Genomic diversity of cultivable Lactobacillus populations residing in the neonatal and adult gastrointestinal tract

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Abstract

The objective of this study was to investigate the cultivable Lactobacillus population in adult and infant faecal material to identify strains shared across a number of individuals. A range of lactobacilli isolated on Lactobacillus-selective agar from faeces of 16 infants and 11 adults were genetically fingerprinted and further characterized by 16S rRNA gene sequencing. The relatedness of all the Lactobacillus strains isolated to known species was also determined both genetically and phenotypically. This study revealed that the human intestine is initially colonized by only a few (1–2) different cultivable strains whereas in adults the pattern becomes more complex with a higher diversity of strains. The adult samples contained three genetically distinct Lactobacillus strains in some cases, while infant samples generally harboured only one dominant Lactobacillus strain. Moreover, the species in general appeared to differ with Lactobacillus rhamnosus and Lactobacillus casei/paracasei found mainly in adults, whereas Lactobacillus gasseri and Lactobacillus salivarius were more commonly isolated in infant samples. The data reaffirm the differences in Lactobacillus populations both between individual subjects and between the infant and adult, with an overall change in the diversity and complexity from early stages of life to adulthood.

Introduction

The human gastrointestinal (GI) tract is heavily populated by bacteria with individuals hosting a characteristic gut biota that is critical for normal development of the intestine and immune functions for defence against infections. The numerically predominant species of bacteria are obligately anaerobic and are represented by both gram-positive and gram-negative genera (Tannock, 1994). It is estimated that more than 400 species of bacteria reside in the human gut, while only 30–40 species represent 99% of the microbiota found in any one human subject (Kimura et al., 1997). At birth, the newborn infant intestine is sterile, but is colonized rapidly thereafter by microorganisms from the mother and the surrounding environment. Neonatal human intestine contains large numbers of facultative anaerobes such as streptococci and coliforms that decline during weaning as obligate anaerobes such as Bacteroides and clostridia establish and predominate in the intestine (Zhang et al., 2005). The influence of intestinal bacteria on human health can be considered harmful, beneficial or neutral. Lactobacillus species are believed to be beneficial and can contribute to digestion, stimulation of immunity and inhibition of pathogens (Fuller, 1989). At the time of writing, the genus Lactobacillus includes 54 recognized species and some subspecies (Robinson, 2002), many of which have been isolated from faecal sources. In human faeces, lactobacilli generally account for less than 1% of the bacterial community and it has been reported that they were not detected in faecal samples collected from c. 27% of Americans (Finegold et al., 1983; Sghir et al., 2000). Particular Lactobacillus species are reported to be persistent in the intestinal tracts of some humans, undetectable in others or exhibiting temporal changes in strain composition (McCARTNEY et al., 1996; Kimura et al., 1997). Lactobacillus acidophilus, Lactobacillus salivarius, Lactobacilli casei, Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus reuteri and Lactobacillus brevis are the most commonly isolated species from the human intestine (Mitsuoka, 1992). Lactobacillus acidophilus, L. salivarius and L. fermentum are usually found in infant faeces and also in adults (Cooperstock & Zedd, 1983; Benno & Mitsuoka, 1986). The extent to which lactobacilli colonize...
the intestines of newborn infants and children is controversial. There is little information available regarding how and when the gut of infants becomes colonized with lactobacilli, although it is believed that some infants may receive lactobacilli at birth from the mother’s vagina (Matsumiya et al., 2002).

There is now convincing evidence that certain species of bacteria in the human gut, including bifidobacteria and lactobacilli, positively contribute to human health, providing the basis for probiotics, i.e. healthy bacteria that positively influence human health. The potential health-promoting effects achieved by the consumption of dairy products containing probiotic organisms, such as Lactobacillus species, have therefore resulted in intensive research efforts in recent years. Probiotic lactobacilli have been associated with antitumour activity, reduction of serum cholesterol, alleviation of lactose intolerance, stimulation of the immune system, enhancement of resistance against pathogens, and prevention of traveller’s and antibiotic-induced diarrhoea (for reviews, see Mitsuoka, 1992; Sanders, 1994; Lee & Salminen, 1995). However, to deliver these health benefits, they must survive and persist within the GI tract. The ability to colonize and persist within the intestine is also one of the main criteria for selecting probiotics (Bernet et al., 1994).

