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Diversity of *Bifidobacterium* and *Lactobacillus* spp. in Breast-Fed and Formula-Fed Infants as Assessed by 16S rDNA Sequence Differences

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A qualitative molecular monitoring approach based on PCR and denaturing gradient gel electrophoresis (DGGE) was used to study the diversity of dominant bacteria, bifidobacteria and lactobacilli in vaginally delivered full-term infants. Seven breast-fed and six formula-fed infants participated in the study. 16S rDNA targeted primers were used for the specific PCR amplification of fragments from bacteria, bifidobacteria and lactobacilli from faecal samples that were collected before and after weaning at the age of approximately 1 and 7 months, respectively. The PCR fragments were subsequently resolved in a sequence-dependent manner by DGGE. In addition, cloning and sequence analysis of the PCR fragments was used to identify the species from which they originated. Based on the number of fragments in the DGGE profiles it was estimated that breast-fed and formula-fed infants harboured bacterial communities of equal complexity. There was no conspicuous difference in the distribution of *Bifidobacterium* or *Lactobacillus* species between breast-fed and formula-fed infants. The most frequently found representatives of these genera were *B. infantis* and species belonging to the *L. acidophilus*-group in both groups of infants. The predominant *Bifidobacterium* and *Lactobacillus* populations in most infants consisted of only one or two species. *Key words*: bifidobacteria, lactobacilli, 16S rDNA, denaturing gradient gel electrophoresis, infant, intestinal microbiota.

INTRODUCTION

Microbial colonisation of the human GI-tract starts at birth when the new-born infant comes in contact with the microbes from the mother and surrounding environment. Colonisation proceeds in several stages until a complex, diverse and stable microbiota resembling that of an adult is developed (1, 2). The first 1–2 weeks of life are considered as the initial period of bacterial colonisation, during which diverse bacteria transit through infants GI-tract and can be found in the faeces. Facultative anaerobic bacteria such as *Escherichia coli* and streptococci colonise the gut during this period. During the next stage anaerobic bacteria such as bifidobacteria, *Bacteroides* and clostridia are found in the faeces of infants. In breast-fed infants bifidobacteria predominate, whereas in formula-fed infants a more diverse microbiota develops (1, 2). Once dietary supplementation begins, the difference in the faecal microbiota between breast-fed and formula-fed infants disappears. The last stage represents the period of conversion to adult microbiota after the weaning is completed. Factors that influence the microbial succession and colonisation include both host-related (3) and external factors such as the mode of delivery (vaginal or caesarean), microbial load of the surrounding environment (hygienic conditions), type of feeding (breast- or formula-feeding) and possible antibiotic therapy of the infant (1, 4).

Early culture-based and microscopic investigations showed that bifidobacteria were the predominant microorganisms in faeces of breast-fed infants but not in formula-fed infants (5, 6). However, several other culture based studies have shown that both groups of infants have equal frequency and level of bifidobacterial colonisation by the age of approximately 1 month or earlier (7–9). Conflicting results have also been obtained regarding other groups of bacteria such as *Bacteroides*, clostridia, enterococci, lactobacilli and enterobacteria in breast-fed and formula-fed infants. Culture-based studies have shown a more diverse microbiota in formula-fed infants in comparison to those

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breast-fed (6, 10). Moreover, in some studies formula-fed infants were found to have more complex faecal short-chain fatty acid (SCFA) profiles than breast-fed infants, which indicates a more complex microbiota in the aforementioned group (11, 12). In contrast, earlier studies based on cultivation showed no difference in the composition of anaerobic and facultative anaerobic bacteria between breast-fed and formula-fed infants (9, 13).

New microbiological, biochemical and molecular biological methods for detection of different bacterial groups and species have been developed in order to describe the microbial communities more accurately (1, 14). Modern molecular ecology techniques based on direct detection and sequence comparison of nucleic acids (DNA or RNA) have been applied in numerous studies to characterise adult human GI-tract microbiota (14–16), but only in a few to describe infant microbiota (17, 18). A widely used approach is to detect the target molecules 16S rDNA or rRNA by using fluorescent *in situ* hybridisation (FISH), dot blot hybridisation, and PCR-based techniques (14–16, 18). Recently, culture-independent study based on FISH was reported that supported the predominance of bifidobacteria in breast-fed infants in comparison to formula-fed infants and suggested a more diverse microbiota in the latter group (18).

