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Malin Olsson

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Transmission of lactobacilli and bifidobacteria from mother to infant

Överföring av laktobaciller och bifidobakterier från moder till barn

Malin Olsson

Supervisor: Hans Jonsson

Assistant Supervisor: Johan Dicksved

Examiner: Stefan Roos

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Abstract

The bacteria in our intestine affect us in many different ways, for example, they protect us against other harmful microorganisms, produce essential vitamins and they are also though to affect our immune system. Allergies and other immunological diseases are very common in developed countries compared with developing countries. This has given rise to the hygiene hypothesis, which suggests that less exposure to microorganisms increases the risk of developing immunological diseases. Therefore some bacteria might decrease the risk of developing allergies. People, living according to an anthroposophic lifestyle have been seen to have less prevalence of allergies. By living according to this lifestyle they eat lots of fermented food which sometimes contains bifidobacteria and lactobacilli, have limited use of antibiotics etc. These characteristics may be important factors why anthroposophic individuals have less prevalence of developing allergies.

Through this study, lactobacilli and bifidobacteria were analyzed in fecal samples from 16 mothers and their infants to see if there were any similarities between mother and child and if the infant get colonized with the mothers' fecal lactobacilli and bifidobacteria. The anthroposophic lifestyle was also investigated, comparing anthroposophic infants with infants being sensibilized. The amount of bacteria was calculated by using culturing techniques and the bacteria were typed through rep-PCR. Sequencing of the 16S rRNA genes was also done on a few lactobacilli and bifidobacteria to identify which species that were transferred.

The results indicated that the infants did have more bifidobacteria and lactobacilli than their mother but that there was high variation between the samples. The results from rep-PCR showed that there occurred transmission from the mother to her infant. But that the infants' microbiota is very unstable and that these bacteria from the mother only in some cases persist over time and are able to establish in the infants microbiota. No clear difference was seen between infants living according to an anthroposophic lifestyle and infants being sensibilized. The results from the sequencing showed that the species which was the most common *Lactobacillus* and *Bifidobacterium* among the samples were *Lactobacillus rhamnosus/paracasei* and *Bifidobacterium longum.*

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1 Introduction

Humans live in symbiosis with many microorganisms and one example is the bacteria living in the intestine, the gut microbiota. (Guarner, 2007) The bacterial composition can affect the host in both harmful and protective ways (Sekirov et al, 2010), for example the microbial fermentation contributes to the host energy uptake, produce essential vitamins and protect the host from pathogens. (Hsiao et al, 2008) There have also been some indications that the microbiota plays an essential role for immune system development. (Dicksved, 2008)

The prevalence of allergy has increased remarkably in developed countries these last decades in comparison with developing countries. (Noverr and Huffnagle, 2005) There have been studies indicating that disruption of the microbiota increase the potential to develop allergies and this is particularly important when the microbiota establish in newborn infants. (Dicksved, 2008)

1.1 The hygiene hypothesis

The hygiene hypothesis means that our modern society and lifestyle have created a very clean environment and that this in turn increases the risk of developing immunological diseases. This is in contrast to what we have believed before, that "over cleanliness" and less exposure to microorganisms is considered good and prevents us from getting sick. But according to this hypothesis, less exposure to pathogens decrease the protection against developing allergies and other immunological diseases. It has also been indications that some environments protect individuals from immunological diseases which confirm this hypothesis. Examples of this kind of environment factors are older siblings, poor hygienic conditions during the first years and pets. (Guarner, 2007)

It has been demonstrated that there is an association between the exposure of microbes and the likelihood to develop immunological disorders. Therefore, in developed countries, asthma and allergies are more common than in developing countries. The hygiene hypothesis indicates that infections can protect us against developing immunological diseases. However, there has been studies where infection instead of protecting against allergies have shown to trigger them. (Guarner, 2007) Therefore, the development of tolerance is dependent on the bacterial strains colonizing the intestines. Some bacterial strains might increase tolerance meanwhile other might not. Also if some strains are reduced in amount that might delay tolerance development. (Alm et al, 2002)

1.2 Mechanism

The use of antibiotics and other factors can lead to changes of the normal immunological tolerance mechanism. The regulatory T cells are involved in many processes and one of them is that they can trigger pro-inflammatory cytokines which in turn gives an anti-inflammatory effect and affect the immunological tolerance. (Noverr and Huffnagle, 2005) There is no difference in number of regulatory T cells between allergic and non-allergic individuals. The difference is instead in their functional activity (Guarner, 2007). Infections can affect the regulatory T cells by increasing their activity and amount. During infections these regulatory T cells drive the self protective immune response. This is a way to explain the hygiene

hypothesis by infections increasing tolerance through increasing the amount of regulatory T cells. (Guarner, 2007)

1.3 The gut microbiota

The gut microbiota is the term for all microorganisms living in the intestine of a host. The microbial communities in the gut are highly complex and variable between individuals. (Guarner, 2007) The microbiota is very complex in the human gastrointestinal tract and contains most likely over 1000 different species. (Vaughan et al, 2002) The composition of the bacterial community in the gut has been shown to depend on several factors such as host genotype, diet and transmission at birth. (Guarner, 2007)

The intestine are completely sterile in infants at birth but are colonized during and after delivery. (Noverr and Huffnagle, 2005) The infants are first exposed to the bacteria from the mother's vagina and feces (Guarner, 2007) and from the surroundings. The microbial community is very variable the first months for infants and most of the bacteria are facultative anaerobes. When food is introduced the gut gets more and more colonized with obligate anaerobes (Dicksved, 2008) and develops into a bacterial community. The bacterial composition of this community is then dependent on the host lifestyle. (Noverr and Huffnagle, 2005) When the child reaches two years of age the microbiota is stabilized and resembles the microbiota of adults. (Wall et al, 2009) The amount of lactobacilli in infants is similar to that of adults but infants' exhibit higher counts on bifidobacteria than adult humans have. (Vaughan et al. 2002) Major factors which affect the microbial composition are the birth delivery mode, if the infant is delivered by cesarean section, antibiotic use, diet, if the infant are breast-fed or get supplement. (Noverr and Huffnagle, 2005) The factors which affect the composition of the microbial community and how it affects the hosts are yet not fully understood. (Dicksved, 2008)

1.4 Atopy

There have been studies showing that infants having allergies differ in their microbiota from healthy infants. (Guarner, 2007) It has been shown that non-atopic infants have higher proportions of bifidobacteria than the atopic infants. (Dicksved, 2008) Lower counts of some bifidobacteria have also been associated with allergies. (Guarner, 2007) In addition, allergy has been associated with higher amount of aerobic microorganisms and decreased amount of anaerobic microbes, especially lactobacilli. (Sekirov et al, 2010) This is an indication that lactobacilli and bifidobacteria can be beneficial for preventing the development of allergies. (Dicksved, 2008)