Determination of the diversity of a population to strain level necessitates the use of methods suitable for handling large numbers of isolates. Various molecular genetic methods have been used to compare and identify Lactobacillus isolated from food and the GI tract of humans. These include ribotyping (Ferrero et al., 1996; Tynkkynen et al., 1999; Chagnaud et al., 2001), total DNA–DNA hybridization (Bringel et al., 1996; Klein et al., 1998; Song et al., 2000), 16S rRNA gene sequencing (Tannock, 1999; Vaughan et al., 1999; Burton et al., 2003), randomly amplified polymorphic DNA (RAPD) PCR (Klein et al., 1998; Tynkkynen et al., 1999), multiplex RAPD PCR (Khaled Daud et al., 1997) and pulsed-field gel electrophoresis (PFGE) (Klein et al., 1998; Tynkkynen et al., 1999). Genomic DNA fingerprinting using RAPD has been found to be useful for differentiating between closely related bacteria. The genomic diversity of lactobacilli was characterized by RAPD PCR in a study by Tailliez et al. (1996). Furthermore, RAPD has been successfully used to distinguish between L. acidophilus and the closely related species Lactobacillus crispatus, Lactobacillus amylivorans, Lactobacillus gallinarum, Lactobacillus gasseri and Lactobacillus johnsonii as well as between individual strains of L. acidophilus (Du Plessis & Dicks, 1995). Available literature indicates that for many genera, PFGE is more effective than ribotyping, sodium dodecyl sulfate polyacrylamide gel electrophoresis or RAPD PCR for discriminating strains (O’Riordan & Fitzgerald, 1997). Indeed, PFGE is considered best for strain identification because of its sensitivity, consistency and accuracy, based on accumulating evidence from previous studies. Carbohydrate fermentation has been used as a method to identify Lactobacillus for many years (Yin & Zheng, 2004). However, several Lactobacillus species have only minor differences in the fermentation pattern and some species have a highly variable phenotype, which makes reliable identification difficult. Therefore, the use of a phenotypic identification method in combination with a genotypic method is desirable.

The first objective of this study was to examine the diversity of the cultivable Lactobacillus population in neonatal and adult stool samples using RAPD PCR and PFGE for isolation of dominant strains in both population groups and their eventual exploitation as probiotics. The second objective was to investigate the carbohydrate fermentation characteristics of the Lactobacillus strains isolated and their relationship to genotypic characteristics.

Material and methods

Subjects and faecal sampling

Faecal material was sampled, using swabs, from 16 infants (five preterm and 11 term infants) aged from 3 days to 3 months (Table 1). In most cases, faecal swab samples were taken that allowed isolation of strains but not accurate enumeration of bacteria. Samples were stored at −20 °C and processed within 5 h of sampling. The swabs were vortex-mixed in Maximum Recovery Diluent (MRD; Oxoid Ltd.) and serially diluted to enumerate CFUs per swab. Some infant sample swabs were streaked directly onto agar plates. All infant samples were collected at the neonatal unit of the Erinville Hospital, Cork, with fully informed consent from all parents. Faecal material was also obtained from 11 healthy adults, aged from 8 to 42 years (Table 1).

Growth conditions and selection of colonies

Serial dilutions of faecal samples were pour-plated onto Lactobacillus selective agar (Becton Dickinson Co.) for selective outgrowth of lactobacilli. Agar plates were incubated anaerobically (anaerobic jars with Anaerocult A gas packs; Merck) at 37 °C for 72 h. To analyse the dominant Lactobacillus population of each subject, 12 colonies were randomly selected from each sample and subcultured in MRS broth for 24–48 h. Ten colonies are believed to provide adequate representation of the major bacterial strains cultured on selective medium (Hartley et al., 1977).