Combination of PCR amplification of 16S rDNA and rRNA fragments with temperature or denaturing gradient gel electrophoresis (TGGE and DGGE, respectively) can be used to achieve sequence-specific separation of PCR-fragments obtained from bacterial communities in gastrointestinal samples (19, 20). This approach has been used to follow the colonisation of new-born infants in considerable detail (17). It is also possible to target separate genera or groups of bacteria with specific PCR primers in order to obtain DGGE community-fingerprints of certain bacterial populations (19, 21). In this study we used a culture-independent molecular approach based on DGGE of 16S rDNA amplicons to compare the diversity of the microbiota in breast-fed and formula-fed infants before and after weaning. Cloning and sequencing of 16S rDNA derived PCR fragments was used to identify the predominant *Bifidobacterium* and *Lactobacillus* species present in faeces of breast-fed and formula-fed infants.

MATERIALS AND METHODS

Infants, faecal samples and DNA extraction

Thirteen vaginally delivered infants (seven breast-fed and six formula-fed) were recruited to the study (Table I). Two infants, S and D are Dutch, whereas all other infants are Scottish. All infants were in general good health and were not treated with antibiotics during the sampling period. The weaning of infants (introduction of solid food) took place from weeks 12–21 after birth. The infants continued taking breast or formula milk (Milumil for infant 49 and Aptamil for all others, Milupa, GmbH & Co.KG, Friedrichdorf, Germany) after weaning during the whole sampling period with the exception of infants S and D from whom breast-milk was withdrawn at 1.5 and 6.5 months, respectively. Faecal samples were collected before and after weaning, at the age of approximately 1 and 7 months and kept frozen at −20°C until the analysis. Isolation of DNA from faecal samples was performed as described elsewhere (20).

Primers

All primers used in the study are targeted on the 16S rRNA gene (Table II). Bacterial PCR products were produced with primers 968-GC-f and 1401-r, *Bifidobacterium* genus-specific PCR was performed with primers Bif164-f and Bif662-GC-r and a nested PCR with primer sets 7-f and 0677lab-r, followed by 124-GC-f and 515-r was used to detect lactobacilli and related bacteria. A 40 bp GC-clamp was attached to the 5′ end of Bif662-GC-r, 124-GC-f and 968-GC-f primers (see Table II) in order to facilitate the analysis of the PCR products by DGGE. Primers T7 and Sp6 labelled with IRD800 were used for sequencing. All primers were purchased from MWG-Biotech (Ebersberg, Germany).

Table I

Infants and faecal samples

Breast-fed				Formula-fed			
Code	Sex	Weaning age (weeks)	Sampling times (month)	Code	Sex	Weaning age (weeks)	Sampling times (month)
1	F	15	1 and 7	9	F	12	1 and 7
2	M	13	1 and 8	13	F	12	1 and 7
33	F	21	1 and 6	24	F	16	1 and 7
40	M	14	1,5 and 8,5	28	F	14	1 and 7
52	F	12	1 and 7	49	F	13	1 and 6
S	F	17	1 and 7	54	F	12	3 weeks and 7
D	M	19	1 and 7				

The infants coded with numbers participated also in the study by Savage et al. (31), where the coding is corresponding.

Table II

Primers used in the study

Primer	Sequence 5′–3′	Use	Target	Ref.
968-GC-f	CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGG-AACGCGAAGAACCTTA	PCR	Bacteria	(36)
1401-r	CGGTGTGTACAAGACCC	PCR	Bacteria	(36)
Bif-164-f	GGGTGGTAATGCCGGATG	PCR	Bifidobacteria	(19, 37)
Bif-662-GC-r	CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG-CCACCGTTACACCGGGAA	PCR	Bifidobacteria	(19, 37)
7-f	AGAGTTTGAT C/T A/C TGG CTCAG	PCR	Lactobacilli	(21)
0677-lab	CACCGCTACACATGGAG	PCR	Lactobacilli	(21)
124-GC-f	CGCCGGGGGCGCGCCC-CGGGCGGGGCGGGGGCACGGGGGGCACGG-ATCCGGAC-GGGTGAGTAACACG	PCR/nested	Lactobacilli	(21)
515-r	ATCGTATTACCGCGGCTGCTGGCAC	PCR/nested	Lactobacilli	(21)
Sp6	GATTTAGGTGACACTATAG	Sequencing	pGEM-^{T}	Promega
T7	TAATACGACTCACTATAGGG	Sequencing	pGEM-^{T}	Promega

