Molecular Genetic Study of Species and Strain Variability in Bifidobacteria Population in Intestinal Microflora of Breast-Fed Infants and Their Mothers

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Qualitative and quantitative composition of enteric bifidoflora was studied in a group of 13 mother–infant pairs. Pure cultures of Bifidobacterium strains were isolated from feces and their species were identified by PCR with species-specific primers or by partial sequencing of 16S rDNA. The strains were compared by REP-PCR. The most incident Bifidobacterium species in mothers were *B. longum* and *B. adolescentis*. The infants were mainly colonized by *B. bifidum* and *B. longum*. The mother and her baby were colonized by the same Bifidobacterium species in 9 of 13 cases. In 5 (38.5%) of these cases, these pairs of strains were identical by their REP-PCR profiles. These strains belonged to *B. longum* in one case, *B. bifidum* in 3 cases, and *B. adolescentis* in 1 case. Our results support the hypothesis on early colonization of infants with maternal bifidobacterium strains.

Key Words: *enteric microflora; bifidobacteria; REP-PCR*

The formation of normal enteric microflora in humans starts from the earliest stages of life. It is assumed that the maternal passages serve as the first source of bacteria colonizing the gastrointestinal tract in the infant, as passing them the newborn swallows their contents together with vaginal microflora. After birth, colonization of the infant intestine is continued with not only bacteria from the mother, but also with microorganisms from the environment [2,3,9,10]. However, the hypotheses on the contribution of maternal bacterial strains to primary colonization of the infant gastrointestinal tract remain not sufficiently confirmed by research findings. This, in turn, is explained by low informative value of microbiological methods mainly based on studies of morphological, physiological biochemical, and cultural characteristics of maternal and infant bacterial strains.

Introduction of PCR-based molecular biological methods in practical microbiological studies promotes effective identification and molecular typing of microorganisms [4,6,7].

We carried out a comparative analysis of bifidobacterium strains in mother–infant pairs by the REP-PCR method (amplification of repeating elements of genome DNA) in order to detect their genetic identity.

MATERIALS AND METHODS

Study of the species and subspecies composition of bifidoflora was carried out in 13 mother–infant pairs. Two infants were aged 11 and 9 months, the rest 2-5.5 months (mean age 3.2 months). Two mothers were aged 19 and 42 years, the rest 24-30 years (mean age 26.2 years). All mothers and infants were clinically healthy by the moment of the study, without a history of gastrointestinal infections. The infant were breast fed from birth.

Feces were collected with a sterile spatula into a container and transported to a bacteriological laboratory. Serial dilutions were prepared from the material.

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Aliquots (0.1 ml) from 10^7 and 10^9 dilutions of the studied material were inoculated in Petri dishes with Bactofoc medium (Hydrobios). The dishes with cultures were incubated in microanaerostates (OXOID) with gaseous mixture (85% N_2, 10 H_2, 5% CO_2) at 37°C for 72 h. Primary screening of bifidobacterium strains was carried out by evaluating the morphology of the resultant colonies and gram-staining of the material from the colonies. All types of colonies suspected to belong to Bifidobacterium genus were reinoculated in order to obtain pure cultures: first in solid nutrient medium, then in TPY liquid nutrient medium for subsequent preparation of lyophilized stock culture. Lyophilization of pure bacterial cultures was carried out in sucrose (10%) and gelatin (1%) solution in an SB1 lyophilizer (Chemlab). All subsequent manipulations with pure Bifidobacterium cultures were carried out after their isolation from lyophilized stock.

Genome DNA was isolated from pure Bifidobacterium cultures using Genomic DNA Purification Kit (Fermentas) in accordance with the instruction. The quality and volume of the resultant DNA were evaluated by electrophoresis in 1% agarose gel.

Species identification of bifidobacterium strains was carried out by PCR with 9 pairs of species-specific primers (*Bifidobacterium longum*, *B. breve*, *B. bifidum*, *B. longum bv. infantis*, *B. adolescentis* genotype A, *B. adolescentis* genotype B, *B. catenulatum*, *B. angulatum*, and *B. dentium*) as described previously [1]. Strains not identified by the PCR method were identified by sequencing 16S rRNA gene fragment as described previously [8]. Genotyping of bifidobacteria by the REP-PCR method was carried out according to the previously described protocol [8].

PCR products were visualized by gel electrophoresis of 10-µl sample in 1.5% agarose gel prepared in 1X TAE buffer containing 0.5 µg/ml ethidium bromide.