RAPD PCR

Genomic DNA was extracted from the isolates and RAPD PCR was performed, using the random primer, R1 (5′-ATGTAACGCC-3′ synthesized by Sigma-Genosys Ltd.).
Details of procedures for genomic DNA isolation and RAPD PCR conditions are as previously described (Simpson et al., 2002). Initially, three different primers, R1 (5'-ATGTAACGCC-3'), R2 (5'-GTGATGTGCTGGTGTTATGTTTA-3') and P5 (5'-CAATCTGATC-3') (Fitzsimons et al., 1999; Gardiner et al., 2003) were tested against 12 isolates from five neonates to identify the most informative primer.

**PFGE**

High-molecular-weight DNA was isolated from stationary-phase cultures using procedures previously described (Simpson et al., 2003). The restriction enzymes *ApaI* or *XbaI* (New England Biolabs) were used and the DNA fragments resolved with a contour-clamped homogeneous electric field CHEF-DR III pulsed-field system (Bio-Rad Laboratories) at 6 V cm⁻¹ for 18 h with a 1 to 15-s linear ramp pulse time, with 0.5 × Tris base-borate-EDTA running buffer maintained at 14 °C. Gels were stained in distilled water containing 0.5 μg ethidium bromide mL⁻¹ for 30 min and destained in distilled water for 60 min. PFGE gels were visualized by UV transillumination and the size of the PFGE fragments was estimated by comparison with a low-range PFGE marker, ranging from 2.03 to 194 kb (no. N0350S, New England Biolabs).

**Carbohydrate fermentation profile**

All the strains were tested for their ability to ferment 49 different carbohydrates using API 50CH strips and API CHL medium (bioMérieux). The following type strains were included: *Lactobacillus rhamnosus* LMG 6400T, *L. plantarum* LMG 6907T, *L. casei* LMG 6904T, *L. gasseri* LMG 9203T, *L. salivarius* LMG 9477T, *L. johnsonii* DSM 10533T, *Lactobacillus ruminus* DSM 20403T, *L. crispatus* LMG 9479T and *L. acidophilus* ATCC 4356T. All the strains were cultivated on MRS plates in anaerobic jars for 24 h. Grown colonies were harvested and resuspended in suspension medium (bioMérieux). The turbidity of the suspension was determined according to the manufacturer’s instructions. The cell suspension was applied into API 50CH strip wells, coated with sterile paraffin oil to ensure anaerobiosis, and then incubated at 37 °C. Readings were taken after 24 h and...
verified after 48 h. Fermentation of carbohydrates in the carbohydrate medium was indicated by a yellow colour except for aesculine (black). Colour reactions were scored against a chart provided by the manufacturer. Strains were identified by comparison of biochemical profiles from API 50 CH test strips with the API database (bioMérieux). Species identification was also made by comparison of the results with known carbohydrate fermentation profiles of lactobacilli (Sneath et al., 1986).

16rRNA gene sequencing

Two 16S rRNA gene primers, CO1, for the 5' end (5' - AGTTTGATCCTGGCTCAG-3'), and CO2, for the 3' end (5' - TACCTTGTTACGACT-3'), were used to generate an approximate 1.5-kb product, using PCR conditions described previously (Simpson et al., 2003), which was partially sequenced using the primer CO1 by MWG sequencing service. Comparison of the 16S rRNA gene sequences obtained using the BLAST program allowed assignment of a strain to a particular species. In general, when 16S rRNA gene similarity values exceed 97%, the strains are considered to belong to the same species (Stackebrandt & Goebel, 1994).