PCR amplification

PCR reactions were performed as described previously (19–21) using *Taq* DNA polymerase kit from Life Technologies (Gaithersburg, MD, US). The reaction mixture consisted of 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 3 mM $MgCl_2$, 0.2 mM dNTP, 0.2 μM of each primer, 1.25 U of *Taq* polymerase and 1 μl of appropriately diluted template DNA in a final volume of 50 μl. The PCR thermocycling program with primers 968-GC-f and 1401-r was the following: 94°C for 5 min; 35 cycles of 94°C for 30 s, 56°C for 20 s, 68°C for 40 s; and 68°C for 7 min. The reactions were subsequently cooled to 4°C. The annealing temperature was set at 62°C with primers Bif164-f and Bif662-GC-r and at 66°C with primers 7-f and 0677-lab-r. For the nested PCR, the *Lactobacillus*-like 7-f to 0677-lab-r PCR products were purified with the QIAquick PCR purification kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and eluted into 50 μl of milliQ water (autoclaved, sterile-filtrated and UV-treated). One microlitre of the resulting eluent was used as a template in the subsequent PCR with primers 124-GC-f and 515-r and using the same thermocycling program as described above for primers 968-GC-f and 1401-r. The size and amounts of PCR products were estimated by analysing 5 μl samples by 1.2% agarose gel (w/v) electrophoresis and ethidium bromide staining.

DGGE analysis of PCR products

DGGE analysis of PCR amplicons was performed essentially as described previously (22, 23) using the DCode or DGENE System apparatus (BioRad, Hercules, CA, US). Polyacrylamide gels (8% w/v, acrylamide:bisacrylamide—37.5:1) in 0.5 × TAE with a denaturing gradient were prepared with a gradient mixer and Econo-pump (Bio-Rad). The following denaturing gradients were used: 40–50% for bacterial, 45–60% for bifidobacterial and 30–60% for lactobacilli PCR products, respectively. A 100% denaturant corresponds to 7 M urea and 40% (v/v) formamide. PCR amplicons were separated by electrophoresis at a constant voltage of 85 V and a temperature of 60°C for 16 h. The DNA fragments were visualised by $AgNO_3$ staining and developing as described previously (24).

Analysis of the DGGE gels

DGGE gels were scanned at 400 dpi and analysed using the Molecular Analyst 1.12 software (BioRad). Similarity indexes of compared profiles were calculated from the densitometric curves of DGGE profiles by using the Pearson's product–moment correlation coefficient. For the assessment of the diversity of 16S rDNA sequences in DGGE, a manual check was performed to ensure that all fragments in a gel track were found by the software. The surface area of fragments was calculated and the fragments constituting less than 1% of the total surface area of all fragments were omitted from the account of fragments for the diversity assessment. Student's *t*-tests were used for the statistical analysis of the data.

Cloning of the PCR products

The PCR products were purified with the QIAquick PCR purification kit (Qiagen) according to manufacturer's instructions and cloned in *E. coli* JM109 by using the pGEM-^{T} vector system (Promega, Madison, WI, US). Colonies were picked and transferred into 20 μl of TE, boiled for 15 min to lyse the cells and the cell lysates were used to screen the transformants by the *Bifidobacterium*- or *Lactobacillus*-specific PCR followed by DGGE analysis. Clones for subsequent sequence analysis were selected according to the migration position of the clone PCR fragment in DGGE in comparison to the fragments in the original sample DGGE profile. Plasmid DNA of selected transformants was isolated using QIAprep spin miniprep kit (Qiagen).

