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The *Lactobacillus* and *Bifidobacterium* Microflora of the Human Intestine: Composition and Succession

Gerhard Reuter

Department of Food Hygiene, Veterinary Faculty,
Free University of Berlin, Bruemmerstr. 10, D - 14195 Berlin (Dahlem), Germany

Abstract

***Lactobacillus* and bifidobacterial cultures are increasingly used as probiotics in pharmaceuticals and in foods. The selection of strains is performed often for technological rather than for microecological reasons. Detailed reports about species and strains composition of these microorganisms in the intestinal microflora of man are rare. Our investigations were performed with samples originating from infants and adults, taken from faeces and from upper sections of the intestinal tract including mouth and stomach, and from caecum and colon. Post mortem cases as well as test subjects under physiological conditions were analyzed using an automatic capsule system sampling at defined times in different parts of the intestinal tract. The fate of selected strains after oral intake was studied, too. Furthermore, influences of the microflora originating from food were considered.**

The identification of autochthonous (indigenous) and allochthonous (transient) species could be achieved with descriptions of new species in the genera *Lactobacillus* and *Bifidobacterium*. *L. gasseri* and *L. reuteri* proved to be predominant autochthonous *Lactobacillus* species in infants as well as in adults. Both species were occasionally present even in the stomach. This was also the case with an anaerobic lactic acid bacterium, previously named *Catenabacterium catenaforme*, later classified as *L. ruminis*, a non-motile variant of this species.

The bifidobacterial microflora differed in composition between infants and adults and in different stages of the host's life. Up to 5 species or special strains of bifidobacteria could be present in different, individually fixed, combinations. Species typical for infants were *B. bifidum, B. infantis*, *B. breve*, and *B. parvulorum*. Typical for adults were 4 different variants of *B. adolescentis*. *B. bifidum* and *B. longum* could often be found in both groups, but in lower numbers. *B. longum* showed some oxygen tolerance whereas *B. bifidum* and *B. adolescentis* required strict anaerobic and fastidious conditions for cultivation.

The autochthonous *Lactobacillus* and *Bifidobacterium* microflora in man will remain stable life-long. With lactobacilli, however, some successions may be caused by transient species derived from food or from the oral cavity, thus giving the impression of an altered microflora. Nevertheless *L. gasseri, L. reuteri, L. ruminis,* and to some degree, *L. salivarius,* may be present as autochthonous species all of the time. With bifidobacteria, a decreasing tendency in counts and in multiple composition in elderly people exists. Furthermore, this microflora is also influenced by consumption habits, which are probably caused by geographical circumstances.

Introduction

The gastrointestinal microflora is a very complex community. Within the gastrointestinal tract, different habitats have to be recognized, e.g. mouth, stomach, small intestine (especially lower jejunum and ileum) large intestine (caecum, colon) and rectum. Normally, near stability exists in these habitats. The balance is influenced primarily by the host's individuality. This means that interpersonal (individual) variations exist. Each person will have an individually fixed microflora as far as qualitative structure and the quantities of lactobacilli and bifidobacteria are concerned (Reuter,1963 1965a, b). This fact is of great interest, as more than 400 species within the intestinal microflora can be identified and may attain population levels nearly as high as 10^{12}/g in the colon (Mitsuoka, 1982, 1992; Tannock, 1999a).

An indigenous microflora can be recognized, consisting of autochthonous species (e.g. species, which are able to colonize the mucosal surface of the gastrointestinal tract due to special adhesion factors including compatibility with the immunological system of the host). These microorganisms have to be distinguished from allochthonous species, which may be present in the intestine, too, but which may only have a transient character. The presence of these strains in the intestinal tract will last for a limited time, probably only a few days (Tannock, 1999a). Especially with lactobacilli, a strict distinction between these two groups must be considered, due to the fact that large amounts of lactobacilli are enriched in fermented foods which will be transferred day by day through the stomach and small intestine into the large bowel. This supplementation depends on individual consumption habits (Reuter, 1965a). Some species will not survive stomach and duodenal passage easily, while others will resist very well. This may be the cause of many misinterpretations of the "normal" indigenous *Lactobacillus* microflora of man as described in the literature even in recent times, where even *L. plantarum* is mentioned as an autochthonous *Lactobacillus* species in the intestine (Ahrné *et al*., 1998).

Some reports in the literature ignore the differences of the intestinal microflora between infants on one hand,

*For correspondence. Email x; Tel. 030-83852796; Fax. 030-83852792.

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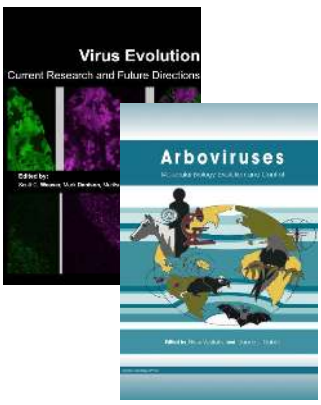

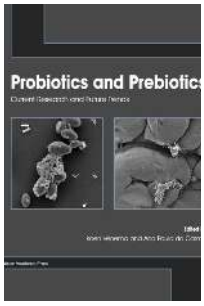

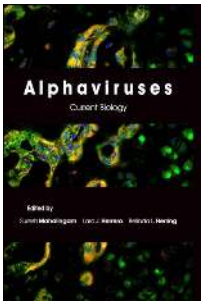

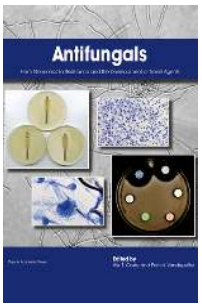

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and adults and elderly people on the other. In these age groups, lactobacilli and bifidobacterial populations are substantially different in structure and quantities. While lactobacilli are changing only quantitatively, probably after they have randomly colonized immediately after birth, the development of the bifidobacteria microflora is influenced by nutritional factors also (e.g. in-breast fed or formula-fed infants) or by the age of an individual.