1.5 LAB (Lactic acid bacteria)

Among the LAB, strains of bifidobacteria, lactobacilli and leuconostoc are the most common in the human microbiota. Bifidobacteria doesn't belong to the lactic acid bacteria (LAB) but it has similar features to those bacteria belonging to LAB. Therefore bifidobacteria is often thought of as a LAB. (Vaughan et al, 2002). It has been seen that infants being breastfeed exhibit higher counts of *Bifidobacterium*, although this bacterium decrease into adulthood. (Goin, 2010) Bifidobacteria and lactobacilli are the ones which are most studied because they have been proven to promote human health. Therefore these are also often included as

probiotics. (Vaughan et al, 2002) Probiotics contain living microorganisms which can reach the intestine in active state and exhibit positive health effects. (Goin, 2010) What have been established is that these bacteria are beneficial for the host in protection against pathogens and the maturation of the immune system. (Vaughan et al. 2002)

1.6 Anthroposophic lifestyle

Children living according to an anthroposophic lifestyle eat a lot of fermented food with living lactic acid bacteria. (Noverr and Huffnagle, 2005) They also have a limited use of antibiotics as well as anti-pyretics and vaccines. Infants which have been treated with antibiotics have shown to exhibit a lower count of lactic acid bacteria. (Alm et al, 2002) Interestingly, studies have shown that individuals living according to an anthroposophic lifestyle have a reduced risk of developing allergies compared with individuals living in the same area. Ingestion of LAB may be one factor important for the decreased the risk of developing allergies. Anthroposophic individuals have shown to have higher counts of lactic acid bacteria. (Noverr and Huffnagle, 2005) The diversity of Lactobacilli where also shown to higher in anthroposophic infants. (Alm et al, 2002)

1.7 Rep-PCR

Repetitive sequence based PCR (rep-PCR) is a fingerprinting method. (Jernberg et al. 2007) Dispersed repetitive sequences can be found in both prokaryotes as well as in eukaryotes. The rep-PCR method uses this as a target for amplification. The primers bind to these specific repetitive sequences and through PCR they are amplified. The PCR product can then be separated through electrophoresis by mass and charge which give rise to unique rep-PCR DNA fingerprint patterns. (http://www.microbe-environmental.com/services/reppcr) The band patterns visualized on the gels are specific for each strain or clone which enables typing. (Jernberg et al. 2007)

1.8 Aim

In this study, the aim was to examine the abundance and stability of selected bacterial groups in infants and their mothers. Lactobacilli and bifidobacteria was quantified by culture techniques and typed using rep-PCR. Transmission of bacterial strains from the mother to the infant was also examined and the persistence of these strains was followed over time. Two different lifestyles were analyzed including those which are living according to an anthroposophic lifestyle and a control group which have infants being sensibilized. This was made to see if there were any differences in amount or transmission between these two groups. Sequencing was also done on some commonly found isolates to determine identity and to see the effectiveness of the method used.

2 Methods

2.1 Experimental setup and sampling

The feces samples from mother-child pair were obtained as a part of the ALADDIN (Assessment of Lifestyle and Allergic Disease During Infancy) cohort. The collections of data in ALADDIN started 2004.

(http://www.vidarkliniken.se/info/forskning/forskningsprojekt/allergi/alladin/) In the current study, a total of 16 mother-child pairs were selected. The mothers were sampled approximately 1 week before delivery and two months after delivery. From the infants, samples were collected at four time points; 3-6 days, 3 weeks, 2 months and 6 months after birth. For 4 mother-child pair the whole sample series were analyzed to see if and when the transmission from mother to child was detectable. For the other 12 mother-child pairs only the mother sample 1 week before delivery, infant samples 3 weeks and 6 months after birth were analyzed. Of the 16 pairs, seven were living according to an anthroposophic lifestyle and the other nine showed signs of sensitization. Five of the infants living according to an anthroposophic lifestyle were delivered in the home, all other infants where delivered at the hospital and no infant was delivered with caesarean section. All infants were breast feed but two of them got formula the first week, both of them being sensibilized infants. Two infants exhibit gut symptoms after two months and two had colic. One of the mothers having a sensibilized infant got antibiotic during pregnancy.

2.2 Culturing conditions

To be able to quantify the amount of bifidobacteria and lactobacilli, dilution series were done and the colony forming units (CFU) were counted and calculated. From each sample 0,1 g feces were weighted and diluted with PBS in 10x steps and spread on agar plates. Bifidobacteria were grown on MRS medium supplemented with 50mg/l mupirocin (P.J Simpsons, 2003) to make the medium selective and lactobacilli were cultured on Rogosa medium. The plates were incubated under anaerobic conditions at 37°C for three days.

2.3 Typing by rep-PCR

Seven colonies from each sample were selected and analyzed by rep-PCR. The primer used for the PCR was GTG5 5´ - GTGGTGGTGGTGGTG - 3´. In each reaction, 8,8 µl green Taq mix was added to 8,8 µl nuclease free water and 1 µM primer. As templates, colonies where suspended in 20 µl sterile water and from the suspension 2 µl where added to the PCR reaction. The PCR program was as follows: 95°C for 7 min; 90°C, 30s; the cycling conditions for 30 cycles was; 95°C, 1 min; 40°C, 1 min; 65°C, 4 min then 65°C for 16 min; 4°C, ∞. The PCR products were analyzed on an 1% agarose gel with a 1 kb size marker and were run for approximately 1 hour at 100 V. To see if there was any overlap of strains between the pairs, the software GelCompare II was used to analyze the results. All band visualized on the gel was marked and the band patterns were compared with each other. A line was drawn at 75% similarity were the patterns was considered to be the same ribotype.

2.4 Identification by 16S rRNA sequencing

Sequencing of the 16S rRNA genes from 20 bifidobacteria and 20 lactobacilli isolates were done to determine which bacteria that was dominant by culturing. By sequencing, the effectiveness of the Rep-PCR method could also be evaluated (see if two similar band patterns are the same species). The amplification of the 16S rRNA gene was done directly on the bacterial suspensions used earlier. PuReTaq Ready To Go PCR beads (GE healthcare) was used for the PCR reaction. To the beads, 0,5 µl of the bacterial suspension, 1 µl of the primers F8m 5´-AGAGTTTGATCCTGGCTCAG-3´ (10 pmol/µl) and 926r 5´-CCG TCA ATT CCT TTG AGT TT-3´ (10 pmol/µl) and 22,5 µl nuclease free water, was added. The PCR program

was as follows; 95 °C for 5 min; the cycling conditions for 30 cycles was; 95°C, 30s; 55°C, 30s; 72°C, 1 min, then 72°C for 10 min; 4°C, ∞. The PCR products were then checked on a 1% agarose gel with a 1 kb size marker and were run for approximately 40 min at 100 V. The PCR products were then sent directly for PCR product purification and sequencing at Macrogen Inc.

3 Results

3.1 Analysis of lactobacilli

Fecal bacteria were isolated on agar plates and CFU/g was calculated. The amounts of lactobacilli CFU are summarized in figure 1. Sample A and B are from the mothers and C to F are samples from the infants. All four mother-child pairs from which the whole sample series were analyzed are shown in the figure. Samples 73 and 122 are from sensibilized infants and 139 and 144 are two of the infants living according to an anthroposophic lifestyle. All A samples have approximately the same amount of lactobacilli but the B samples have more variety. Two out of three B samples had higher amount of lactobacilli than the A samples. In sample A and C from group 139 and C from group 73 there were no growth. There was a very high variation among the D samples, ranging from very low amount of lactobacilli to very high. In the E and F samples the variance was lower.