Sequence analysis

Sequencing of the cloned PCR fragments was carried out using purified plasmid DNA and sequencing primers T7 and Sp6 complementary to the adjacent sequences of the pGEM-^{T} cloning site. Sequencing reactions were performed with the Sequenase sequencing kit (Amersham, Slough, UK) according to the manufacturer's instructions. The sequences were analysed with automatic LI-COR DNA sequencer 4000L (Lincoln, NE, US) and corrected manually. Sequence alignment of the complementary strands was carried out using DNASTAR SeqMan program (Madison, WI, US). Similarity searches of 16S rDNA sequences derived from faecal clones were performed using the BCM Nucleic acid sequence search service available on the internet (http://www.searchlauncher.bcm.tmc.edu).

RESULTS

DGGE profiles of predominant bacterial communities

In this study the diversity of the microbiota in breast-fed and formula-fed infants before and after weaning was compared. The faecal samples were collected at 1 and 7 months of age. In order to assess the diversity and changes of the bacterial populations in the infant faecal samples, PCR fragments generated with primers 968-GC-f and 1401-r were analysed by DGGE (Fig. 1). Differences seen in the faecal DGGE profiles between the samples taken before and after weaning reflected changes occurring in the faecal microbiota. The changes varied in both groups of infants from a complete or almost complete change of the fragments in the profile (infants 24 and 33, Fig. 1) to appearance and disappearance of some fragments in the

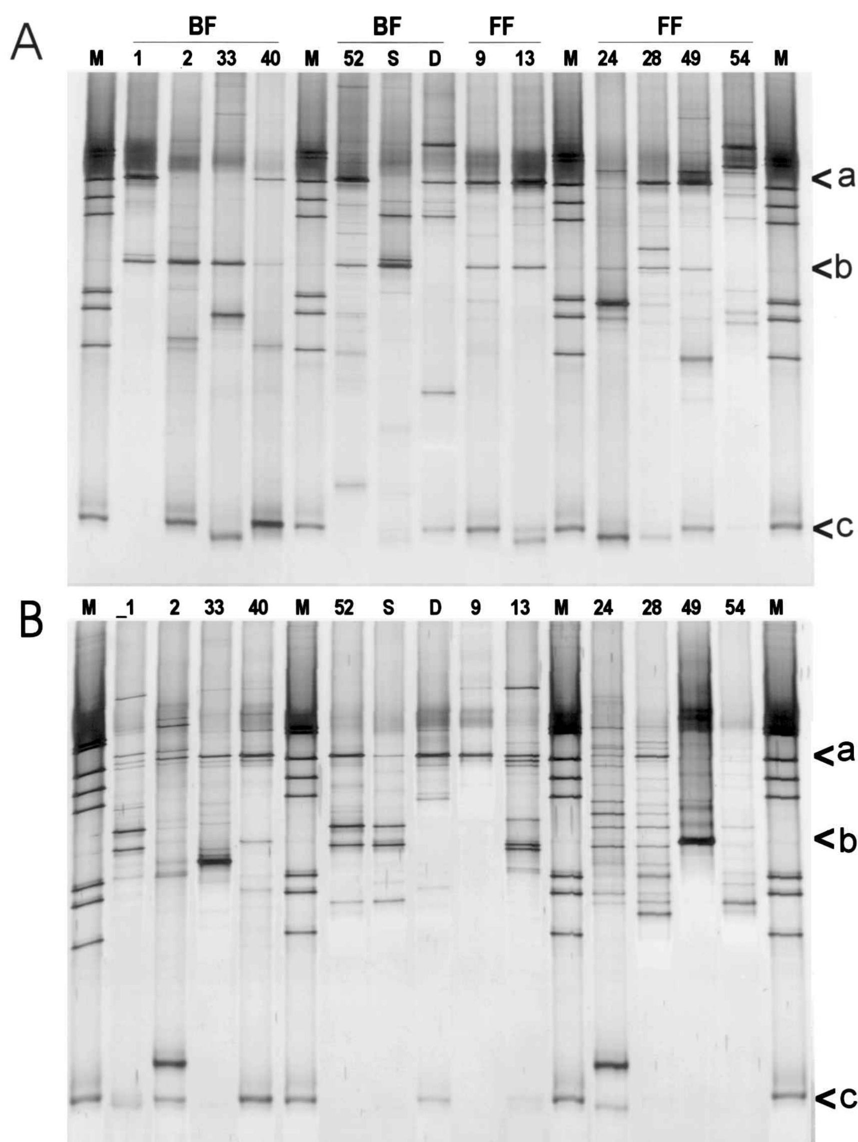

Fig. 1. Bacterial DGGE profiles of faecal samples from breast-fed (BF) and formula-fed (FF) infants at approximately 1 month (A) and 7 months (B) of age. Lanes are marked with the code of the infant (see also Table I). The lane containing the marker is indicated with M. Positions where fragments were found in the majority of samples are labelled with a, b and c, and marked with arrowheads.