Results

Isolation and molecular characterisation of Lactobacillus strains

The isolates were obtained from stool samples taken from infants with a range of different case histories and from healthy adults (Table 1). In all cases, the samples were cultured on Lactobacillus selective agar (LBS). Lactobacillus isolates were obtained from seven of 16 infant samples and from seven of 11 adult samples (Table 1). Most samples containing lactobacilli were from full-term infants (five of seven lactobacilli-containing samples). RAPD analysis was performed in an initial attempt to distinguish and group Lactobacillus strains within each sample. Lactobacillus isolates from a single subject showing the same band pattern on RAPD analysis were regarded as belonging to the same strain. RAPD PCR was performed on 292 isolates obtained from the adults and infants. Initially, three different RAPD primers, R1, R2 and P5, were tested against 12 isolates from five infants to identify the most informative primer. The suitability of each primer was scored on the basis of distribution of primer binding sites, reproducibility of fingerprint pattern and intensity of the DNA bands. Greatest discrimination was found with primer R1, which was subsequently used in the genetic screen. Under the conditions described, primer R1 generally generated ~5–12 DNA fragments ranging in size from 100 bp to ~3 kb per isolate. Discrimination was only considered when isolates were less than 70% similar. Figure 1a shows an RAPD PCR gel of all isolates from faecal sample 20 (an 8-year-old). RAPD PCR grouped these isolates into three genotypes, as indicated in lanes 1, 7 and 16. Subsequently, PFGE was performed on the same isolates from faecal sample 20, following genomic digestion with the restriction enzyme Apal (Fig. 1b). As a result of this, PFGE also grouped these isolates into three genotypes, as indicated in lanes 1, 7 and 16.

PFGE strain discrimination

To confirm the genetic groupings of all infant and adult isolates exhibiting distinct RAPD PCR fingerprints and to make a final grouping, PFGE analysis was performed using the enzyme Apal, which has previously been shown to discriminate lactobacilli (Roy et al., 2000). Apal restriction of the genomic DNA from the infant isolates showing different RAPD patterns generated eight different Lactobacillus macro restriction patterns, termed A–H (Fig. 2a). Apal restriction of the isolates showing different RAPD patterns from the 11 adults resulted in 11 different Lactobacillus macro restriction patterns, 1–S (Fig. 2b). A combination of PFGE and RAPD PCR profiling revealed that most of the infant samples contained one dominant strain type (Table 2). Only one of the infants harboured multiple strains, with sample 8 (a 3-month-old) having two genetically distinct strains associated with PFGE patterns G and H (Fig. 2a and Table 2). Five out of seven adults that harboured Lactobacillus contained a single dominant Lactobacillus strain each. Two samples from the adult group, sample 20 (an 8-year-old) and sample 26 (a 35-year-old), contained three genetically distinct Lactobacillus strains each, associated with PFGE patterns J, K, L and P, Q, R, respectively (Fig. 2b and Table 2). PFGE analysis also showed that isolates from two individuals with the same Apal restriction pattern were found on only one occasion. Subsequent analysis with XbaI to differentiate the isolates did not allow any discrimination between two of the isolates (Fig. 2c). Thus, PFGE analysis demonstrated eight genetically distinct Lactobacillus strains isolated from the 16 infants and 11 genetically distinct Lactobacillus strains from the 11 adults.

Comparison between genotypic and phenotypic analysis

Carbohydrate fermentation analysis was performed on all strains showing different PFGE macro restriction patterns. The results obtained with API 50 CH were mostly consistent with the PFGE profiling. However, some strains that appeared to have distinctly different PFGE patterns shared identical carbohydrate fermentation profiles and so could not be distinguished by the API 50 CH system, e.g. the L. salivarius strains associated with PFGE patterns C, F (Fig. 2a) and Q (Fig. 2b). The L. salivarius strain isolated...
from one of the infants (PFGE pattern C, Fig. 2a) that showed a similar genetic fingerprint to the type strain *L. salivarius* LMG 9477\(^T\) (PFGE pattern 5, Fig. 2b) gave an identical carbohydrate fermentation profile to the type strain. Furthermore, the *L. gasseri* strain isolated from neonatal sample 3 and adult sample 20, associated with related PFGE patterns A (Fig. 2a) and L (Fig. 2b), yielded identical carbohydrate fermentation profiles. These strains also seemed to be genetically related to the type strain *L. gasseri* LMG 9203\(^T\) (PFGE pattern 4, Fig. 2b), but showed distinctly different fermentation profiles to *L. gasseri* LMG 9203\(^T\). Apart from this, all the strains that shared less similar PFGE patterns demonstrated distinctly different carbohydrate fermentation profiles of the 49 carbohydrates tested. The *Lactobacillus casei/paracasei* strains that appeared to be the same strain in one of the adults (sample 27) and in the 3-month-old baby (sample 8), based on identical PFGE patterns, showed identical carbohydrate fermentation profiles. All the *L. rhamnosus* and *L. casei/paracasei* strains isolated in this study showed a similar carbohydrate fermentation pattern within the species, with a difference of only two to six sugars between the strains in respective species. Finally, results for strains whose species were determined by 16S rRNA gene sequencing were in complete accordance with those obtained with the API 50 CH system (Table 3).

