breve</i> ss. <i>parvulorum</i> b		+	2	0	2	8
<i>B. adolescentis</i> a		+	5	3	16	49
<i>B. adolescentis</i> b		+	2	1	3	12
<i>B. adolescentis</i> c		+	8	3	5	25
<i>B. adolescentis</i> d		+	1	1	3	14
<i>B. longum</i> a		+	5	2	4	12
<i>B. longum</i> b		++	14	12	10	37
<i>Bifidobacterium</i> -other			7	4	13	26

^a Br: Breast-fed infant. ^b Bo: bottle-fed infant. ^c Number of specimens. ^d Frequency of isolation. ^e Number of specimens harbored.

15 healthy elderly persons with a median age of 84 years in a rural area whose inhabitants tend to be long-lived (Yuzurihara, Uenohara, Yamanashi Prefecture) was compared with the microflora of individuals with a median age of 68 years in an urban area (Tokyo). The diet of the elderly persons in the Yuzurihara area is characterized by a high intake of dietary fiber. Total numbers of anaerobic bacteria were significantly smaller in the elderly persons in the Yuzurihara area than those of the individuals in the Tokyo area. A significantly large number of bifidobacteria, but not of lecithinase-negative clostridia, was observed in the elderly persons in the Yuzurihara area. Large numbers and high incidences of bacilli and lecithinase-positive clostridia (mainly *C. perfringens*) were found in the elderly persons in the Tokyo area (Table 13) [48]. Differences in the fecal microbiota between elderly persons in the Yuzurihara area and those in the Tokyo area might be due to a difference in the intake of dietary fiber.

Influence of diseases

a) Colorectal cancer and stomach cancer

The fecal microbiota of 54 healthy adults was compared

with that of 44 patients with colorectal cancer and that of 39 patients with stomach cancer. In the patients with colorectal cancer, the *Eubacterium* and *Bacteroides melaninogenicus* counts were significantly higher, and in the patients with stomach cancer, the *Bifidobacterium* and *Bacteroidaceae* counts were significantly lower and those of *C. perfringens* and *Streptococcus* were significantly higher. In addition, the detection rates of *Micrococcaceae* and *Pseudomonas* were significantly higher [49].

b) Ulcerative colitis and Crohn's disease

Comparing the fecal microbiota of patients with ulcerative colitis and Crohn's disease with those of healthy adults, it was found that total bacterial counts, especially those of *Bacteroidaceae*, *Eubacterium*, *Peptococcaceae* and *Pseudomonas*, were significantly decreased in both groups of patients. In addition, the *Lactobacillus*, *Streptococcus* and *Pseudomonas* counts were significantly decreased and that for *Enterobacteriaceae* was significantly increased in patients with ulcerative colitis [49].

c) Dementia senilis

Examination of the fecal microbiota of 7 patients (aged 61 to 90 years old) with dementia senilis showed

Table 13. Comparison of the fecal microbiota of aged persons in Yuzurihara and Tokyo