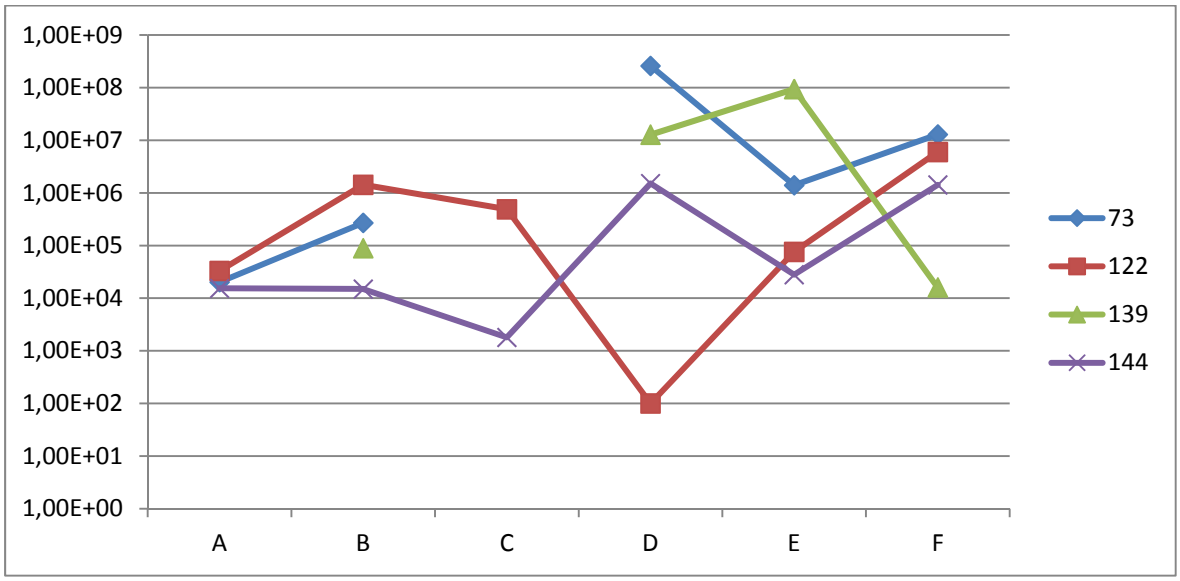

Figure 1: Amount (CFU/g) of lactobacilli from mother one week before delivery (A), two months after delivery (B), infant 3-6 days after birth (C), 3 weeks after birth (D), 2 months after birth (E) and 6 months after birth (F).

Lactobacilli counts from the other mother-child pairs, in total 12 pairs are summarized in figure 2. For each pair three time points were analyzed; mother sample one week before delivery (A), infant sample 3 weeks (D) and 6 months after birth (F). The A samples had very similar amount of CFU while D and F samples showed a large variation. In five D samples; 291, 50, 179, 43 and 52 there were no bacterial growth. The five first pair samples; 244, 291, 212, 208 and 50 are those living according to an anthroposophic lifestyle and the other seven pairs are control groups.

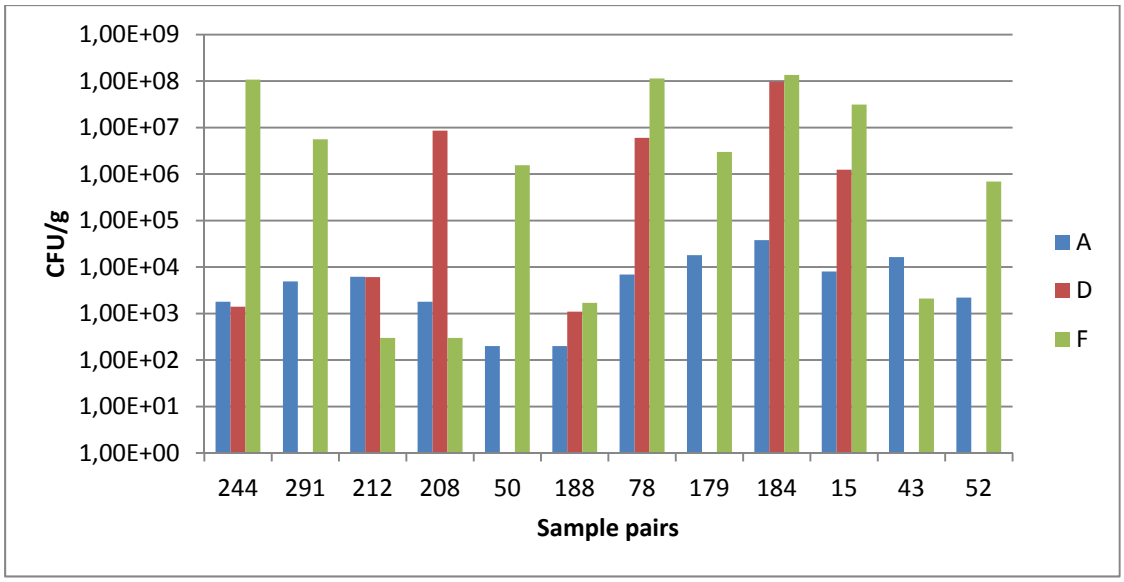

Figure 2: Amount of lactobacilli (cfu/g) from the 12 mother-child pairs analyzed at three time points; mother 1 week before delivery (A), child samples 3 weeks after birth (D) and 6 months after birth (F).

3.2 Analysis of bifidobacteria

The four samples from which the whole time series was analyzed for bifidobacterium are shown in figure 3. Sample A and B are from the mothers and C to F is samples from the infants. Samples 73 and 122 are from sensibilized infants and 139 and 144 are two of the infants which are living according to an anthroposophic lifestyle. In the pairs 73 sample A, 122 sample A, C, D and E, 139 sample C and 144 sample B bifidobacteria were detected.

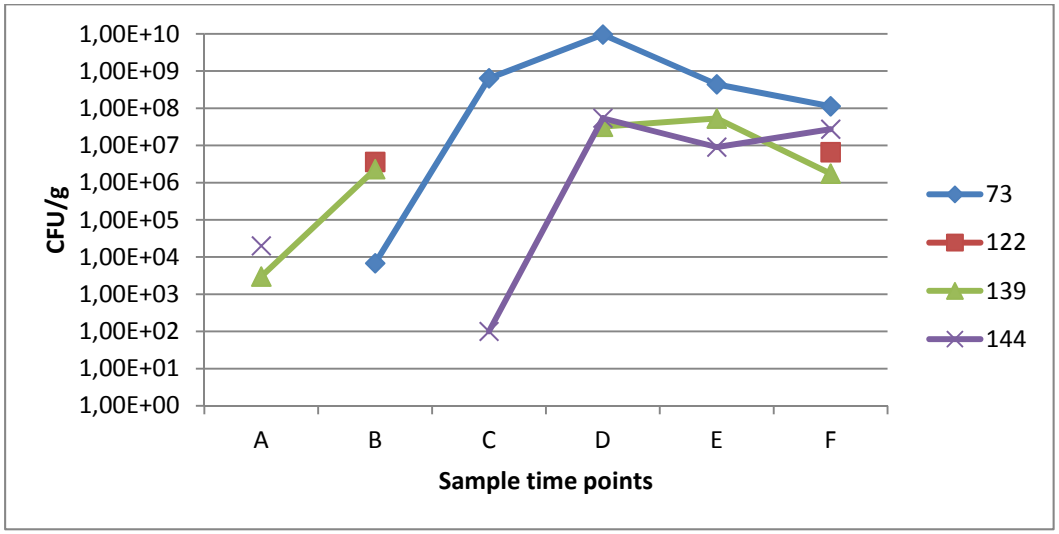

Figure 3: Amount of Bifidobacterium (cfu/g) from mother 1 week before delivery (A), two months after delivery (B), infant 3-6 days after birth (C), 3 weeks after birth (D), 2 months after birth (E) and 6 months after birth (F).