**16rRNA gene sequencing**

Genetically distinct strains exhibiting different PFGE patterns were investigated further by 16S rRNA gene sequencing, with similarity values exceeding 97% indicating similar species (Stackebrandt & Goebel, 1994). Among the adult samples analysed, the most commonly encountered *Lactobacillus* species were *L. rhamnosus* and *L. casei/paracasei*, each of which was present in four out of seven *Lactobacillus*-containing adult samples. Among the other *Lactobacillus* species present in the adult faecal samples were *L. gasseri*, *L. salivarius* and *L. ruminus* (Table 2). The most commonly encountered *Lactobacillus* species in the infant samples were *L. gasseri* and *L. salivarius*, which were present in four out of seven *Lactobacillus*-containing samples. Among the other *Lactobacillus* species present in the infant faecal samples were *L. brevis*, *L. reuteri*, *L. rhamnosus* and *L. casei/paracasei* (Table 2). Interestingly, the *L. casei/paracasei* strains found in one of the adults (sample 27) and in the 3-month-old...
baby (sample 8) gave identical PFGE patterns following restriction with *ApaI* and remained indistinguishable following restriction with *XbaI* (Fig. 2c). In this respect, this strain was found in 100% of isolates from one sample (sample 27) and in the other case it was present in 80% of isolates, while the remaining 20% were identified as *L. rhamnosus* (sample 8, Table 2).

The *L. gasseri* strain isolated from neonatal sample 3 and adult sample 20, associated with PFGE patterns A (Fig. 2a) and L (Fig. 2b), shared a close genetic relationship given that their PFGE fingerprints were very similar, differing by only five fragments in the low-molecular-weight region. According to Tenover et al. (1995), four- to six-band differences can be explained by simple insertions or deletions of DNA or by the generation or loss of restriction sites. Adult samples 23 and 25 each consisted of a dominant *L. casei/paracasei* strain, associated with PFGE patterns N and O (Fig. 2b), which indicate a close genetic relationship.

Some nonlactobacilli strains were also isolated on the *Lactobacillus*-selective medium, including enterococci and bifidobacteria, indicating that LBS was not totally selective for lactobacilli. For example, *Bifidobacterium longum/infantis* was isolated in five out of 16 infants and in four out of 11 adult samples. *Enterococcus faecalis* was the dominant strain in four

![Fig. 2. Lactobacillus macro restriction pattern following genomic digests of neonatal isolates with *ApaI* (a, PFGE patterns A–H) and *Lactobacillus* pattern of adult isolates together with the type strains of recognized *Lactobacillus* species (b, PFGE patterns I–S and 1–9, respectively), obtained by PFGE. PFGE pattern S is shared by adult sample 27 and one of the strains in neonatal sample 8 (b). *XbaI* restriction to differentiate strains sharing pattern S (samples 8 and 27) (c), PFGE patterns A1 and A2. Molecular weight (MW) markers are shown in the outside lanes of all the gels.](image-url)
The overall aim of this study was to determine differences between cultivable Lactobacillus populations of infants and adults. The study revealed that the human intestine is initially colonized by only a few (1–2) different strains, with greater complexity and diversity and different prevailing species in adults. Some reports in the literature ignore differences in the intestinal microbiota between infants on the one hand and adults and the elderly on the other. In these age groups, lactobacilli populations are substantially different in relation both to species and to numbers, which is an important issue when selecting probiotics.

Lactobacillus was only isolated in seven out of 16 infants, as found previously for lactobacilli in newborn infants. For example, Ahnne et al. (2005) isolated lactobacilli from only 21% (23/112) of stool samples from 1-week-old infants and Gronlund et al. (1999) isolated faecal lactobacilli from c. 20% of infants born vaginally and aged 3 days. Mackie et al. (1999) found lactobacilli in neonates at $10^5$ CFU g$^{-1}$ faeces, while in infants of 1 month and older numbers increased to $10^6$–$10^8$ CFU g$^{-1}$ faeces. In our study, lactobacilli were isolated less frequently from preterm infants, in agreement with Hall et al. (1990), who observed decreased numbers of lactobacilli in preterm infants. Bacterial colonization of intestines of preterm infants may differ from that of term infants because methods of neonatal care, such as treatment with antibiotics or nursing in incubators, may delay or impair colonization.