Fecal microflora	No. ^a (frequency) ^b in	
	Aged persons in Yuzurihara	Aged persons in Tokyo
<i>Bacteroidaceae</i>	10.8 ± 0.4 (15/15)	10.9 ± 0.2 (15/15)
<i>Mitsuokella</i>	8.6 ± 0.5 (3/15)	0 (0/15)
<i>Eubacteria</i>	10.2 ± 0.3 (15/15)	10.5 ± 0.7 (15/15)
<i>Peptococcaceae</i>	10.2 ± 0.4 (15/15)	10.3 ± 0.4 (15/15)
<i>Gemmiger</i>	9.4 ± 0.2 (3/15)	9.4 ± 0.8 (2/15)
<i>Bifidobacteria</i>	9.6 ± 0.5 (13/15)	9.1 ± 0.5 ^c (11/15)
<i>Veillonella</i>	6.5 ± 2.0 (15/15)	5.6 ± 1.8 (12/15)
<i>Megasphaera</i>	9.0 ± 2.0 (4/15)	9.4 ± 0.6 (2/15)
Curved rods	9.7 ± 0.6 (8/15)	9.7 ± 0.1 (2/15)
Lecithinase-positive clostridia	6.6 ± 1.6 (8/15)	7.2 ± 1.8 (13/15)
Lecithinase-negative clostridia	8.7 ± 0.7 (15/15)	9.7 ± 0.6 ^d (15/15)
<i>Lactobacillus</i>	6.7 ± 1.8 (15/15)	7.2 ± 1.8 (15/15)
Total anaerobes	10.9 ± 0.3 (15/15)	11.1 ± 0.2^c (15/15)
<i>Enterobacteriaceae</i>	8.5 ± 1.0 (15/15)	8.4 ± 0.8 (15/15)
<i>Streptococcus</i>	7.1 ± 1.5 (13/15)	6.7 ± 1.0 (15/15)
<i>Staphylococcus</i>	3.3 ± 0.4 (5/15)	3.8 ± 0.9 (3/15)
<i>Pseudomonas</i>	3.0 (1/15)	3.0 ± 1.1 (3/15)
<i>Corynebacteria</i>	0 (0/15)	2.9 (1/15)
<i>Bacilli</i>	3.3 (1/15)	5.4 ± 1.6 (11/15) ^c
Yeasts	4.2 ± 1.0 (13/15)	4.8 ± 1.5 (12/15)
Total aerobes	8.6 ± 1.0 (15/15)	8.3 ± 0.5 (15/15)
Total bacteria	10.9 ± 0.3 (15/15)	11.1 ± 0.2^c (15/15)

^a Bacterial counts are expressed as the mean ± standard deviation of log10 per gram (wet weight) of feces.

^b Frequency occurrence is expressed as the number of subjects with the organism detected/number of subjects examined.

^c Statistically significant at the p<0.05 level when compared with elderly persons in the rural area (Student's t test for the bacterial counts and chi-square test for the frequency of occurrence).

^d Statistically significant at the p<0.001 level when compared with elderly persons in the rural area.

that the total bacterial counts were moderately lower and the counts of patients with dementia senilis were significantly higher, as compared with our earlier study examined healthy 72 senile persons (aged 65–86 years old). This result suggested that the fecal microbiota is related to dementia senilis [49].

SIGNIFICANCE OF THE INTESTINAL MICROBIOTA IN HEALTH AND DISEASE

Relationships between the intestinal microbiota and the host

The intestinal flora possess diverse enzymes that perform extremely varied types of metabolism in the intestine, converting substances into compounds that

can be beneficial or detrimental to the host (Fig. 8) [50]. In general, metabolism within the tissues of an organism, particularly hepatic metabolism, involves oxidation and biosynthesis with glucuronic acid and sulfuric acid conjugation. This leads to production of polar, water-soluble substances. In contrast, metabolism by the intestinal flora primarily involves reduction and hydrolysis. The harmful bacteria may form substances that are noxious to the host. Among these are certain putrefactive substances (ammonia, hydrogen sulfide, amines, phenols, indoles, etc.) and secondary bile acids. These substances may injure the intestine directly and are also partially absorbed, potentially contributing throughout the host's life to aging and geriatric diseases such as cancer, arteriosclerosis, hypertension, liver disorders, autoimmune diseases and immunosuppression. These substances may affect nutrition, physiologic function, drug efficacy, carcinogenesis and aging, as well as the host's resistance to infection.

Intestinal bacteria influence both the health and disease of the host. I have postulated three groups of intestinal bacteria: the first group consists of organisms symbiotic to the host and constitutes the predominant flora, the second group consists of ubiquitous organisms such as *E. coli* or *Streptococcus* group but with counts in the normal host not being predominant, and the third group of bacteria consists of true pathogenic bacteria, sometimes producing infection, with counts that are normally low (Fig. 9) [50].