profile (infants 13 and 40, Fig. 1). The similarity indexes between the DGGE profiles of pre- and post-weaning samples from each infant were calculated. The similarity indexes of the breast-fed infants (mean 51.3; SD 26.0) and the formula-fed infants (mean 46.2; SD 23.8) did not differ significantly ($p = 0.72$) showing an equal extent of profile changes in both groups.

In order to compare the relative diversity of the predominant bacterial populations in breast-fed and formula-fed infants, the numbers of predominant individual fragments in the DGGE profiles were compared. No significant difference was found in the number of fragments in the DGGE profiles between breast-fed and formula-fed infants at approximately 1 or 7 months of age (p values 0.98 and 0.95, respectively). These results suggest that the diversity in predominant species or strains do not differ significantly between the two groups of infants before or after weaning.

The DGGE profiles from different infants had some fragments in common (Fig. 1). The uppermost fragment (a) in common to the infants was cloned and sequenced from the 1 month samples of infants 1, 9 and 13. The sequences of these fragments showed highest similarity (98, 96 and 97%, respectively) to that of *Ruminococcus gnavus* 16S rDNA sequence. The lowest fragments (c) from the 1 month samples of infants 9 and 13 were identified as *Bifidobacterium* spp. (96 and 97% similarity). The common fragment appearing in the middle part of the gel (b) was cloned and sequenced from the 1 month sample of infant 1 and it appeared to have originated from *E. coli* (similarity 99%).

Detection and identification of bifidobacteria

In order to find out whether the changes detected in the dominant bacterial population were also reflected to the bifidobacterial populations, the samples were analysed using genus-specific PCR combined to DGGE and cloning and sequencing of the specific PCR fragments. Bifidobacteria were detected in five out of seven breast-fed infants and in all six formula-fed infants at 1 month of age. At 7 months of age bifidobacteria were detected in six out of seven breast-fed and five out of six formula-fed infants. One breast-fed infant (coded 52) lacked bifidobacteria at both sampling times. The bifidobacterial DGGE profiles from all positive samples are shown in Fig. 2. Sequence-specific separation of the amplified bifidobacterial 16S rDNA fragments revealed that in most infants the predominant *Bifidobacterium* population consisted of one or two dominant species (Fig. 2). Comparison of bifidobacterial profiles before and after weaning was possible in the case of ten infants when PCR products were obtained from both 1 and 7 month faecal samples. Three breast-fed (33, S and D; Fig. 2) and three formula-fed (9, 24 and 54; Fig. 2) infants showed no or minor changes in the position or relative intensity of fragments in their bifidobacterial