Seven out of 11 adults harboured Lactobacillus species. Earlier studies based on plate counts showed that Lactobacillus strains were present in the GI tract of 70% of humans that consumed a Western-like diet but were not detectable in the remainder of subjects (Tannock, 1991). Furthermore, Finegold et al. (1983) detected lactobacilli in 73% of 62 healthy Americans when a single faecal sample from each was examined. Lactobacillus species in general appeared to differ between adults and infants, with L. rhamnosus and L. casei/paracasei being found mainly in adults, whereas L. gasseri and L. salivarius were more commonly isolated from neonatal samples. Lactobacillus gasseri and L. salivarius were predominant single strains in two neonates each, while other Lactobacillus strains found in neonates were L. brevis, L. reuteri, L. rhamnosus and L. casei/paracasei. Ahrne et al. (2005) found L. gasseri and L. rhamnosus to be the most commonly isolated species in the first 2 months of life. Furthermore, L. gasseri is common in the vaginal flora of women (Vasquez et al., 2002), and vaginal lactobacilli may transiently colonize newborn infants. The most commonly encountered Lactobacillus species obtained in adult faecal material were L. rhamnosus and L. casei/paracasei, which were each present in four out of seven Lactobacillus-containing adult samples. Ahrne et al. (1998) found that L. rhamnosus is one of the dominant Lactobacillus species in healthy individuals and 26% of the individuals in their study harboured this species. Among the other Lactobacillus species present in the adult faecal samples were L. gasseri, L. salivarius and L. ruminus. It was evident that some of the

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Table 2. Distribution of PFGE patterns among faecal samples and numbers of isolates sharing the same pattern

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>PFGE pattern</th>
<th>No. of Lactobacillus isolates</th>
<th>Species identity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neo 3</td>
<td>A</td>
<td>12</td>
<td>Lactobacillus gasseri</td>
</tr>
<tr>
<td>Neo 4</td>
<td>B</td>
<td>2</td>
<td>Lactobacillus reuteri</td>
</tr>
<tr>
<td>Neo 5</td>
<td>C</td>
<td>12</td>
<td>Lactobacillus salivarius</td>
</tr>
<tr>
<td>Neo 7</td>
<td>D</td>
<td>12</td>
<td>Lactobacillus brevis</td>
</tr>
<tr>
<td>Neo 8</td>
<td>G</td>
<td>2</td>
<td>Lactobacillus rhamnosus</td>
</tr>
<tr>
<td>Neo 14</td>
<td>E</td>
<td>12</td>
<td>Lactobacillus casei/paracasei</td>
</tr>
<tr>
<td>Neo 15</td>
<td>F</td>
<td>12</td>
<td>Lactobacillus salivarius</td>
</tr>
<tr>
<td>Ad 19</td>
<td>I</td>
<td>8</td>
<td>Lactobacillus casei/paracasei</td>
</tr>
<tr>
<td>Ad 20</td>
<td>J</td>
<td>2</td>
<td>Lactobacillus rhamnosus</td>
</tr>
<tr>
<td>Ad 21</td>
<td>M</td>
<td>9</td>
<td>Lactobacillus ruminus</td>
</tr>
<tr>
<td>Ad 23</td>
<td>N</td>
<td>12</td>
<td>Lactobacillus casei/paracasei</td>
</tr>
<tr>
<td>Ad 25</td>
<td>O</td>
<td>12</td>
<td>Lactobacillus casei/paracasei</td>
</tr>
<tr>
<td>Ad 26</td>
<td>P</td>
<td>4</td>
<td>Lactobacillus rhamnosus/casei</td>
</tr>
<tr>
<td>Ad 27</td>
<td>Q</td>
<td>15</td>
<td>Lactobacillus salivarius</td>
</tr>
<tr>
<td>Ad 27</td>
<td>S (same pattern as H)</td>
<td>12</td>
<td>Lactobacillus casei/paracasei</td>
</tr>
</tbody>
</table>

*According to 16S rRNA gene sequencing and API 50 CH system analysis.