The bacterial cell components are known to stimulate immunity. The intestinal microbiota is known to be important in the development of many lymphoid cells in the gut-associated lymphoid tissue (GALT) and the induction of a mucosal immune response. Since lymphocytes stimulated in GALT subsequently migrate to other lymphoid tissues, the intestinal microbiota may also influence the proliferative response of spleen and lymph node cells.

Lactic acid bacteria possess various immunological functions, including mitogenic activity, adjuvant activity, macrophage activation, antibody production enhancement, interferon production and antitumor effects. Furthermore, both the cell wall and cytoplasm of lactic acid bacteria were found to have induced mitogenic responses of spleen cells.

On the other hand, some intestinal bacteria have potential pathogenicity. Aging; the administration of antibiotics, immunosuppressive agents, anticancer agents or adrenocortical (steroid) hormones; stresses; or radiation therapy can lead to decreased resistance of the host, and some intestinal bacteria can then invade

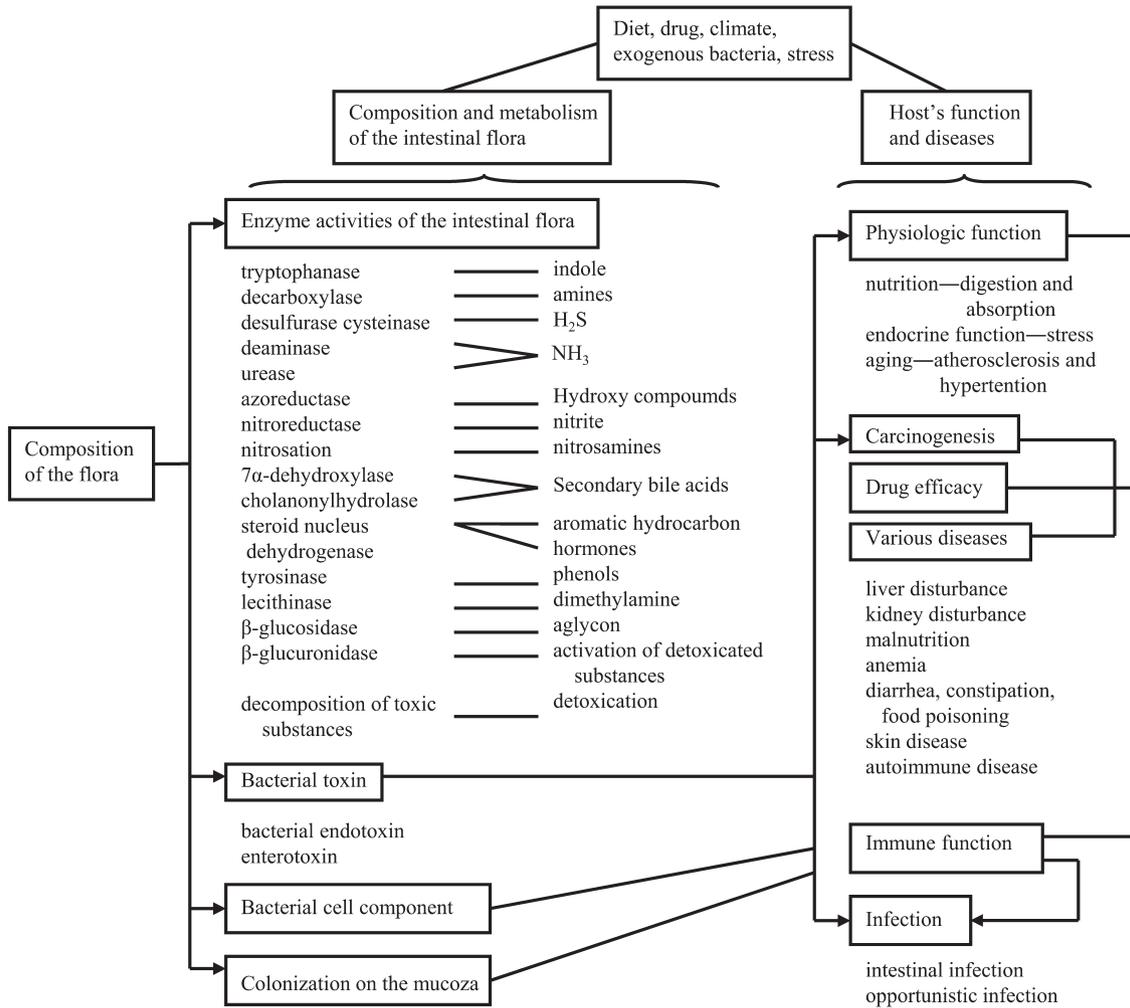


Fig. 8. The metabolic activities of intestinal bacteria.