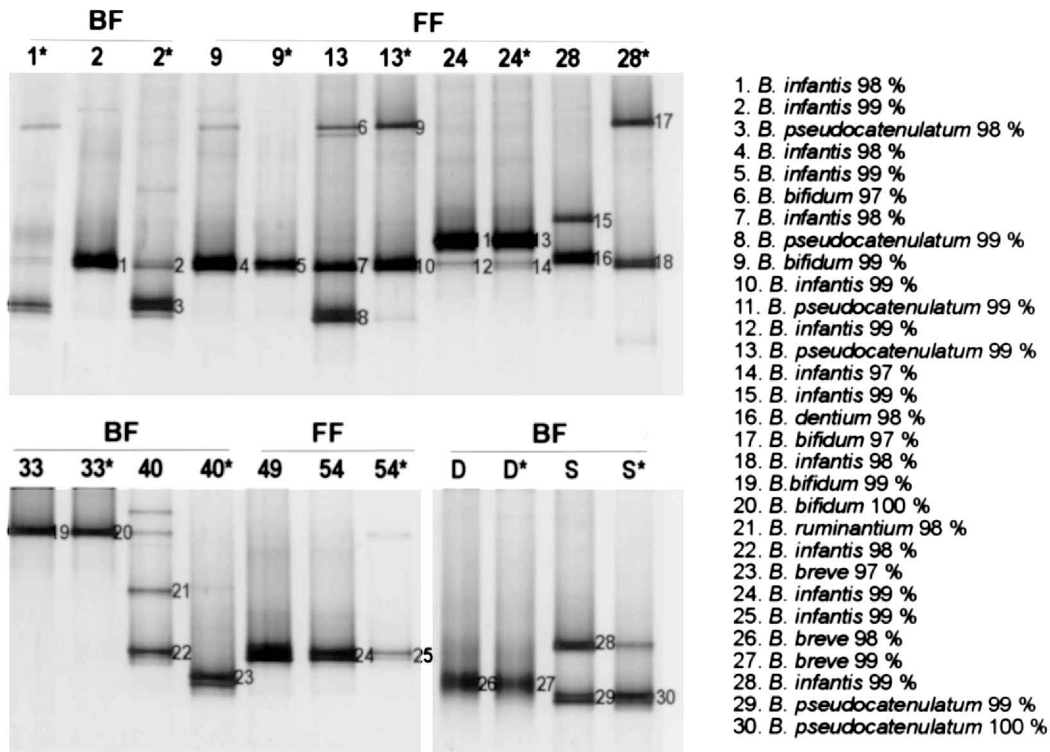

Fig. 2. Bifidobacterial DGGE profiles of faeces and PCR fragments identified by cloning and sequencing from breast-fed (BF) and formula-fed (FF) infants at approximately 1 and 7 months of age. Lanes are marked with the code of the infant (see also Table I) and the approximate 7 month sample is indicated with an asterisk.

profiles before and after weaning. Two infants (2 and 13; Fig. 2) showed a change of some fragments in their *Bifidobacterium* DGGE profiles, whereas two infants (28 and 40; Fig. 2) showed a complete change in the composition of their *Bifidobacterium* population. Although the DGGE profiles of dominant bacterial populations changed in all infants during the weaning at least to some extent (Fig. 1), the specific bifidobacterial profiles remained practically unaltered in half of the infants (Fig. 2).

The predominant *Bifidobacterium* species of most samples were identified by subsequent sequence analysis of the 16S rDNA PCR fragments. The PCR fragments were first cloned into *E. coli* and clones with an insert that produced a PCR fragment corresponding to a predominant fragment in the original DGGE profile of the faecal sample were selected for sequencing. Comparative sequence analysis with the databases revealed that all fragments had high similarity (97% or higher) to the 16S rDNA sequences of known *Bifidobacterium* species (Fig. 2). Most of the sequenced fragments resembled typical infant *Bifidobacterium* species, such as *B. infantis*, *B. bifidum*, *B. breve*, and *B. dentium*. Four infants were also found to harbour *B. pseudocatenulatum* like species, which is found more typically in adults. In general, no prominent difference was found in the distribution of bifidobacterial species between breast-fed and formula-fed infants in the faecal samples from either 1 or 7 months. Based on sequencing of the PCR amplicons, *B. infantis* was the most frequently found species in both groups of infants (Fig. 2). *B. breve* was detected in two of the breast-fed infants, but not in the formula-fed infants, and *B. dentium* was detected in one formula-fed infant. *B. ruminantium*, which is not considered to be a human-associated *Bifidobacterium* species was found in a faecal sample from one breast-fed infant. However, it remains debatable whether the amplified 16S rDNA fragment derived from *B. ruminantium* or its closely related species *B. dentium* or *B. adolescentis* because an unequivocal species identification cannot be obtained based only on the 16S rDNA sequence data.