In general, microbiological analyses of the human small intestine are difficult due to sampling problems. Many methods were tested dating from the distant past (1922-1925) up to recent times (1960-1970) (Lerche and Reuter, 1962; Reuter, 1975). The main problem seemed to be obtaining specimens correlating with the physiological status of the intestinal tract. Trials have been carried out during abdominal surgery procedures or in post mortem cases for a long time. This way of sampling does not appear to be quite realistic in general, however. Prior to surgery, the ingestion of food has been interrupted for some days, and in this fasting period, the intestine was cleaned up, emptied, or even pre-treated with antibiotics or other pharmaceuticals. In post mortem cases, the effect of clinical pre-treatment or the time elapsing between death and dissection may have caused alterations of the microflora. Better results were achieved with the application of transient capsules in different modifications, obtaining samples during passage through the intestinal tract by different mechanisms. Sampling with intubation and aspiration methods were other methods employed. Drainage sampling after ileostomy was sometimes used. Disadvantages exist with every method. Intubation methods may have been accompanied by an irritating effect causing changes in the physiology of the intestine, and consequently, in the microecology. Automatic capsules not connected with an intubation system sometimes produced problems such as recovery from the faeces at variable times after intake. Ileostomy-aspirates could have been influenced by oxygen and bowel motility factors.

The microecological balance in the different habitats of the intestine can have been disturbed considerably, however, depending on internal (intrinsic) and external (extrinsic) influences. Functional disturbances such as alterations of gastric acidity or bile secretion can influence the microflora fundamentally as well as the administration of pharmaceuticals with antimicrobial efficacy. Occasionally, usually suppressed components of the indigenous microflora can allow potential pathogens or toxinogenic strains to colonize and to multiply thus causing diarrhoea, flatulence, or different variations of colitis (haemorrhagic, pseudomembranous etc.)

One way to re-establish the ecological balance of a disturbed habitat in the intestinal or urogenital tract, is to apply components of the indigenous microflora by oral intake or rectal or vaginal instillation. These procedures have been applied for a very long time, but became more common again in the last decades of the past century as probiotic cultures were propagated for that purpose world-wide (Reuter, 1997; Tannock, 1999a).

The selection of suitable species or strains as probiotic cultures, however, is a very critical step (Bonaparte and Reuter, 1997). The descriptions of strains used by producers of probiotic cultures are often incomplete or even false. The choice of strains has often been done at random from culture plates. Occasionally, the selection of probiotic strains was determined preponderantly by technological reasons (e.g. viability, productivity, viability of the cultures) even if used in a pharmaceutical preparation (Reuter, 1969; Reuter *et al*., 2001). Probiotic cultures are often named as *L. acidophilus,* neglecting the fact that this term characterizes a group presently comprised of six separate species with few of them being relevant to human autochthonous microflora (Fujisawa *et al*., 1996). *L. fermentum* was often reported, not recognising that this species must be differentiated from other phenotypically similar ones such as *L. reuteri*, *L. oris*, and *L. vaginalis*. *L. fermentum* has its natural habitats in food, especially in dairy products, while the others are autochthonous species in humans. *Bifidobacterium bifidum* has been often cited as a component of probiotic food, disregarding the fact that this species requires the most strict anaerobic conditions for growth of all bifidobacteria of humans. Maintenance of viability of this species is not possible for the required shelf life in probiotic foods (Reuter, 1990). *B. longum* has been preferred because of its less demanding requirements for anaerobic conditions in foods and in pharmaceuticals (Dreher *et al*., 1991), taking into account that in many cases the species which was actually used, belonged to the related species *B. animalis* because of its higher tolerance to acidic conditions. Furthermore, a false declaration of strains of this last species was propagated by creating a new species taxon, *B. lactis*, which must be considered non-valid (Cai *et al*., 2000). The choice of strains as probiotic cultures should therefore be made more thoroughly with bifidobacteria than with lactobacilli.

The selection of strains out of the *L. casei* group is a special field of probiotic research (Tannock *et al*., 2000). Since *L. casei* and *L. rhamnosus* strains are transient organisms in the intestine, their use is justified mainly by technological reasons. Further interpretations are given in a recent paper (Reuter *et al*., 2001). A recent report upon adhesion properties of novel probiotic strains was published by Oweland *et al*. (2001).

I. Composition of the Microflora of the Human Small Intestine and the Fate of Cultures after Oral Intake

To provide representative sampling, different series of investigations both from post mortem cases and from samples obtained with an automatic capsule system from children 10-14 years of age as well as from adults mainly in the 20-40 years age group, were performed. Samples from faeces were investigated in parallel as far as possible (Lerche and Reuter, 1961, 1962; Reuter, 1961, 1963, 1965b, 1975; Hirtzmann and Reuter, 1963; Hirtzmann, 1965).

The selection of post mortem cases was made directly by the investigator. Non-physiological influences could be minimized due to the selection of cases of sudden death caused by heart attack or traffic accidents . Samples were taken by excision of ligated parts from the intestine containing ingesta, from the upper jejunum, the upper and the lower ileum, the caecum, and the descending colon

Table 1. Microecology (log_{10}/g) in different habitats of the intestine of 9 adults. Samples obtained from selected cadavers (≤ 2 days p.m., n = positive samples) (according to Lerche and Reuter, 1962; Reuter, 1975)

	jejunum		ileum		caecum/colon desc.	
	n	log_{10} (med)	n	log_{10} (med)	n	log_{10} (med)
yeasts and fungi	4	4.5-7.0 (6.7)	2	4.9-7.6	2	6.8-7.4
micrococci	3	4.7-7.2 (6.7)	1	6.7	1	6.4
streptococci*	4	6.1-8.0 (7.6)	6	5.9-8.0 (7.8)	8	6.7-8.2 (7.7)
enterobacteria	1	7.9	2	5.9-8.0	2	5.7-7.9
E. coli	4	7.3-7.8 (7.6)	5	4.8-8.7 (7.5)	8	6.9-8.6 (7.9)
bifidobacteria	0	–	2	7.4-8.0	5	7.6-10.2 (8.6)
bacteroides	3	6.3-9.4 (7.8)	3	7.8-9.4 (8.2)	6	6.4-11.2 (8.8)
lactobacilli**	7	5.6-9.4 (7.7)	6	6.0-8.4 (7.6)	9	6.2-10.2 (8.0)
L. gasseri	7	5.5-9.3 (6.9)	6	5.8-7.5 (6.8)	8	6.1-9.2 (6.9)
L. reuteri	5	5.8-8.4 (7.4)	5	5.8-7.5 (6.8)	8	5.5-7.4 (6.7)
L. salivarius	3	5.6-8.6 (7.8)	4	5.5-8.2 (6.7)	4	5.8-7.2 (6.3)
L. casei	1	8.3	1	6.3	5	5.5-6.8 (5.8)
L. plantarum	1	8.7	1	6.3	3	5.5-7.0 (7.0)
L. buchneri	2	3.4-7.5	1	6.0	0	–