the viscera through the gastrointestinal tract and exhibit pathogenicity. These organisms can cause a so-called opportunistic infection: sepsis; endocarditis; brain, hepatic or pulmonary abscesses; cystitis; or vaginitis.

The composition of the intestinal flora reflects intestinal metabolism. This also has a variety of effects on the organism. The ingredients of the food ingested each day and substances secreted and excreted by the intestine are converted into various substances. This therefore has a profound influence on factors such as nutrition, drug effects, physiologic function, aging, carcinogenicity, immunity and infection within the host.

Microbial populations in the gastrointestinal tract are known to form a barrier against the proliferation of exogenous pathogens. Thus, host susceptibility to specific enteric infections is influenced by the nature of the intestinal microbiota. One of the mechanisms may

be related to the colonization of the indigenous flora, which prevents colonization of the invader by competing effectively for essential nutrients or attachment sites on the epithelium. Another mechanism to prevent colonization of exogenous pathogens is production of bacteriocidal or bacteriostatic agents.

Usefulness of gnotobiotics and human flora animals

Gnotobiotic animals associated with human intestinal bacteria and ex-germfree animals associated with human fecal flora provide useful models for studying the role of the intestinal flora in carcinogenesis and ageing.

a) Liver tumor experiment

The effect of intestinal bacteria on liver tumorigenesis in gnotobiotic C3H/He male mice monoassociated, diassociated or polyassociated with various intestinal bacteria has been investigated [51]. As shown in Table