Detection and identification of lactobacilli

Lactobacilli form a well-studied bacterial group and are found in variable but usually low numbers in infants (1, 18). To analyse the effect of diet and time on the diversity of lactobacilli, a similar approach as for bifidobacteria was used consisting of a group-specific PCR combined to DGGE and cloning and sequencing of the specific PCR fragments (21). Lactobacilli were detected by PCR in eight of the 13 infants, five breast-fed and three formula-fed (Fig. 3). Subsequent DGGE analysis revealed that the predominant *Lactobacillus* population, like the predominant *Bifidobacterium* population, consisted of one or two dominant species or strains (Fig. 3). *Lactobacillus*-specific PCR products were obtained from both the 1 and 7 month samples from four infants, 28, 40, D and S (Fig. 3). In the two infants 28 and 40 (Fig. 3), the major *Lactobacillus* fragment in the 1 month sample was replaced with another fragment in the 7 month sample. It is noteworthy, that for the same infants complete changes of the bifidobacterial DGGE profiles were observed (Fig. 2). In the other two infants, D and S (Fig. 3), the *Lactobacillus* population appeared more stable; the same predominant fragment was present in both 1 and 7 month samples, but also some fragments appeared or disappeared in the 7 month sample as compared with the 1 month sample. Infants D and S did not have any major changes in their *Bifidobacterium* DGGE profiles.

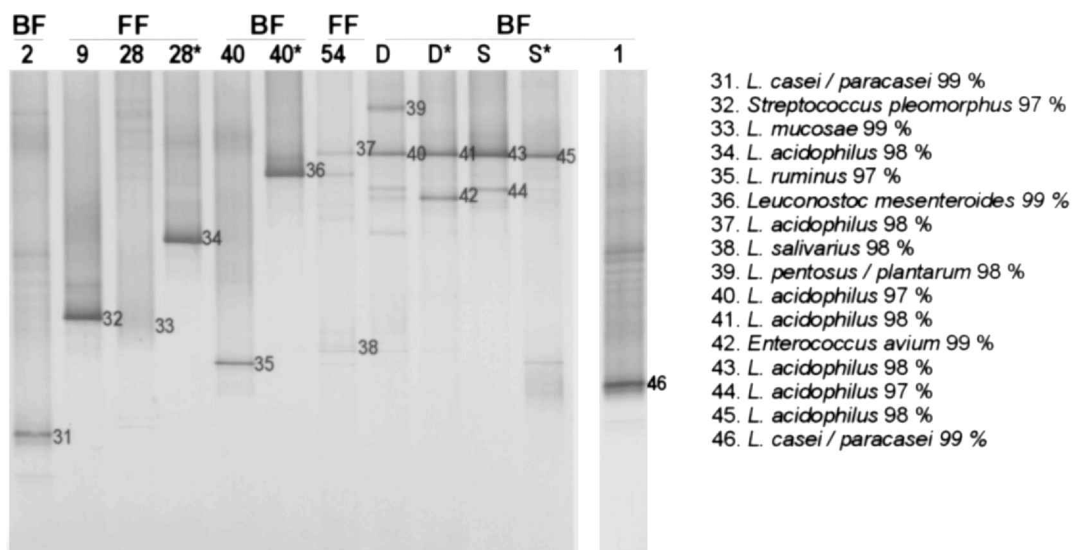

Fig. 3. Lactobacilli DGGE profiles of faeces and PCR fragments identified by cloning and sequencing from breast-fed (BF) and formula-fed (FF) infants at approximately 1 and 7 months of age. Lanes are marked with the code of the infant (see also Table I) and the approximate 7 month sample is indicated with an asterisk.

The most commonly detected *Lactobacillus* species was *L. acidophilus* (*sensu lato*). The species *L. acidophilus*, *L. johnsonii* and *L. gasseri* share 16S rDNA sequences with high similarity, thus, the PCR fragments with highest similarity to *L. acidophilus* may have originated from any of these three species. Other species found with the *Lactobacillus*-group specific are listed in Fig. 3. In general, the *Lactobacillus*-group primers had a good specificity for the target group, which includes *Lactobacillus*, *Pediococcus*, *Weissella* and *Leuconostoc*, but occasionally PCR fragments from other bacteria such as streptococci and enterococci were also amplified (Fig. 3).