For the other 12 mother-child pairs the amount of bifidobacteria is shown in figure 4. For each pair, three time points were analyzed; mother sample one week before delivery (A), infant sample 3 weeks (D) and 6 months after birth (F). There was no bacterial growth in pair 208 sample D but in all others. The five first pair samples; 50, 244, 291, 212 and 208 are those which are living according to an anthroposophic lifestyle and all others are control groups. The control groups are those which are sensibilized. The amount of bifidobacteria in the A samples ranges from very low to quite high amounts. From the figure we can see that in most

of the pairs the A (mother samples) have lover amounts of bifidobacteria than the D and F samples (infant samples). What also is shown is that the amounts of bifidobacteria were very high in most F samples.

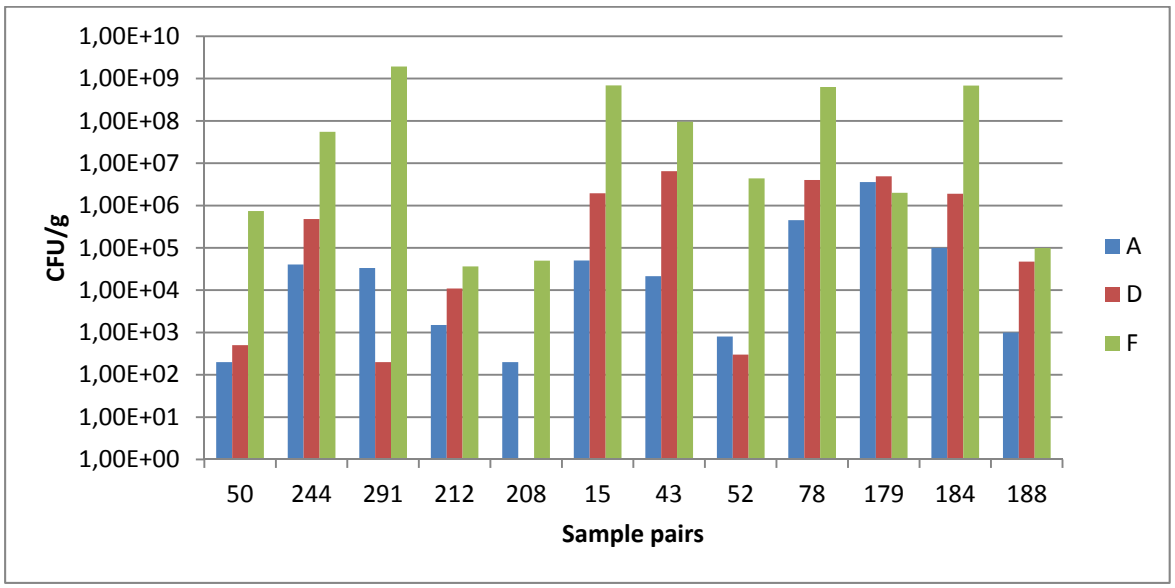

Figure 4: Amount of bifidobacteria in the 12 mother-child pairs at three time points; mother 1 week before delivery (A), child samples 3 weeks after birth (D) and 6 months after birth (F).

3.3 Rep-PCR

From the agar plates, 7-10 colonies representing each sample were picked and analyzed by rep-PCR. Rep-PCR give rise to different band pattern for different strains. The banding pattern can then be compared between isolates to see if they are the same strain. The four samples for which the whole time series were analyzed are summarized in figure 5 and 6. In figure 5 the different types of lactobacilli are shown and in figure 6 the different *Bifidobacterium* are shown. Those rep-PCR types which had an overlap between samples was given an own number (pile) and those rep-PCR types which were only seen in one sample are stacked together as unique. The sizes of the piles are related to the abundance found in the samples. The mother-child pairs 144 and 139 are those which live according to an anthroposophic lifestyle.

Between the two mother samples (A and B) there were rep-PCR types which were found in both samples. There was also some variation seen between them, some Rep-PCR types were only found in one sample. In pairs 73, 122 and 144 there have probably been transmission of lactobacilli from mother to child. But only in pair 122 (and 73) the strains seems to persist over time. Between the infant samples there is considerable variation but some strains persist. In figure 6 we can see that there is overlap from mother to child in all four pairs. In 139 the overlap is only seen in sample F which is after the infant reached the age of 6 months. In the other infant samples none of these strains were detected. In pair 122 one strain dominated in sample C but in sample F other strains were taking over. In sample 144 only one strain was dominating in the infant samples which is the same strain seen in the mother. In pairs 73 sample A, 122 samples A, D and E, 139 sample C no band pattern were retrieved.