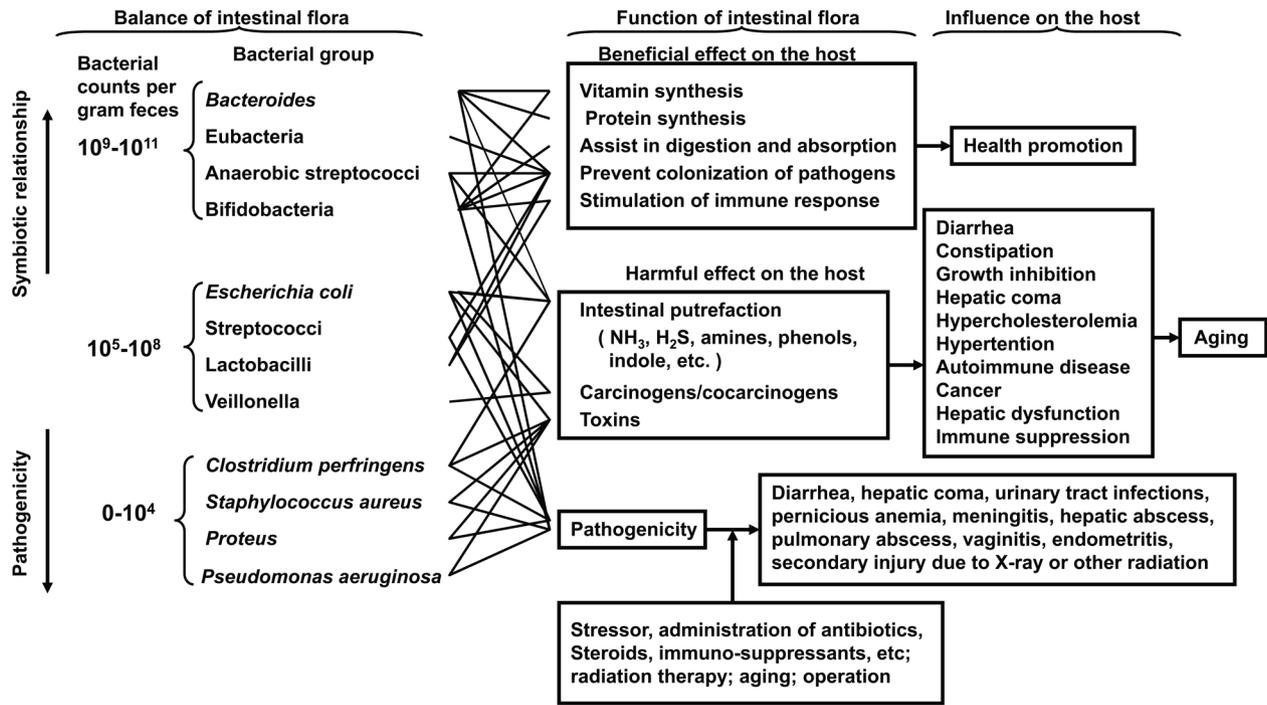


Fig. 9. Relationship between intestinal bacteria and the host.

14, the incidence of liver tumors was higher in most of the gnotobiotics and conventional mice than in the germfree mice. Liver tumors were observed in 100% of mice associated with a bacterial combination of *E. coli*, *S. faecalis* and *C. paraputrificum*, while they were only observed in 30% of germfree mice and 75% of conventional mice. However, this tumor-promoting effect of intestinal bacteria was suppressed by 46% by addition of *B. longum* to the promoting combination and by 65% by addition of *L. acidophilus*. 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 stimulate immunity of the host or to detoxify carcinogens.

b) Aging experiment

The effect of intestinal flora on life span has also been studied. Germfree, conventional and gnotobiotic (GB) CF#1 female mice were produced. GB-1 were associated with *E. coli*, *Enterococcus faecalis*, *Bacteroides vulgatus*, *Eubacterium aerofaciens*, *Bifidobacterium longum* and *C. perfringens*, and those associated with the same combination of intestinal bacteria without *B. longum* (GB-2) or *C. perfringens* (GB-3) were produced and maintained until their natural deaths [49] (Table 15). The verage life spans were longest in germfree mice (96.3

weeks) and shortest in conventional (78.2 weeks) mice. Of the gnotobiotics, the average life spans were shorter in GB-2 (80.7 weeks) than in GB-1 and GB-3 (87.1 weeks). These findings suggest that the presence of *B. longum* may be related to longevity in gnotobiotic animals.

In the two gnotobiotic studies reported here, almost all of the bacteria inoculated were established in high numbers in the intestine of gnotobiotic mice, and the bacterial numbers differed significantly from the numbers of the same bacteria in the human intestine, but it was not possible to monocontaminate animals with *L. acidophilus*. Thus, it is not easy to extrapolate from an experiment in laboratory animals to humans.

CONCLUSION

In 1953, our group began research on the intestinal microbiota. At first, we made it possible to cultivate and isolate intestinal bacteria for comprehensive investigation. Then, we developed a classification and identification system of intestinal anaerobes and clarified the characteristic composition and habitat of the intestinal microbiota and lactic acid bacteria of humans and animals. Based on these results, we have carried out ecological research on the intestinal microbiota of humans and animals, and I have put forward a hypothesis

Table 14. Incidence of liver tumors in gnotobiotic C3H/He male mice associated with human intestinal bacteria