RESULTS

A total of 104 bifidobacterium strains were isolated from 13 mother–infant pairs by primary screening (61 from mothers and 43 from infants). The incidence of bifidobacteria was 100% in both groups. The mean levels of bifidobacteria was 10.2±0.6 log CFU/g material in infants and 9.4±0.6 log CFU/g in mothers. For species identification, each strain was tested with nine pairs of species-specific primers. The majority of strains were identified by this method. For 12 strains, no positive reaction of DNA with any of the primer pairs was found. Three of these were identified by partial sequencing of 16S rDNA and 9 isolated strains were not identified. The mean number of Bifidobacterium species per sample identified with consideration for the selected resolution level was 2.7±1.1 in mothers and 1.9±1.0 in infants.

Thus, 95 of 104 isolated cultures were identified: 17 of these were *B. longum*, 29 *B. adolescentis*, 4 *B. catenulatum*, 7 *B. bifidum*, and 1 *B. breve* in mothers and 13 *B. longum*, 13 *B. bifidum*, 4 *B. longum bv. infantis*, 2 *B. breve*, 1 *B. angulatum*, 2 *B. adolescentis*, and 2 *B. dentium* in infants. The incidence of bifidobacterium species in the mothers was as follows: 92.3 and 76.9% *B. longum* and *B. adolescentis*, respectively, 46.2, 30.8, and 7.7% *B. bifidum*, *B. catenulatum*, and *B. breve*, respectively (Table 1). A different distribution of bifidobacterium species was seen in infants. The most incident were *B. bifidum* and *B. longum* (61.5 and 53.8%, respectively), *B. longum bv. infantis* and *B. breve* were more rare (30.7 and 15.4%), the incidence of *B. adolescentis* and *B.*

TABLE 1. Quantitative Levels and Incidence of Predominating Bifidobacterium Species in Enteric Microflora of Infants and Their Mothers (*M*±*m*)

Bifidobacterium species	Infants (*n*=13)		Mothers (*n*=13)	
	log GFU/g	abs/%	log GFU/g	abs/%
B. bifidum	9.5±0.7	8/62	8.9±0.6	6/46
B. longum	9.7±0.8	7/54	8.5±0.8	12/92
B. breve	9.5±1.0	2/15	8.6	1/8
B. catenulatum	<8.0	0	8.8±0.8	4/31
B. adolescentis	9.8±1.2	2/15	9.4±0.5	10/77
B. longum bv. infantis	9.7±0.6	4/31	<8.0	0
B. dentium	8.3	2/15	<8.0	0
B. angulatum	9.8	1/8	<8.0	0