* : including enterococci
** : including anaerobic Lactobacillus sp. (*Catenabacterium catenaforme* = *L. ruminis*, non-motile)
(med) : median-value of logarithms

(Lerche and Reuter, 1962). Microbiological analyses were performed no later than 2 hours after sampling.

The automatic capsule used was about 2.4 cm in length and 0.85 cm in diameter, with a weight of 4.1 g. Most test persons, including children, were able to incorporate the capsule without difficulties. Each capsule opened and closed at defined times for about half an hour during the passage through the intestinal tract. The capsule's location in the intestinal tract during the opening stage was checked by clinical radiographs at the end of the opening phase. The functionality of the capsules concerning mechanisms and tightness was tested by Hirtzmann using dyes and disinfection solutions(1963, 1965) before and after their application.

The bacteriological analyses included a quantitative cultivation procedure with ten-fold dilution steps after homogenization of the samples, the application of a set of polyvalent media, supplemented blood agar, and various selective media (Reuter, 1961; Reuter, 1963, 1985). The aerobic as well as anaerobic microflora was analyzed, with species and biotype identification from multiple subcultures. The lactobacilli and bifidobacteria, and other important groups or species, could be identified by classical methods according to the state of knowledge at that time (Lerche and Reuter, 1961, 1962; Reuter, 1963, 1964). The quantitative identification of *Bacteroides*-species and of veillonellae in correlation to bifidobacteria was possible, too. "coryneforms" isolated at that time should be regarded as identical to peptococcaceae nowadays (Mitsuoka, 1982). The lower range of cultivation was about log_{10} 6.0/g for bifidobacteria and lactobacilli, with selective plates even two log cycles lower.

Results of 9 Post Mortem Cases Selected from 30 in Total (Series 1)

In 14 of 30 cases examined at autopsy, the small intestine was overgrown by gram-negative bacteria or by enterococci. These cases were mainly based on septic and severe chronic diseases or on elderly people suffering from different dysfunctions. These were not considered further for judging the lactobacillus and bifidobacterial microflora of the small intestine. Of the remaining 16 cases, 9 showed a remarkable *Lactobacillus* microflora in the small intestine. These cases consisted of middle-aged adults who were sudden or accidental death victims, without preceeding long-term illnesses. Dissection was performed within 2 days of death. The microbiological results of these cases therefore were considered as a plausible interpretation of the microecological situation in the upper intestine before death (Table 1). In cases where ingesta was present, the jejunum contained lactobacilli in considerable counts. The median value was log_{10} 7.7/g. Predominant strains belonged to the species *L. acidophilus* and *L. fermenti*, according to the existing taxonomic system of that time. Their classification must be seen nowadays as corresponding to the new species *L. gasseri* (= *L. acidophilus*, biotype I, formerly) and *L. reuteri* (= *L. fermenti*, biotype II b, formerly). *L. salivarius* appeared less frequently. Enterobacteria, micrococci, enterococci, yeasts and fungi occurred occasionally. *E. coli* and *Bacteroides* strains might have been detected through their retrograde spread from the ileum after death. The presence of an additional strain of enterobacteria might have been caused by a transient strain.

The ileum contained enterococci more frequently, and bifidobacteria in single cases. Lactobacilli showed a similar distribution as in the jejunum. In the large intestine (caecum/descending colon) enterococci and *E. coli* were present on a regular basis, accompanied by the autochthonous lactobacilli: *L. gasseri*, *L. reuteri*, and *L. salivarius*. Although in different frequency, some transient lactobacilli (*L. casei, L. plantarum, L. buchneri*) were present in all parts of the small intestine.

Results with the Automatic Capsule in Children, Aged 6-12 Years (Series 2)

The intestinal microflora of 24 hospitalized children, 9 of which were considered as physically healthy, yet most of them being mentally retarded, was analyzed. Data were reported by Hirtzmann and Reuter (1963). Repeated sampling followed from 10 children selected out of this group. In total, 47 tests were evaluated. On thirty eight occassions, the capsule opened in the ileum, and 9 times in the caecum. In most cases the passage time through the intestinal tract took between 2 and 3 days (Table 2).

In ileal samples, lactobacilli were the most frequent microorganisms, followed by *E. coli*, enterococci, bifidobacteria, and aerobic bacilli. An anaerobic lactic acid bacterium could be recognized and was determined according to the existing taxonomic system of Bergey's Manual (1957) as *Catenabacterium catenaforme*. This

Table 2. Microecology (log_{10}/g) in the ileum and caecum of 24 children aged 6-12 years. Samples obtained by automatic capsule method (according to Hirtzmann and Reuter, 1963; Reuter, 1965a, b).