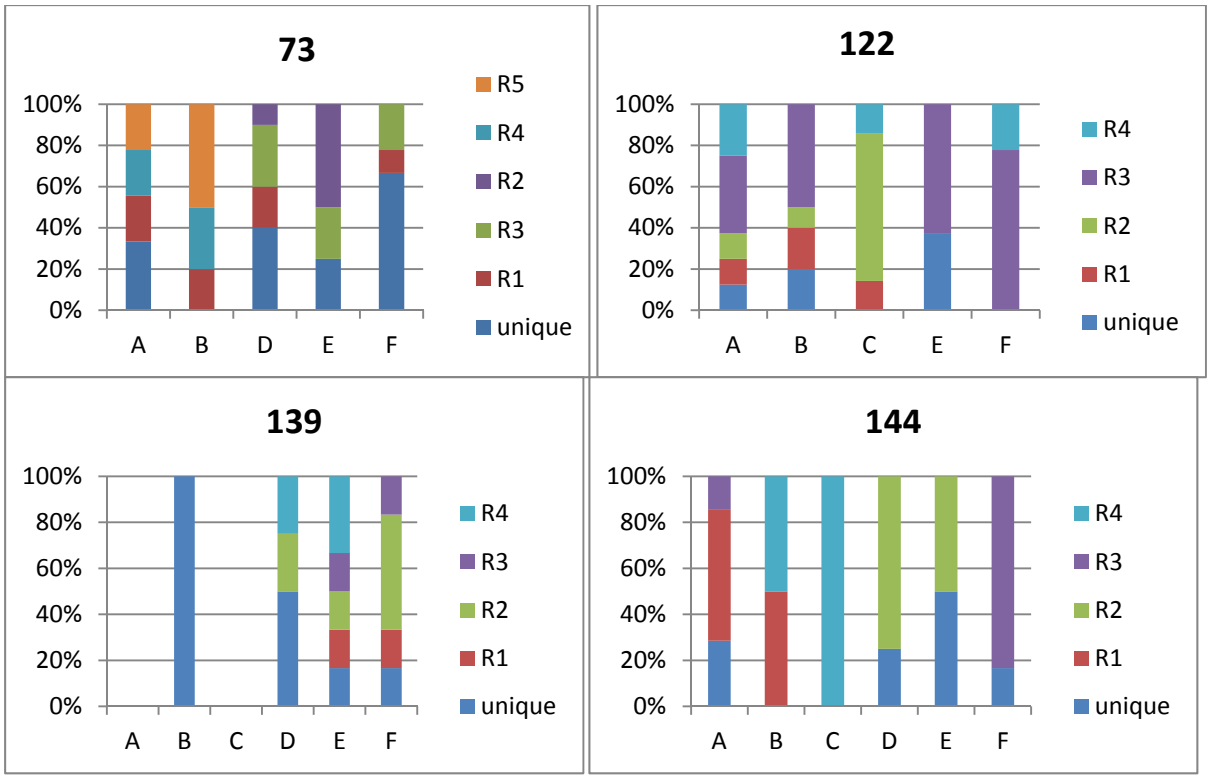

Figure 5: Strains of lactobacilli detected in the whole sample series of four mother-child pairs. The amount (%) of different strains is shown for each sample. The stack representing unique are those ribotypes which were only found in one sample.

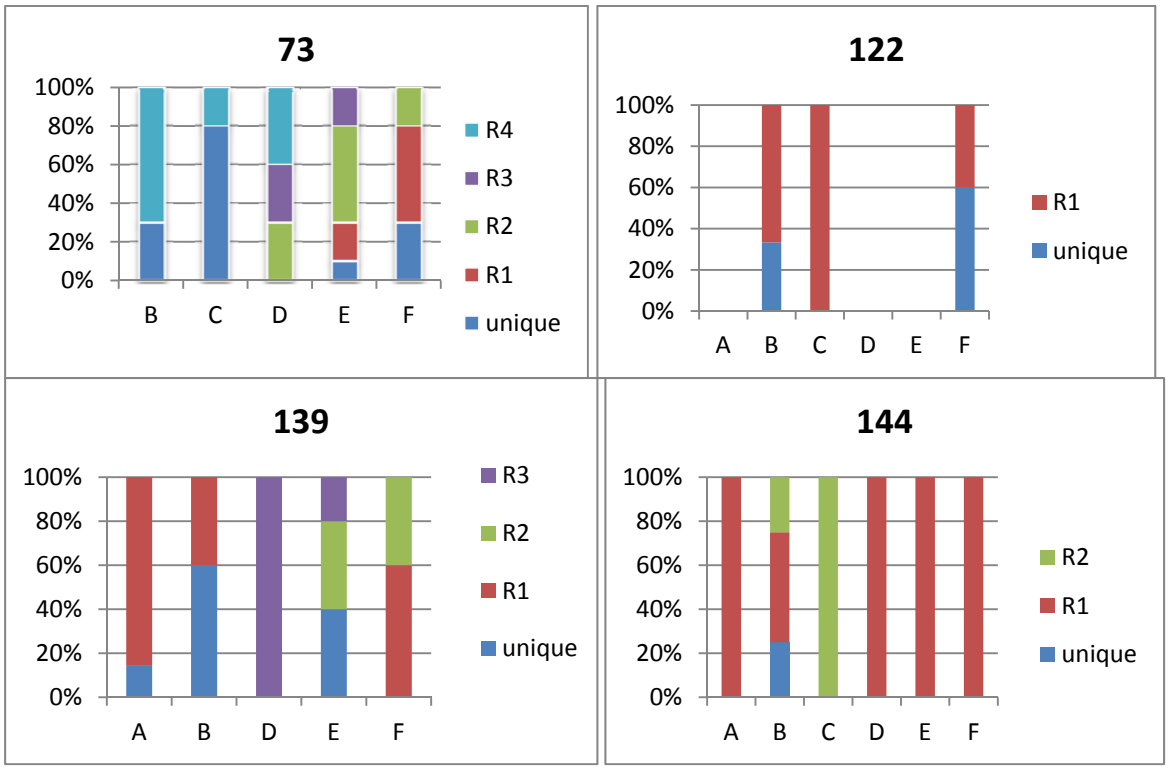

Figure 6: Strains of bifidobacteria detected in the whole sample series of four mother-child pairs. The amount (%) of different types is shown for each sample. The stack representing unique are those ribotypes which were only found in one sample.

3.4 Analysis of lactobacilli at three time points by rep-PCR

The rep-PCR profiles from the 12 mother-child pairs, including the samples A, D and F, are shown in figure 7. Those pairs which live according to an anthroposophic lifestyle are 244, 291, 50 and 212. The other pairs are those infants being sensibilized. In pairs 43, 52, 188, 291, 50 and 179 the rep-PCR didn't work for one of the three samples. And for pair 208 none of the samples worked. In pairs 52, 43, 188, 184 and 50 only unique strains were found, and thus no overlap between the samples was detected. In pairs 212 and 15 there were transmission from mother to child but the bacteria did not persist over time. In 179, 78, 244 and 291 there were also transmission and the strains persisted over time.