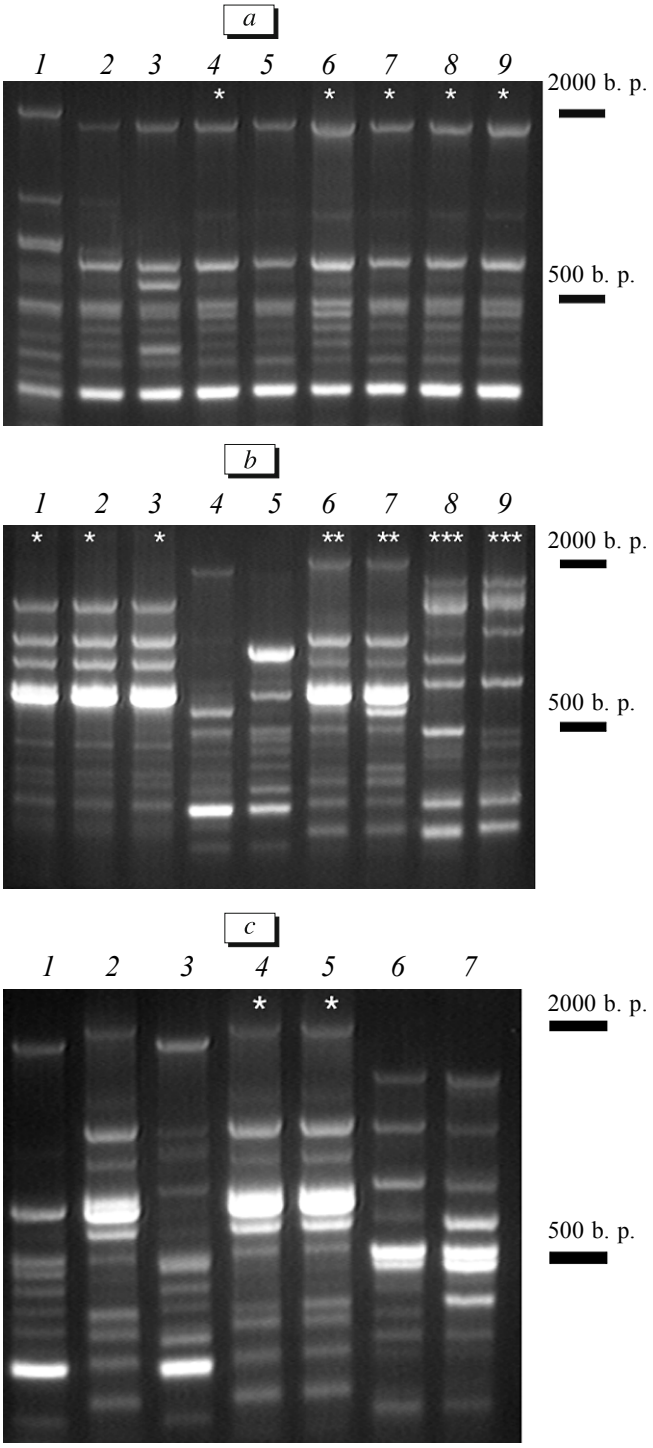

Fig. 1. Gel electrophoresis of REP-PCR products of genome DNA of bifidobacterium strains isolated from mother–infant pairs. Asterisks show identical profiles of electrophoretic bands of REP-PCR DNA for infant (P) and maternal (M) strains. The number of asterisks (*b*) differentiates the compared pairs. *a*) pair No. 1, *B. longum* strains. *1*) M7; *2*) P22; *3*) P29; *4*) M1; *5*) P18; *6*) M3; *7*) P23; *8*) P19; *9*) P25. *b*) pair No. 9, *B. bifidum* strains. *1*) M11; *2*) M15; *3*) P2. Pair No. 9, *B. longum* strains. *4*) M13; *5*) P1. Pair No. 10, *B. bifidum* strains. *6*) M2; *7*) P8. Pair No. 12, *B. adolescentis* strains. *8*) M2; *9*) P11. *c*) Pair No. 1, *B. longum* strain P17; *2*) pair No. 8, *B. bifidum* strain P2; *3*) pair No. 10, *B. longum* strain M6. Pair No. 13, *B. bifidum* strains: *4*) M5; *5*) P2. Pair No. 13, *B. breve* strains: *6*) P4; *7*) P1.

dentium was similar (15.4%), and the most rare species was *B. angulatum* (7.7%). Species identification of isolated strains showed that in some cases two or more strains of the same species were isolated from the same patient.

In 9 of 13 mother–infant pairs, the same bifidobacterium strains were identified (a total of 34 strains). In both mothers and infants, *B. longum* were found in 6 mother–infant pairs, *B. bifidum* in 4 pairs, *B. adolescentis* and *B. breve* in 1 pair. In order to clear out whether the maternal and infant strains were genetically identical, the REP-PCR method was used, a rapid and reliable method for inter-strain differentiation of prokaryotes, including the lactic acid bacteria [5]. In cases when the REP-PCR profiles of the studied bifidobacteria isolated from mothers and infants had 100% identity by electrophoretic band number and weights, they were regarded as belonging to the same bacterial strain, and *vice versa*, when electrophoretic separation of PCR products showed different profiles of the bands, the isolated strains were regarded as different strains of the same bifidobacterium species (Fig. 1). We found identical bifidobacterium strains in the intestines of 5 (38.5%) mother–infant pairs. In three cases, identical bifidobacterium strains belonged to *B. bifidum*, in one case *B. longum*, and in one case *B. adolescentis*. In four cases, the age of infants colonized with bifidobacteria genetically identical to maternal strains varied from 2 to 5.5 months and in one case was 11 months.

Hence, more than one-third of infants were colonized with enteric bifidobacteria genetically identical to the strains isolated from mothers. These results support the hypothesis on the contribution of maternal bifidobacterium strains to early colonization of the infant intestine. On the other hand, no bifidobacteria genetically identical to maternal strains were detected in the microflora of the greater part of infants and hence, their source is unknown. This can be due to the fact that our study was focused on comparative analysis of bifidobacterial strains predominating in the intestine of mothers and their infants, with the concentrations of no less than 10^8 CFU/g material, while the populations of bacteria colonizing the intestine in lower concentrations were neglected. In addition, as was mentioned above, the maternal passages could be the sources of primary colonization of the infant intestine. The microflora of maternal passages includes, in addition to lactobacilli predominating in this ecological niche, bifidobacteria. All these data imply that further studies in this direction should be based on comparison of not only the strains predominating in the intestine, but also of minor groups of bifidobacteria and numerous sources of potential colonization of infants with normal microflora.

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