	ileum (n = 38)		caecum (n = 9)	
	n	log_{10}/g (med)	n	log_{10}/g (med)
yeasts and fungi	4	3.0-8.0 (4.9)	1	5.4
bacilli	20	3.0-5.3 (3.7)	2	3.0-3.6
micrococci	7	3.0-8.4 (3.9)	1	4.8
streptococci*	26	3.0-6.7 (6.4)	6	4.4-6.9 (6.0)
enterobacteria	2	3.9-4.8	1	3.9
E. coli	29	3.0-7.0 (4.1)	9	4.0-7.5 (6.3)
bifidobacteria	23	4.0-7.0 (6.7)	9	6.0-8.6 (7.6)
bacteroides	5	5.3-8.2 (6.8)	7	6.9-8.9 (7.8)
coryneformes**	3	5.4-8.0 (7.0)	2	7.0-8.5
veillonella	0	-	2	5.8-6.4
clostridia	1	6.3	1	6.7
lactobacilli	36	3.0-8.5 (6.8)	8	4.5-9.0 (5.8)
anaerobic lactobacilli***	21	4.0-8.0 (6.0)	5	5.3-6.8 (5.5)

* : including enterococci
** : may be *Peptococcaceae*
*** : *Catenab. catenaforme* → *L. ruminis*, non-motile
(med) : median-value of logarithms

species seemed to be a regular component of the indigenous microflora. It was later classified as *L. ruminis* (Sharpe, 1974; Sharpe and Dellaglio, 1977), but as a nonmotile variant, whereas strains from the rumen of cattle principally showed motility. Micrococci, yeasts and fungi occurred sporadically as well as bacteroides and coryneforms (nowadays peptococcaceae) in the distal ileum. Quantitatively, the bifidobacteria and lactobacilli showed the highest median values followed by those of "catenabacteria" and enterococci. The counts of *E. coli* were low, and bacilli showed the lowest quantities. The variation in appearance and in counts of yeasts and fungi might have been caused by prior oral intake.

Samples from the caecum qualitatively showed the predominance of bifidobacteria, and bacteroides species, followed by *E. coli*, lactobacilli and enterococci. The counts of bacilli were reduced, veillonellae occurred sometimes. The median values of counts of bifidobacteria and enterococci were markedly increased compared with those of the ileum. *Bacteroides* populations were predominant. A selected group of 5 children tested continuously (5 and 7 times) over a period of 3 to 5 weeks yielded information about the personal characteristics of the microflora in the small intestine of each individual. Definite strains of lactobacilli or bifidobacteria appeared regularly in one or the other child, and, in addition, nearly in equal quantities during the entire investigation period. In 3 children, bacilli could be isolated from the ileum on a regular basis; in the other two not at all. Also frequency and quantities of enterococci appeared to be related to the individual host. In two children the "catenabacteria" were an obligatory part of the individual microflora. The opportunistic occurrence of other strains, such as some other *Lactobacillus* species, micrococci, yeasts and fungi may have been caused by oral intake with food just prior to sampling. The results have been reported in detail previously (Reuter, 1965a, 1975).

Table 3. Microorganisms other than lactic acid bacteria from the small intestine and caecum of healthy adults under non- restricted diet. Samples obtained by the automatic capsule method (n = number of positive samples, brackets = total number of samples).

test person	age and sex	capsules passage: days	capsules total	**jejunum** / n = 13 (18) upper 1 (3)	jejunum middle 6 (9)	jejunum lower 6 (6)	**ileum** / n = 38 (38) upper 5 (5)	ileum middle 18 (18)	ileum lower 15 (15)	**caecum** n = 6 (6)
1	36 m	1 - 2	5				M C Kl Pr	C	2 C	C Bc Ba V
2	46 f	1 - 2	6		Y		2 Y C		3 Y M C	
3	57 m	1 - 2	5	Y Bc	Y M Kl Bc			2 Y C Kl		Y C Kl Ba Cor
4	34 m	3	6			Bc		C	4 C [Ba]	
5	35 m	1 - 3	4			2 C		2 C Bc		
6	32 m	1 - 2	10			∅	Bc	2 (Bc)	4 C (Bc) [Mc]	2 C Bc Cor
7	29 f	2 - 4	3			∅		2 C (Bc)		
8	52 f	2	1	∅						
9	34 m	2	2					C Ci	C	
10	48 f	2	2		Y			Y		
11	26 f	1 - 2	2		∅			∅		
12	24 f	5	2		∅			C		
13	28 f	3	1		2 (Bc)			C		
14	28 f	5	1					C		
15	27 m	1 - 4	5		Kl					
16	21 f	1 - 2	3		Bc	C		C Ba		2 Y C Ba Cor
18	36 m	1 - 3	1	∅			Kl Bc		Ba Cl	
19	21 f	2	3					C Bc		

∅ = negative
( ) = not in all cases
[ ] = in a single case

Y = Yeasts
M = Moulds
C = *E. coli*

Ci = *Citrobacter*
Kl = *Klebsiella*
Pr = *Proteus*

Bc = *Bacillus*
Ba = *Bacteroides*
V = *Veillonella*

Cl = *Clostridium*
Cor = Coryneformes (= *Peptococcaceae*)
Mc = *Micrococcus*

Table 4. *Lactobacillus* species from the intestine of healthy adults under nont--restricted diet. Samples obtained by the automatic capsule method and from faeces in parallel.

test person	age and sex	not ingested with food (*autochthonous*)				ingested with food (*transient*)			
		stomach	small intestine	caecum	feces	stomach	jejunum	ileum	feces
1	36 m		Lg Lr Ls	Lg	aL			Lc Lf Lp	Lbr Lp
2	46 f		Lg Lr		Lg Lr				
3	57 m	Lr	Lg Lr Ls	Ls	aL				
4	34 m	Lr	Lr		Lr			Lf	
5	35 m	Lr	Lr		Lr				Lbu Lp
6	32 m	aL	aL	aL	aL				Lbu Lp
7	29 f		aL		aL Lr				Lp
8	52 f	aL Lr			aL Lr				Lbu Lp
9	34 m		aL		aL Lr				
10	48 f	aL	aL Lg		aL Lg				Lbr Lp
11	26 f							Lbu	Lbu Lc Lp
12	24 f		aL						Lc Lp
13	28 f		aL Lr					Lbu	Lbu Lc
14	28 f								Lc Lf Lp
15	27 m				Lg Lr				
16	21 f			Lr	Lr		Lc Lp	Lc Lp	Lc Lp
17	57 f				aL				Lp
18	36 m		aL Lr		aL Ls			Lp	Lbu Lf Lp
19	21 f				aL Lg Lr			Lp	Lbu Lc Lp
20	55 m					Lp			Lf Lp
21	31 f	aL			aL				
22	21 f				aL Lr	Lf			Lbu Lf

m =male f = female Lg = *L. gasseri* Ls = *L. salivarius* Lbu = *L. buchneri* Lp = *L. plantarum*
aL = anaerobic *Lactobacillus* Lr = *L. reuteri* Lf = *L. fermentum* Lbr = *L. brevis* Lc = *L. casei*