DISCUSSION

In this study the diversity of dominant bacteria was monitored by amplifying 16S rDNA fragments from bacteria and subsequently separating the amplicons in a sequence-specific manner by DGGE. The abundance of fragments in DGGE profiles representing different 16S rDNA sequences reflects the bacterial species diversity. The diversity of fragments in DGGE profiles of 13 infants indicated that there was no significant difference in the complexity of the predominant bacterial community between breast-fed and formula-fed infants before or after weaning. Tannock (25) and Orrhage and Nord (4) compared the results from numerous original cultivation studies and concluded that differences in the infants' intestinal microbiota due to breast-feeding or formula-feeding are minor and apply mainly to clostridia that are found more frequently and in higher numbers in formula-fed infants. The results of our molecular study are in agreement with this observation. This deviation from the original idea that bifidobacteria are found mainly in breast-fed infants may be due to numerous factors. Firstly, the composition of infant formulae has been improved over the years (25, 26), which is likely to have led to a more breast-fed-like colonisation pattern in formula-fed infants. Secondly, delivery with few exposures to microbes due to the use of vaginal antiseptics at the time of birth or caesarean section can lead to an altered intestinal colonisation in infancy (9, 27, 28). Finally, there are significant geographical differences in the composition of intestinal microbiota of infants (13, 29). It is noteworthy that a fragment in the DGGE profile may include different sequences with the same mobility and in some cases one species can produce more than one fragment in DGGE (19). Nevertheless, PCR–DGGE can give a reasonable indication on the bacterial diversity.

Ruminococcus gnavus-like sequences corresponding to an upper dominant band in the DGGE profiles were detected in several of the faecal samples and appears to be one of the major species at least in some infants. This fragment was previously cloned and sequenced also from infant D and was tentatively identified to have derived from *R. gnavus* (96% similarity) (17). The use of alternative universal 16S rRNA primers with modification of the denaturing gradient resulted in different migration for some of the upper bands (data not shown) suggesting that species other than ruminococci may be responsible for some of these dominant bands. Occasionally, fragments from different species migrate to the same position in DGGE (19), so identity confirmation by cloning and sequencing or hybridisation is necessary.

The changes in bacterial communities between 1 and 7 months were comparable in both feeding groups. This disagrees with cultivation studies indicating that breast-fed infants have more intense shifts in their bacterial communities due to weaning (6). The intestinal microbiota in infants of both groups seem to undergo equally intense changes, at least at the level of bacterial species and strains. Interestingly, the bifidobacterial communities remained stable in many infants despite the weaning period. The *Bifidobacterium* species typically found in adults are different from those in infants (30). According to our results, the shifts in the species composition of the bifidobacterial populations do not occur directly in response to weaning, i.e. the species/strain composition of the *Bifidobacterium* populations was not generally affected by the introduction of solid food to the infants diet.

The colonisation frequency of infants by bifidobacteria, as detected by genus-specific PCR, was comparable in both feeding groups or even higher in the formula-fed group. This result is in contrast with the classical idea that breast-fed infants are more frequently colonised by bifidobacteria. Some of the faecal samples in this study have also been subjected to quantitative 16S rDNA dot blot analysis with specific probes for different bacterial groups or genera (31). The dot blot analysis confirmed our finding that two breast-fed infants, coded 1 and 52, and one formula-fed infant, coded 49 were not colonised by bifidobacteria at the age of 1 and/or 7 months (31). Our results are in line with several studies that show equal, either high (7, 8) or low (9, 13, 27), frequency of bifidobacterial colonisation in both groups. Previous observations that the bifidobacterial populations of infants generally consist of one to three species (30, 32, 33) are confirmed by our findings. The most commonly found *Bifidobacterium* species in infants vary from one study to another (7, 30, 32–34). This may reflect divergent distribution of *Bifidobacterium* species in various geographical regions or ethnic groups. It cannot be excluded that variation in the predominant species between different studies may also be due to differences in the identification procedures (phenotypic or genotypic methods). However, in most studies there was no difference in the species distribution between breast-fed and formula-fed babies (7, 32, 33). In this study, *B. infantis* was the most frequently found *Bifidobacterium* species in both feeding groups. Concerning lactobacilli, our molecular results support the findings of previous culture-based studies that *L. acidophilus* (*sensu lato*) is the

most common lactobacilli in infants irrespective of the type of feeding (7, 35).

In conclusion, PCR–DGGE is a useful and rapid method for obtaining a general picture of the microbial diversity in individual samples and particularly feasible for following shifts in populations in time. The possibilities for comprehensive analysis of intestinal microbiota can be further improved by developing of PCR–DGGE methods for other bacterial groups and by using the qualitative tools in combination with quantitative molecular tools such as FISH.

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