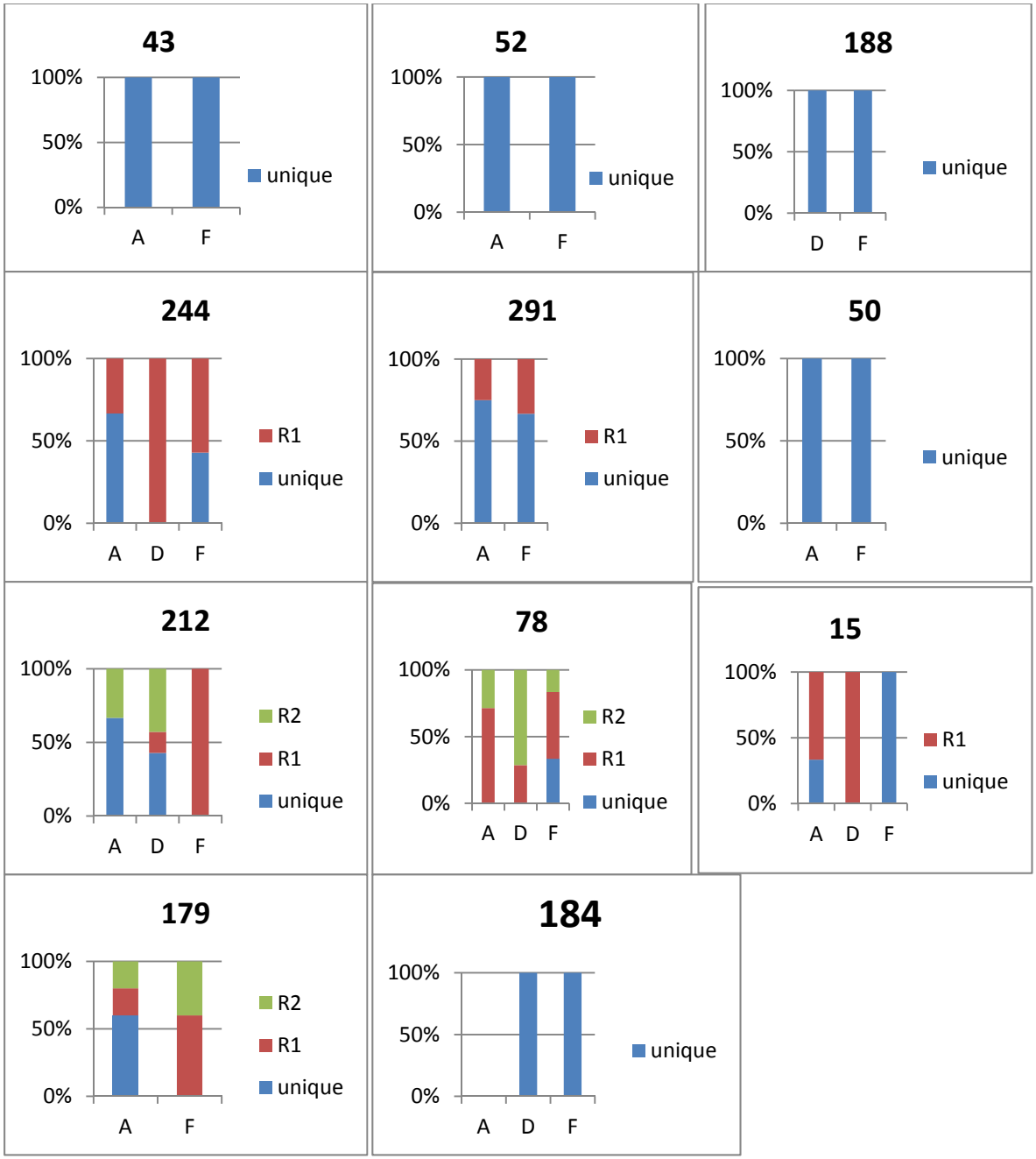

Figure 7: Results from rep-PCR on lactobacilli of the 12 pairs which were analyzed at three time points, mother sample 1 week before delivery (A), infant sample 3 weeks (D) and 6 months (F) after birth. The strains which were found in only one sample are stacked in the unique column. Each strain seen in more than one sample got an own number. There is no overlap of strains between the pairs.

3.5 Analysis of bifidobacteria at three time points by rep-PCR

The 12 mother-child pairs where samples A, D and F were analyzed for bifidobacteria are shown in figure 8. Those pairs which live according to an anthroposophic lifestyle are 244, 291, 208, 50 and 212. The other pairs are those infants being sensibilized. In pairs 244, 291, 208 the rep-PCR didn't work for one of the three samples. For mother-child pair 188 none of the three samples did work. Transmissions were found in samples 43, 52, 212, 50, 78, 179 and 15 and it was only in pair 50 which the strain did not persist over time. In the pairs 244, 208 and 291 only unique strains for each sample were found. When transmission have occurred only one or two strains were found in the infants samples.

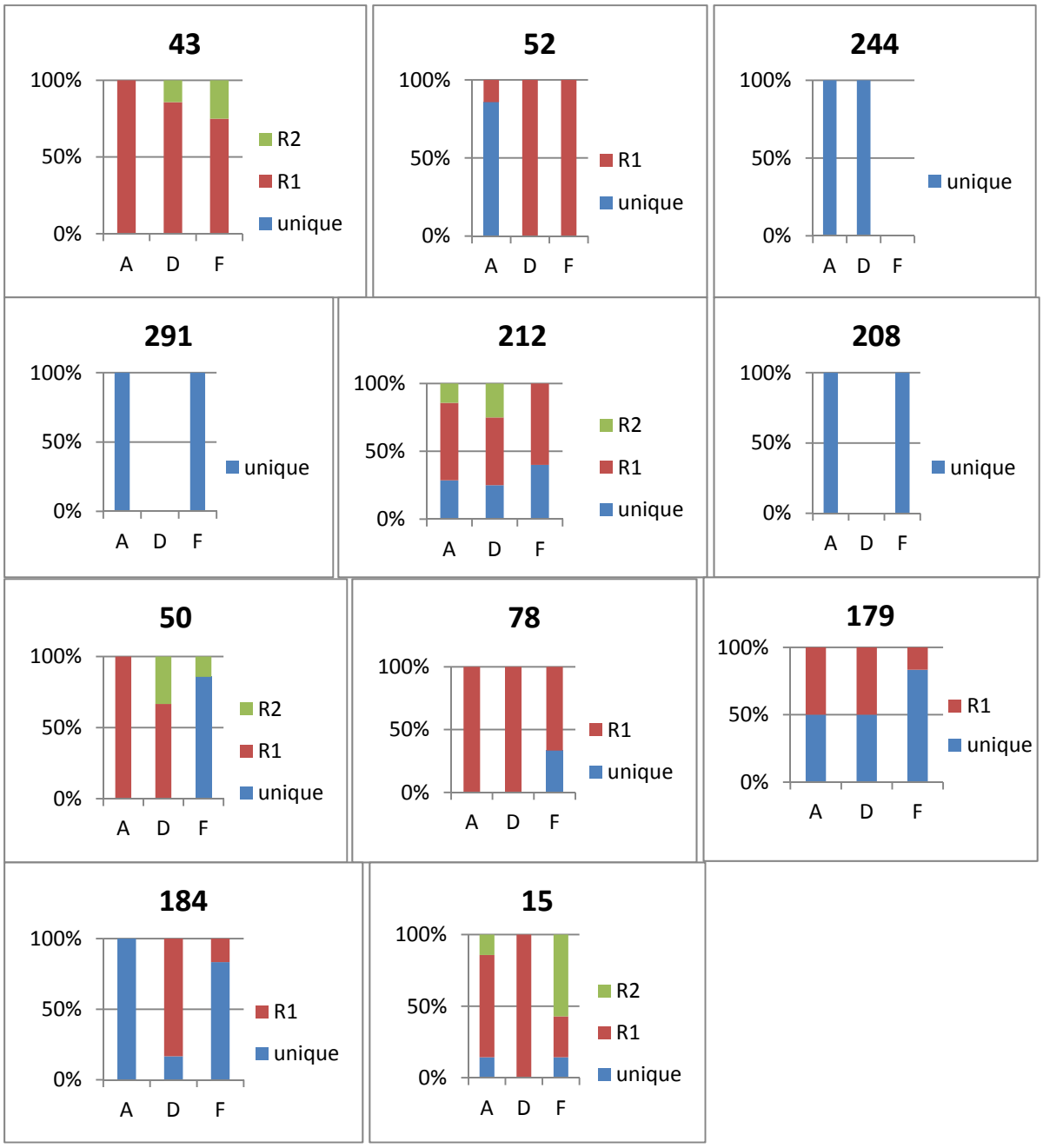

Figure 8: Results from rep-PCR on bifidobacteria of the 12 pairs which were analyzed at three time points, mother sample 1 week before delivery (A), infant sample 3 weeks (D) and 6 months (F) after birth. The ribotypes which were found in only one sample are stacked in the unique column. Each strain seen in more than one sample got an own number.