Results with the Automatic Capsule in 23 adults (Series 3)

The subjects were healthy, 20 to 57 years old, and engaged in the production process of a company. Stool specimens were investigated simultaneously with the contents of the capsules shed in the faeces. The transfer of the capsule through the intestinal tract of adults was markedly slower than that in children, although the latter had been hospitalized. Due to this fact, the capsules remained in the stomach during their opening phases in numerous cases. Twenty one out of 45 stomach samples showed a microbiological content even after a transfer of the capsules through the intestine for 2-4 days (Reuter, 1965b). They contained lactobacilli and "catenabacteria" in about 20% of the cases, followed by bacilli and enterococci. Micrococci, yeasts and fungi occurred in five samples, and enterobacteria in two. Considering median values, the lactobacilli were present in relatively high quantities, bacilli the lowest.

Samples from the jejunum contained predominantly enterococci, qualitatively as well as quantitatively, followed by lactobacilli, yeasts and fungi. *E. coli* occurred in five cases. Ileal samples frequently contained lactobacilli, *E. coli* and enterococci. The median value was highest with *E. coli*. bifidobacteria, "catenabacteria", yeasts and fungi, bacilli, and other enterobacteria occurred in about 20 to 25% of the cases. *Bacteroides* strains occurred three times and micrococci and clostridia two times each.

Samples from the caecum, colon and from the faeces showed a nearly corresponding situation. Typical components were *Bacteroides*, "coryneforms" and veillonellae with a regular appearance of bifidobacteria and *E. coli*. Enterococci and "catenabacteria" were more numerous in the colon than in the faeces. That was also the case with lactobacilli (Reuter, 1965b).

The individual character of the microflora of each subject became obvious again during this series. Yeasts could be repeatedly detected in certain individuals in different parts of the intestinal tract. This was the case with bacilli and klebsiella strains in single cases, too. Some people had only lactobacilli in the small intestine, as did others combined with *E. coli* (Table 3).

The distribution of lactobacilli divided into autochthonous and transient species was reported in detail (Table 4). The species *L. fermentum* and *L. acidophilus* with its special biotypes, which are now classified as *L. reuteri* and *L. gasseri* respectively, and in some cases *L. salivarius*, could be identified as autochthonous .This was also the case with "catenabacteria". Some individuals showed only *L. reuteri* regularly, others only "catenabacteria". In some persons, the same strain was detected throughout the gut, from the stomach down to the faecal sample.

Transient lactobacilli were mainly isolated in the faeces, a few in the ileum, and very few in the jejunum and stomach. This indicated that these bacteria will pass the small intestine quickly and multiply in the colon for a limited time. The most frequently isolated transient strains were *L. plantarum* followed by *L. casei*, *L. buchneri* and *L. brevis*,

Table 5. Cultures re-isolated from different habitats of the intestine of healthy adults after oral intake for 3 days. Samples obtained with capsule method and from faeces in parallel (log_{10} n)

test	cultures	daily	jejunum	ileum			caecum	feces
person	(lyophilized)	dose	middle	upper	middle	lower		
1	*E. coli*	7.08			9.48	9.48	+	+
	L. johnsonii	6.85						
	B. longum	6.85						10.30
2	*E. coli*	7.30		8.30		8.30		++
	L. johnsonii	7.08						
	B. longum	7.08						
3	*E. coli*	7.08			8.30		++	++
	L. johnsonii	6.85						
	B. longum	6.85						
	L. lactis	9.60						
4	*L. gasseri***	9.34						6.00
	B. adolescentis	8.30						6.00
	L. helveticus	8.48						
5	*L. gasseri***	9.18						6.90
	B. adolescentis	8.78						
	L. casei	4.78						3.30
	L. bulgaricus	8.48						
6	*L. gasseri***	9.34				8.20		5.85
	B. adolescentis	8.30						3.48
	L. casei	9.48		3.70				6.48
7	*L. gasseri***	9.30						
8	*L. gasseri***	9.30						5.48
9	*L. gasseri*	8.85				4.11		
10	*L. johnsonii*	9.00						6.48
11	*L. fermentum*	7.60						6.46
12	*L. reuteri*	7.00						
13	*L. lactis*	7.00						
14	Anaer. *lactobacillus**	7.48			7.30			9.85*
15	*L. gasseri*	8.54	4.48					
	Anaer. *lactobacillus**	8.43						
16	*L. gasseri*	8.85						
	L. reuteri	7.40						
17	*L. salivarius*	5.85						

no re-isolations from cultures of *L. bulgaricus*, *L. helveticus*, *L. lactis*, *L. reuteri*, *L. salivarius*

* formerly *Catenab. catenaforme*, now *L. ruminis*, non-motile
** streptomycin-resistent strain (1000μ/ml)
\+ instead of original *E. coli* (S- instead of R-shape)
++ *E. coli* instead of other coliforms

and the food species *L. fermentum*. All these species had been recognized before as components of fermented foods from which they were isolated regularly and in considerable numbers (Reuter, 1965a).

Results with Cultures after Oral Intake (Series 4)

The fate of lyophilized cultures after daily oral intake for three days was tested in a trial with automatic capsules and with faecal sampling. Tested strains were lactobacilli, bifidobacteria, "catenabacteria" and *E. coli* (Table 5). The cultures were administered as a single strain or in combinations of 2 to 4 strains. Test cultures were given preferably to persons which had been repeatedly proven free from the occurrence of the particular strains in their faeces prior to the beginning of the experiment.