Group	Organisms	No. of bacteria established log/g feces	No. of animals ^{a)}	Animals with liver tumor (%)	Tumor nodules per mouse
GF	Germfree C3H/He		139	30	0.4
CV	Conventionalized C3H/He		56	75	1.3
Gb-1	<i>Escherichia coli</i> M66	10.3	13	62	1.5
Gb-2	<i>Streptococcus faecalis</i> M266TA	9.7	18	67	1.0
Gb-6	<i>Bacteroides multiacidus</i> A4052	9.7	28	75	1.3
Gb-16	<i>Clostridium paraputrificum</i> VPI 1586	8.7	20	70	1.2
	<i>Clostridium paraputrificum</i> VPI 6558	8.7			
Gb-13	<i>Bifidobacterium longum</i> E194b	10.1	17	47	0.8
Gb-17	<i>Lactobacillus acidophilus</i> 1-9		27	48	0.7
	<i>Lactobacillus acidophilus</i> 1-65	9.4			
Gb-14	<i>Bacteroides vulgatus</i> M64	10.7	20	25	0.3
Gb-7	<i>Escherichia coli</i>	9.9	16	88	1.7
	<i>Clostridium perfringens</i> MAC521	9.5			
Gb-9	<i>Escherichia coli</i> M66	9.7	20	80	1.5
	<i>Streptococcus faecalis</i> M266TA	9.9			
	<i>Bacteroides vulgatus</i> M64	10.1			
Gb-19	<i>Escherichia coli</i> M66	9.8	19	42	0.7
	<i>Streptococcus faecalis</i> M266TA	10.3			
	<i>Lactobacillus acidophilus</i> F164	8.5			
	<i>Bacteroides vulgatus</i> M67	10.8			
Gb-20	<i>Escherichia coli</i> M66	10.2	10	100	2.8
	<i>Streptococcus faecalis</i> M266TA	10.2			
	<i>Clostridium paraputrificum</i> VPI 1586	9.5			
	<i>Clostridium paraputrificum</i> VPI 6558				
Gb-21	<i>Escherichia coli</i> M66	9.3	13	46	0.7
	<i>Streptococcus faecalis</i> M266TA	10.2			
	<i>Clostridium paraputrificum</i> VPI 1586	9.6			
	<i>Clostridium paraputrificum</i> VPI 6558				
	<i>Bifidobacterium longum</i> E194b	9.8			
Gb-22	<i>Escherichia coli</i> M66	9.9	20	65	1.0
	<i>Streptococcus faecalis</i> M266TA	10.1			
	<i>Clostridium paraputrificum</i> VPI 1586	9.6			
	<i>Clostridium paraputrificum</i> VPI 6558				
	<i>Lactobacillus acidophilus</i> 1-9	8.9			
	<i>Lactobacillus acidophilus</i> 1-65	8.9			

^{a)} Mice were euthanized under ether anesthesia when they were 12 months old.

Table 15. Bacterial strains associated and bacterial number of bacteria (log/g feces) in gnotobiotic (GB) DF#1 mice and differences in the longevity among germfree, gnotobiotic and conventional CF#1 female mice

Bacteria / life span	GF *	GB-3	GB-1	GB-2	CV***
Total counts	0	10.8 **	10.9	10.9	10.7
<i>Bifidobacterium longum</i> E194	0	9.9	8.9	0	7.1
<i>Clostridium perfringens</i> MAC521	0	0	8.9	9.2	8.6
<i>Escheria coli</i> 123	0	9.4	9.5	9.8	4.7
<i>Enterococcus faecalis</i> 1-12	0	10.1	9.8	9.8	5.6
<i>Bacteroides vulgatus</i> M-64	0	9.8	10.3	10.3	10.5
<i>Eubacterium aerofaciens</i> 151	0	10.4	10.6	10.7	9.1
Other bacteria	0	0	0	0	10.1
Average lifespan (weeks)	96.3 ± 14.6	87.1 ± 17.6	87.1 ± 19.9	80.7 ± 21.5	78.2 ± 22.2

*GF: germfree mice. **Log. Number/gram feces. *** Bacterial strains and number of CVs (conventional mice) are indicated as bacterial groups and their numbers in conventional DF#1 mice.

concerning the “relationships between the intestinal microbiota and the host”. Furthermore, using gnotobiotic mice, the effects of intestinal bacteria on longevity and tumorigenesis in mice have been studied, and it was clarified that food exerts a profound effect on the intestinal microbiota and health. At this point of time, I judged that a new interdisciplinary science “*intestinal bacteriology*”, had been established.

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