3.6 Sequencing analysis

20 isolates of bifidobacteria and 20 isolates of lactobacilli were sent for sequencing and the results from the BLAST of the retrieved sequences are shown in appendix as table 1. Some of the bacterial rep-PCR types which were found in more than one sample were chosen for sequencing, also few which were only found in one sample were also sequenced to see if they were unique species. By matching the results from the sequencing with the results from the rep-PCR, B.78A4 is the rep-PCR type R1 (figure 8) which dominated in both mother and child samples. The rep-PCR type (R1) which was found to dominate in almost all samples in pair 144 (figure 6) was shown to be *Bifidobacterium longum* (table 1, B.144B6, B.144A3, B.144D1). The rep-PCR type R1 found in both mother and child sample in pair 179 (figure 8) was shown to be *Clostridium perfringens* (table 1, B.179D1 and B178A1).

As found in the sequencing results (table 1) for example L.50F2 were only found in one sample but are a very common species. The bacteria from type L.73B10 is the strain found in both mother samples (A and B) and child samples 3 weeks and 6 months after birth (D and F). This clone was shown to be a *Lactobacillus rhamnosus* which was a very common bacterium. *Lactobacillus plantarum* was only found to match with one clone which was found in both mother and child samples (figure 7, pair 78: R2). L.122B5 and L.122B8 was also *Lactobacillus rhamnosus* which are the rep-PCR types R1 respective R3 (figure 5) found in both mother and child samples. L.122E2 (figure 5, pair 122: R3) was to confirm that the rep-PCR method was good enough. L.144A2, *Lactobacillus gasseri* which also only was found once are the rep-PCR type R3, found in mother sample 1 week before delivery and child sample 6 months after birth. Then there are L.244A2 and L.244D1, which were taken for the same rep-PCR type (figure 7, pair 244) R1 and they were both identified as *Lactobacillus rhamnosus*. The *Lactobacillius* species found were *Lactobacillus rhamnosus, Lactobacillus paracasei*, *Lactobacillus gasseri* and *Lactobacillus plantarum*. And the only *Bifidobacterium* species found were *Bifidobacterium longum* and *Bifidobacterium scardovii*. Besides these, surprisingly some other species were found; *Clostridium perfringens* and *Enterococcus faecalis.*

4 Discussion

There have been strong indications that the microbiota composition influences the immune system and the development of allergies, especially in infants. Individuals living according to an anthroposophic lifestyle have less occurrence of developing allergies than other individuals living in the same area. Development of the microbiota is of great importance and is therefore target for this study. Fecal samples from mothers and their infants were analyzed for *Bifidobacterium* and *Lactobacillus*. The aim was to find if there occurs transmission of bacteria from the mother's microbiota to the infant and if in that case those bacteria persisted over time. What was also included in this study was quantification of bifidobacteria and lactobacilli. How much bacteria the mother and child had and if there were any samples which had higher or less amount of bacteria compared with the other samples.

When looking on the amount of the whole sample series, sample D from pair 122 (figure 1), show much lower amount of lactobacilli than all other infant samples. Apart from that point,

the amount was more or less similar between the mother samples and the samples of the infant. This agrees with other studies that the amount of lactobacilli is roughly the same between adults and infants. (Vaughan et al. 2002) This also supports earlier observations that the infants are being colonized with lactobacilli very early in life. The amount of lactobacilli varies much between samples and there are no evident difference between those being sensibilized and those living according to an anthroposophic lifestyle. What should be considered is that the amounts of lactobacilli are quite high indicating that this is a relatively abundant bacterium in the microbiota. If considering the other pairs which were analyzed (figure 2) we can see that the mother samples are similar to each other in the amount of lactobacilli but the infant samples have a high variance. The infant at age 3 weeks varied from not detected to very high numbers. What can be concluded is that the F samples (infant after 6 months) mostly had high number of lactobacilli. Eight of twelve pairs have F samples which were much higher than the A samples (mother 1 week before delivery). This is an indication that the infants exhibit higher amount of lactobacilli than their mothers when they reaches the age of 6 months. Again, no evident difference was observed between those living according to an anthroposophic lifestyle and those that are not. But what we can see is that the number of lactobacilli increases for the infant over time.

If looking on the results on CFU of *Bifidobacterium* (figure 3) we can see that the fecal level is lower in the mother samples than the infants' samples. The infant samples are very close together although there is one infant, pair 73 which have higher amount of bifidobacterium than all other but in time decrease to a similar amount as the others. What is surprising is that two out of four C samples did not grow at all because *Bifidobacterium* is known to be one of the first colonizers in the intestine. (Goin, 2010) When looking on the other pairs for which only three time points were analyzed (figure 4) we can see more variation in the mother samples. The D samples (infant two weeks after birth) varies quite much but almost all had *Bifidobacterium*. The F samples (infant 6 months after birth) mostly had quite high amounts of *Bifidobacterium.* What can be concluded from this is that the infant have in most cases more amount of *Bifidobacterium* than their mothers which correlate to previous knowledge. (Vaughan et al. 2002) In some samples both bifidobacteria and lactobacilli could not be detected. What should be considered here is that by cultivation only live bacteria can be detected. Bacteria in low numbers will not be detected either. This means that many bacterial species probably will be lost.

The second part of this study was to find out if there was any transmission of *Bifidobacterium* or *Lactobacillus* from mother to child. In pairs 73, 122, 139 and 144 the whole sample series was analyzed. Transmission of lactobacilli was found in pairs 73, 122 and 144. In pair 139 which are individuals living according to anthroposophic lifestyle, no transmission from mother to child was detected. What should be noted as well was that the other pair having this lifestyle had transmission of two strains. One of them did not persist over time and the other was only detected in the last sample (F) of the infant samples. This may indicate that the strain which was found in sample F might not have been due to transmission from the mother. What should also be considered is that strains might have been present in the other samples but was present in to low numbers to be detected. From the control groups there was

transmission of lactobacilli in both. In pair 73 only one strain in small amount was found in the mother and in the infant samples. But in the other pair, 122 there was transmission of three strains and in the F sample two of them were completely dominating. If looking on the analysis of bifidobacteria (figure 6) we can see that there were transmissions in all pairs. The transmission in pair 73 did not persist in time but all others did. In pair 144 the infant samples all was dominated with the strains found in the mother samples.

Looking on the other pairs which were only analyzed for three time points (figure 7) for lactobacilli there is transmission in pairs 244, 291, 212, 78, 15 and 179. But it was in 244, 291, 78 and 179 the ribotypes persisted over time. Half of them are individuals living an anthroposophic lifestyle and half of them do not, indicating that there is no significant difference. But because seven of the pairs analyzed belonged to the non anthroposophic group there were more of the non anthroposophic infants which did not have any transmission. This means that the transmitted bacteria might persist better in the anthroposophic infants than in the sensibilized infants. But more replicates are needed for verifying this. Considering the analysis for bifidobacterium transmission was found in pairs 43, 52, 212, 50, 78, 179 and 15. In all pairs at least one strain persisted over time but in pairs 50, 179 and 184 there were only small amount of the transmitted strains. For bifidobacteria no significant difference between anthroposophic infant and control were found. But there is an indication that the transmitted bacteria persist better in the sensibilized infants compared with the anthroposophic infants which seems to exhibit more unique strains. A comparison between the pairs were also done (appendix figure 9). A few strains were found in more than one pair. This indicates that not all strains found in both mother and child samples are due to transmission, that the infant in fact got these strains from the environment. But because the same strains were found in so many pairs some of them have to be because of transmission.