E. coli was re-isolated from the ileum in considerable numbers in all 3 tested subjects. *L. gasseri* was re-isolated from the jejunum and the ileum only 3 times in 12 trials and, due to low numbers, only 4 times from the faeces. The identification of this strain was difficult as it could not be separated easily from the autochthonous strains of this species frequently present in lower counts in the test subjects included in the trial. One *L. gasseri* mutant, which had been selected for streptomycin resistance, had reduced resistance during the multiplication in the intestine. Thus, verification with antibiotic selective plates was difficult. The *L. reuteri* strain tested could not be re-isolated. That was also the case with dairy lactobacilli (*L. bulgaricus*, *L. helveticus*, and *L. lactis)*. The data recording these re-isolations are shown in Table 5.

Summarizing the results of these four series mentioned above, it can be stated that the stomach and the small intestine are not free of microorganisms, but rather that they contain a lot of different strains in variable, and in some cases, considerable quantities. The appearance of autochthonous as well as transient components of the microflora in these habitats should be noted. This was mentioned already in several papers, even in the classical literature, but it was questioned in about the same number of publications. It is impossible to cite all the authors engaged on that field. Some early references are cited in the paper of Lerche and Reuter (1962).

It can be concluded that in the small intestine there might exist a more defined individual character of indigenous flora than in the colon. On the other hand, the small intestine's microflora is influenced on a large scale by transient microorganisms from the oral cavity and from ingested food.

In detail, the microflora of the small intestine consists of enterococci and lactobacilli as predominating groups inhabiting the jejunum as well as the ileum. *E. coli*, "catenabacteria" and bifidobacteria do appear in the distal ileum, but not regularly. Strains of *L. gasseri* and *L. reuteri* must be regarded as autochthonous lactobacilli there. However, the *L. gasseri* strains generally isolated in all the series corresponded to the strain which was deposited at ATCC under No. 19992 and reported by Hansen (1968). This strain is not identical with the type strain of *L. gasseri* (ATCC 33323^T) isolated by Gasser from the human vagina. The other frequently detected species was *L. reuteri*. One strain was chosen as the type strain and deposited as ATCC 23272 (Hansen, 1968). This strain had been found regularly in one young female test person throughout all of the intestinal regions. *L. salivarius* may be primarily regarded as a transient microorganism derived from the oral cavity, where it is an autochthonous species. True transient lactobacilli are, in sequence of frequency and quantity: *L. plantarum*, *L. casei*, *L. buchneri*, *L. brevis*, and *L. fermentum* (the food species). A compilation of autochthonous and transient species of lactobacilli in different habitats of humans has been formulated in Tables 6 and 7, which might be considered as orientating material. Other transient organisms included yeasts, bacilli, micrococci, enterobacteria and moulds. An interesting observation seemed to be that in the caecum and colon, counts of "catenabacteria" and enterococci appeared to be increased along with those of lactobacilli, compared with the counts in faeces.

The fact that stomach samples contained autochthonous as well as transient strains has been described by other authors, too. The predominance of *L. fermentum* biotypes I-IV and of *L. acidophilus* biotype I (= *L. gasseri*) were

Table 6. Autochthonous lactobacilli in different habitats of humans (according to Reuter, 1965a, b, 1975; Reuter et al., 2001).

	mouth	stomach	jejunum / ileum	caecum / colon	faeces	vagina
L. acidophilus sensu stricto	+		(+)		•	
L. salivarius, var.saliv. var. *salicinius*	+	•	(+)	•	(+) + (infant)	
L. johnsonii	+	•			•	
L. gasseri		•	(+)	(+)	+	+
L. crispatus	(+)				•	+
L. jensenii					(?)	+
L. casei, *paracasei**	(+)	•			(+)	(+)
*L. rhamnosus**	(+)				(+)	(+)
L. reuteri (incl. *L. oris* *L. vaginalis*)	(+)	(+)	+	(+)	+	(+)
*L. ruminis***		(+)	+	(+)	+	

* : growth at ≤ 20°C
** : formerly *Catenabacterium catenaforme*
frequency:
\+ : frequently
(+) : moderately
≀ : occasionally

reported by Voronina and Lenzner (1968) and Bernhardt (1974). Bernhardt confirmed the presence of lactobacilli in populations up to 10^9/ml aspirate from the stomach with a direct intubation method.

Concerning the fate of test strains after oral intake, it can be seen that *E. coli* persisted for some days in remarkable numbers in the upper intestine. This is in accordance with the results of Shiner *et al.* (1963), who found that the jejunal juice showed no bactericidal effects against enterobacteria. On the other hand, the strains of *L. gasseri* and "catenabacteria" could not be re-isolated regularly, only in certain subjects. Also the test strain of *L. reuteri* and *L. salivarius* failed to do so. *L. salivarius* was mentioned in the literature as bile sensitive and being reduced in count or even incapable of surviving in the small intestine.

Transient lactobacilli, however, multiplied in the caecum and colon for some days. The fact that the thermophilic dairy lactobacilli like *L. bulgaricus*, *L. helveticus*, and *L. lactis* did not survive passage through the stomach and duodenum, seems to be very important for judging products containing these species as probiotics.

Table 7. Transient lactobacilli in different habitats of humans (according to Reuter, 1965a, 1975).

	stomach	jejunum/ ileum	faeces
L. casei, paracasei, rhamnosus	(+)	(+)	+
L. plantarum	•	(+)	+
L. buchneri, brevis	•	(+)	(+)
L. fermentum	(+)	(+)	(+)

frequency:
\+ : frequently
(+) : moderately
≀ : occasionally

II. *Bifidobacterium* Species in the Intestine of Healthy Infants and Adults

Bifidobacteria have been known since Tissier (1900) described a species in infants, which was later named *Lactobacillus bifidus* by Orla-Jensen (1924) (Table 8). Since that time numerous papers were published concerning the ecology and importance of bifidobacteria in the intestine of man, primarily in infants. But in many investigations, the demonstration of bifidobacteria was performed by microscopy rather than by cultivation and identification of subcultures. Important progress was made in the 1960s. Paediatricians and microbiologists detected different types of bifidobacteria in infants (e.g. Dehnert (1957) in Germany). But cultivation of this intestinal microflora was performed mostly as deep agar cultures. It was still difficult to obtain surface cultures on plates because of the strict anaerobic requirements of these microorganisms. A remarkable improvement was achieved by using adequate anaerobic systems combined with surface plating (Reuter, 1961). These systems enabled the culturing of the comprehensive anaerobic microflora of faeces of adults on polyvalent plates (e.g. on blood agar base). These procedures were later optimized by Mitsuoka's technique in Japan and made it possible to separate many different variants characterized by special features of oxygen tolerance, nutritional requirements, and ecological specificities.

Table 8. *Bifidobacterium* species. in the intestine of infants and adults (according to Lerche and Reuter, 1961; Reuter, 1963, 1971; Mitsuoka, 1969, 1982).

	biotypes Reuter/Mitsuoka	infant	adult
B. bifidum (Tissier, 1900, Orla-Jensen, 1924)	a	•	+
	b	$+^T$	•
B. adolescentis (Reuter, 1963)	$a^{x)}$	•	$+^T$
	$b^{xx)}$	–	+
	$c^{xx)}$	•	+
	$d^{xx)}$	–	(+)
B. longum (Reuter, 1963)	a	•	+
	b	$+^T$	•
B. infantis (Reuter, 1963)	a	$+^T$	–
	b	+	–
B. breve (Reuter, 1963) syn. *B. parvulorum* (Reuter, 1963)	a	$(+)^T$	•*
	b	•	–
	c	•	–

frequency:
\+ : frequently
(+) : moderately
• : occasionally
^{T} : including type strain ^{T} of species (Reuter, 1971)
* : also isolated from the human vagina
$^{x)}$: *B. adolescentis* sensu stricto
$^{xx)}$: *B. adolescentis* sensu lato, including *B. dentium* (b?), *B. catenulatum*, *pseudocatenulatum* (c), *B. angulatum* (d) (Scardovi, 1986; Gavini et al., 2001)

Table 9. Structure of *Bifidobacterium* microflora and other related components in the faeces of healthy adults during a long term investigation period (≥ log_{10}=6.0/g) (according to Lerche and Reuter, 1961).

test person sex/age (years)	days*	*Bifidobacterium* spp. *adolescentis* var. a, b, c,	d	*longum* a	*bifidum* b	anaerobic lactobacillus =*L. ruminis*	*E. faecium* ≥ log 6.0	others (total anaerobic count)xx)
A	0	+	–	–	(+)	(+)	–	+
male	4	+	–	(+)	–	–	–	+
30	6	+	–	–	–	–	–	+
	60	+	–	–	–	(+)	–	++
	82	+	–	–	–	–	–	++
	91	(+)	–	–	(+)	–	–	+
	102	(+)	–	–	–	–	–	+
	109	+	–	–	–	–	–	+
B	-59	+	+	+	+	(+)	–	
female	-58	+	+	+	(+)	(+)	–	
22	0	+	(+)	(+)	(+)	+	–	+
	4	+	(+)	(+)	(+)	(+)	–	+
	60	+	(+)	+	+	(+)	–	+
	82	+	(+)	+	+	(+)	–	+
	91	+	(+)	+	+	(+)	–	+
	102	(+)	+	(+)	(+)	(+)	–	+
	109	+	(+)	(+)	(+)	[+]	–	+
C	0	+	(+)	–	+	(+)	–	++
male	60	+	–	[+]	+	+	–	++
26	82	+	–	(+)	+	–	–	+
	91	+	(+)	(+)	+	(+)	–	+
	102	+	–	(+)	+	(+)	–	+
	109	+	–	(+)	(+)	(+)	–	+
D	4	+	+	–	–	–	–	+
male	6	+	–	+	–	–	–	+
28	60	+	+	+	–	(+)	–	+
	102	+	–	(+)	(+)	(+)	–	+
	109	+	–	–	+	[+]	–	+
F	67	(+)	–	(+)	–	(+)	–	+
female	82	+	–	(+)	–	(+)	–	+
27	91	(+)	–	–	–	–	–	+
	102	(+)	–	–	–	–	+	+
	109	+	–	[+]	–	[+]	(+)	+
E	60	–	–	–	–	–	+	+
male	82	–	–	–	–	–	+	+
31	91	–	–	–	–	–	+	+
	102	–	–	–	–	–	+	+
	109	–	–	–	–	–	+	+
G	82	(+)	–	–	–	[+]	–	+
male	91	[+]	–	–	–	–	–	+
52	102	(+)	–	–	–	–	–	+
	109	(+)	–	–	–	[+]	–	+

≤ log 8.0 : [+] ≥ log 9.0 : + * period: 11.08.-29.11.1960
≥ log 8.0 : (+) ≥ log 10.0 : ++ xx): *Bacteroides*, *Peptococcus* spp.

After culturing and subculturing of bifidobacteria on plates was possible, the description of this very complex part of the microflora could start. This was performed with classical phenotypical methods (e.g. by morphology, physiology, fermentation reactions and demonstration of serological relationships) (Reuter, 1963). A new taxonomic system could be established by creating a genus *Bifidobacterium* (*Taxonomic Subcommittee for Bifidobacteria*) with the description of several new species besides *B. bifidum*, which was the only existing species at that time (Reuter, 1963, 1971). That was followed by an increasing number of new species recognized in humans and other animals (Mitsuoka *et al.*, 1969, 1984; Lauer and Kandler, 1983; Scardovi, 1986). The new concept of *Bifidobacterium* taxonomy was summarized in special chapters of Bergey's Manual (1986) and in the Prokaryotes (1981) by Scardovi and Biavati *et al.* (1991). The predominating species in the intestine of humans are still those which were described at the first stage of taxonomic identification by Reuter and Mitsuoka (Table 8).