When looking on the sequencing results (Appendix table 1) it shows that the medium was not selective enough for *Bifidobacterium* because some of the isolates did not belong to this group. That some other bacterial types also could grow on the same medium. But the medium for lactobacilli looks like it was selective enough, only one bifidobacterium was detected. When looking on the species found there was not so much variation, only a few different species were found. Either these bacterial species are very common in the microbiota or that the 16S rRNA gene is too similar between some bifidobacteria and lactobacilli species. Some clones were sequenced to see if the method used was effective and by the sequencing results it seems that way. Those clones which had similar band pattern were shown in the sequencing results to be the same species (appendix, table 1, L.122B8 and L.122E2).

4.1 Conclusions

The amount of bifidobacteria and possible also lactobacilli is higher in the infants than their mothers. The early samples shows much variation in amount which shows that the infants microbiota is very unstable. But the amount slowly increases in the infants' intestines over time. The conclusions which can be drawn from the Rep-PCR are that there occur transmission of both bifidobacteria and lactobacilli from mother to child. Some of these bacteria found in both mother and child also manage to persist in the infants' microbiota over time and some of them are even shown to dominate. No clear difference between infants

living according to an anthroposophic lifestyle and those infants being sensibilized were detected through this study. What can be possible is that the transmission of bifidobacteria in anthroposophic infants doesn't persist as good as in sensibilized infants, that the anthroposophic infants get a more unique microbiota. But further studies have to be done to be able to confirm this.

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7 Appendix

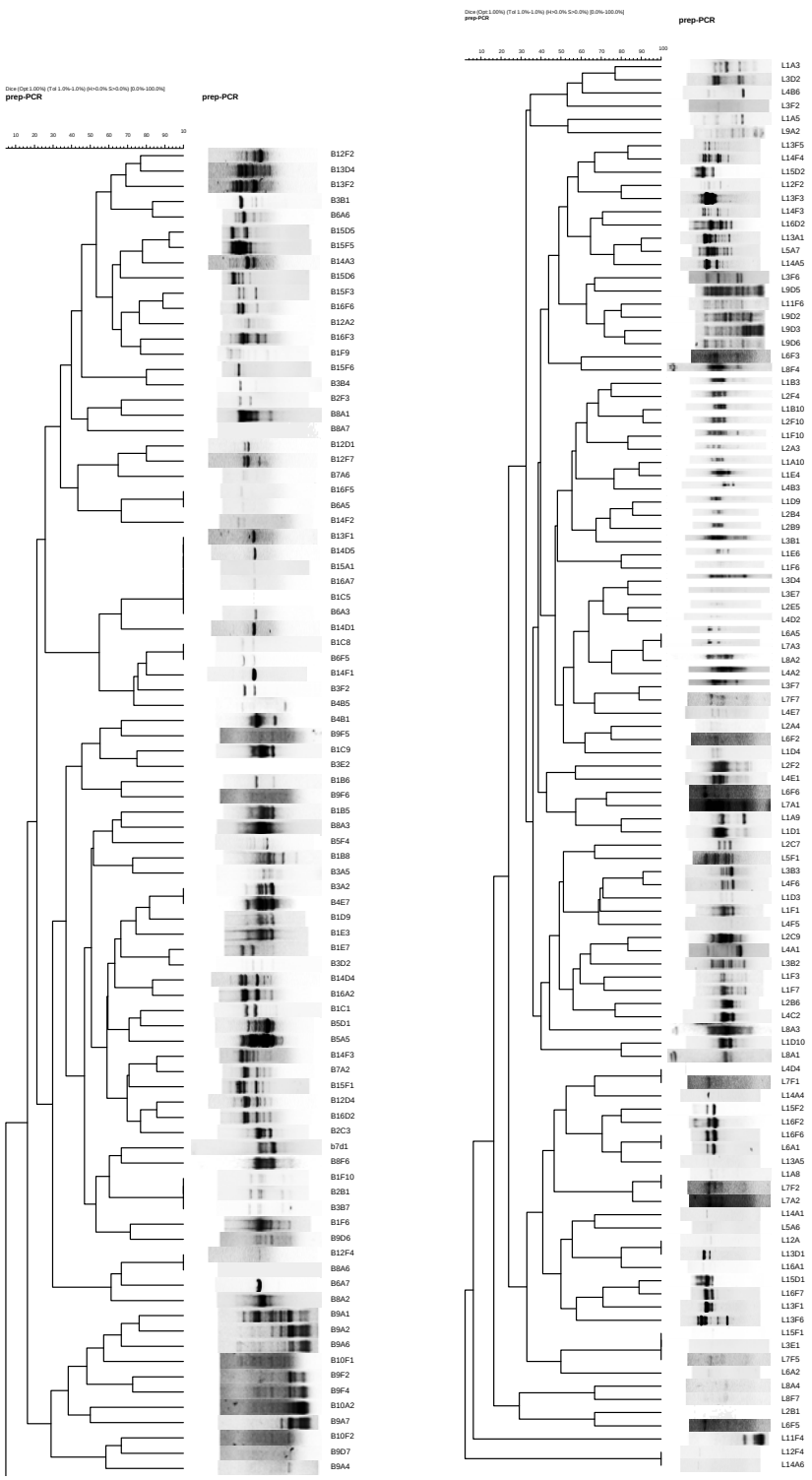

Figure 9: In each pair all different ribotypes were selected and compared with each other to analyze the similarity between pairs.

Table 1: The BLAST results from the sequences retrieved. The first letter stands for which group they should be placed in, *Lactobacilli* (L) or *Bifidobacterium* (B). Then they are divided into the pairs the colonies came from, which sample (A-F) and which colony.

L.50F2	*Lactobacillus rhamnosus*
L.73A8	*Lactobacillus paracasei (Lactobacillus casei)*
L.73B10	*Lactobacillus rhamnosus (Lactobacillus zeae)*
L.73D1	*Lactobacillus rhamnosus*
L.78D1	*Lactobacillus plantarum*
L.122B5	*Lactobacillus rhamnosus (Lactobacillus casei)*
L.122B8	*Lactobacillus rhamnosus*
L.122E2	*Lactobacillus rhamnosus(Lactobacillus casei)*
L.139F4	*Lactobacillus paracasei*
L.144A1	*Lactobacillus paracasei (Lactobacillus casei)*
L.144A2	*Lactobacillus gasseri*
L.144C1	*Bifidobacterium longum*
L.244A2	*Lactobacillus rhamnosus*
L.244D1	*Lactobacillus rhamnosus (Lactobacillus casei)*
B.50F7	*Bifidobacterium scardovii*
B.52D3	*Enterococcus faecalis*
B.73B10	*Bifidobacterium longum*
B.73C1	*Enterococcus faecalis*
B.73C10	*Enterococcus faecalis*
B.73E7	*Bifidobacterium longum*
B.73F6	*Bifidobacterium longum*
B.78A4	*Bifidobacterium longum*
B.122F5	*Enterococcus faecalis*
B.139F2	*Enterococcus faecalis*
B.144A3	*Bifidobacterium longum*
B.144B1	*Clostridium perfringens*
B.144B6	*Bifidobacterium longum*
B.144D1	*Bifidobacterium longum*
B.179A1	*Clostridium perfringens*
B.179D1	*Clostridium perfringens*
B.179D4	*Actinomyces neuii*