Data about the ecology of the bifidobacteria in the intestine of individuals are still very rare because there may

exist an agglomeration of up to 4 or even 5 different species in one habitat. It was difficult to separate them by classical bacteriological methods as no adequate selective agar media existed, which enabled the culturing of all species or strains of bifidobacteria qualitatively and quantitatively. Modern methods using PCR/RAPD or gene probes may produce more accuracy (McCartney *et al*., 1996; Tannock, 1999b; O'Sullivan, 1999; Roy *et al*., 2000) but must be suitable for the identification of all species which may be expected in the sample. Some recent papers showed some vagueness in that concern and came to conclusions, which might not correlate with results obtained by universal classical methods e.g. Sghir *et al*. (2000).

Therefore, our data about composition and succession of bifidobacterial species in the intestine of humans shall be referred to here. They were collected in parallel to the taxonomic research. A comprehensive analysis of each surface culture by subculturing many isolates under microscopic control was the base of these investigations. The first paper was published in 1961 (Lerche and Reuter). The group of bifidobacteria was still named as *Lactobacillus*. The different strains were separated as biotypes. These results, combined with ecological data, are summarized in Table 9. The procedure was as follows: 7 healthy test persons belonging to the staff of the institute, supplied stool samples regularly over a period of more than 165 days and gave information about food consumption habits which might have influenced the successions of strains within the stool microflora. The results were reported in a publication as direct counts (Lerche and Reuter, 1961). Transferring these counts into logarithms and simplifying the results by symbols, shows interesting relationships between different components individually distributed in test subjects (Table 9). From that data it can be seen that a healthy young female showed a very comprehensive bifidobacterial microflora. Representatives of *B. adolescentis* (4 variants) were regularly present in each stool sample. This situation remained stable over the entire investigation period of more than 165 days. Samples from this subject also showed the presence of the specific biotype of *L. fermentum*, later identified as *L. reuteri*, representing the type strain of this new species. Additionally, the presence of an anaerobic species of lactic acid bacteria became obvious and it was identified as *Catenabacterium catenaforme* due to the current taxonomic system and nomenclature at that time. This species seemed to be an autochthonous species of the intestinal tract of humans and was included in the genus *Lactobacillus* as *L. ruminis* by comparative investigations of a submitted strain. But this strain was not motile like the original *L. ruminis* strains (Sharpe, 1974). It was designed as strain 194e = ATCC 25644 (Sharpe and Dellaglio, 1977). Another subject, 31 years of age, showed an abnormal composition of the intestinal gram-positive microflora. No bifidobacteria beyond the level of $\log_{10}$ 6.0/g of faeces could be isolated by the method of cultivation that was utilised. Instead of that a high count of *Enterococcus faecium* was obtained from each sample. This situation remained stable during the whole investigation period. The gram-negative anaerobic count corresponded to that of the other test subjects.

Another male subject of 52 years of age showed a reduced quantity of bifidobacteria and of the anaerobic *Lactobacillus*. That is in accordance with other observations (Mitsuoka, 1982; Gavini *et al*., 2001), where elderly people showed a decrease of that kind of microflora.

The lack of counts in the Table does not necessarily mean that this species might not have been present. It indicates that the counts may be below the detection level (e.g. $\log_{10}$ 6.0/g.). The conclusion from the results in this Table, in general, may be that each healthy adult person will have and maintain its own fixed composition of this part of the gram-positive anaerobic microflora over a long period of life. Inter-personal differences in the composition of the bifidobacterial microflora were confirmed by the investigations of McCartney *et al*. (1996).

In general, the bifidobacterial microflora of adults consisted mainly of strains of the species *B. adolescentis* with the biotypes *a*, *b* and *c*, to a lesser extent also type *d*, which was listed separately due to the dry and rough consistency of colonies growing on blood agar plates. As a biotype *a* strain was chosen as the type strain of the species *B. adolescentis,* the other biotypes *b*, *c*, *d* may correspond to other new species described later on by Scardovi (1986). These relations are pointed out in Table 8, based on indications made by Mitsuoka (1982). This matter is not finished as some overlaps exist (Gavini *et al*., 2001). Another species often detected was *B. longum* with its variant *a* which could be found in adults and was separated from variant *b* which was mostly isolated from infants. The species *B. bifidum* appeared as variant *a* in stools of adults, too, but at lower counts than other *Bifidobacterium* species. Variant *b* of *B. bifidum* was the breast-fed-infant type of this species as described by Tissier (1900). Nevertheless, the dominant bifidobacterial species in infants were *B. infantis*, *B. breve*, *B. parvulorum* etc. (Reuter, 1963, 1971). The existence of biotypes (variants) within these species demonstrates the adaptation to different habitats. This is of importance for the choice of strains for the use as probiotic cultures (Reuter *et al*., 2001).

The differences within the ecological structure of the bifidobacterial microflora between infants and adults are obvious. Successions may occur during life since breast-fed or formula-fed infants may have been the starting base for further development of this part of the microflora. The successions of bifidobacterial species within different periods of human life are not described very well, as only very few long term investigations in the same individuals have been performed (e.g. by McCartney *et al*. (1996)). Several reports describing the structure of the bifidobacterial microflora in different age groups of humans exist. The results are based on separate groups of individuals, however. Also the number of subjects varied due to experimental conditions.

Geographic specificities in the composition of the bifidobacterial microflora have to be considered, too. The most comprehensive surveys had been performed by Mitsuoka and his working group in long term investigations with many reports in the literature including review articles and monographs (e.g. Mitsuoka (1982, 1984, 1992). Recently, publications from the groups of Gavini *et al*. (2001) and Tannock *et al*. (1999b) support the knowledge

about the composition and succession of the bifidobacterial microflora.

The unquestionable conclusion is that the bifidobacterial microflora in elderly people will be reduced quantitatively as well as qualitatively. This fact was mentioned already by Orla-Jensen and by Mitsuoka in their early investigations and can also be seen from the data in Table 9. In general, it can be further stated that the bifidobacterial microflora of humans may contain up to 4 or 5 different species or special strains continuously, and that one or two are quantitatively predominant. *B. longum* and *B. bifidum* are not the predominating species, however. That is a very important fact to be considered in view of the selection of bifidobacterial strains for their use as probiotics.

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