PFGE, pulsed-field gel electrophoresis.

Comparison with type strains within the genus Lactobacillus

Well-characterized Lactobacillus type strains were characterized using PFGE and showed distinct interspecies differences (Fig. 2b, PFGE patterns 1–9). PFGE patterns of some type strains were similar to those isolates cultivated from the infants and adults, suggesting close relationships. For example, the L. gasseri strain isolated from an infant (PFGE pattern A, Fig. 2a) and an adult (PFGE pattern L, Fig. 2b) is possibly genetically related to L. gasseri LMG 9203$^T$ (PFGE pattern 4, Fig. 2b) according to their similar PFGE patterns. Similarly, the dominant L. salivarius strains isolated from one of the infants (associated with PFGE pattern C, Fig. 2a) showed a similar genetic fingerprint to L. salivarius LMG 9477$^T$ (PFGE pattern 5, Fig. 2b).

Discussion

The overall aim of this study was to determine differences between cultivable Lactobacillus populations of infants and adults. The study revealed that the human intestine is...
Table 3. Characterization of genetically distinct strains by 16S rRNA gene partial sequencing and API 50 CH (PFGE patterns A–S, PFGE patterns 1–9: type strains of recognized Lactobacillus species). The percentage value is the similarity of the tested strain with a database strain.

<table>
<thead>
<tr>
<th>PFGE pattern</th>
<th>Strain</th>
<th>% Homology</th>
<th>Carbohydrate fermentation</th>
<th>Identity</th>
<th>API score</th>
<th>% ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Lactobacillus gasseri</td>
<td>100</td>
<td>ND</td>
<td>Lactobacillus gasseri</td>
<td>ND</td>
<td>100</td>
</tr>
<tr>
<td>B</td>
<td>Lactobacillus reuteri</td>
<td>98</td>
<td>ND</td>
<td>Lactobacillus reuteri</td>
<td>98</td>
<td>95</td>
</tr>
<tr>
<td>C</td>
<td>Lactobacillus salivarius</td>
<td>100</td>
<td>ND</td>
<td>Lactobacillus salivarius</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>D</td>
<td>Lactobacillus brevis</td>
<td>100</td>
<td>ND</td>
<td>Lactobacillus brevis</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>E</td>
<td>Lactobacillus gasseri</td>
<td>99</td>
<td>ND</td>
<td>Lactobacillus gasseri</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>F</td>
<td>Lactobacillus salivarius</td>
<td>99</td>
<td>ND</td>
<td>Lactobacillus salivarius</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>G</td>
<td>Lactobacillus rhamnosus</td>
<td>100</td>
<td>ND</td>
<td>Lactobacillus rhamnosus</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>H</td>
<td>Lactobacillus casei/paracasei</td>
<td>99</td>
<td>ND</td>
<td>Lactobacillus casei/paracasei</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>I</td>
<td>Lactobacillus casei/paracasei</td>
<td>100</td>
<td>ND</td>
<td>Lactobacillus casei/paracasei</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>J</td>
<td>Lactobacillus rhamnosus</td>
<td>100</td>
<td>ND</td>
<td>Lactobacillus rhamnosus</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>K</td>
<td>Lactobacillus rhamnosus</td>
<td>100</td>
<td>ND</td>
<td>Lactobacillus rhamnosus</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
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<td>Lactobacillus gasseri</td>
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</tr>
<tr>
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<td>Lactobacillus ruminus</td>
<td>97</td>
<td>ND</td>
<td>Lactobacillus ruminus</td>
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<td>97</td>
</tr>
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*Percentage homology with Lactobacillus species as revealed by 16S rRNA gene sequence analysis.

1Percentage similarity, i.e. how closely the profile corresponds to the taxon relative to all other taxa in the API database.

2ND, not determined, species were not included in the identification database.

3Profile unacceptable according to API database.

PFGE, pulsed-field gel electrophoresis.

strains isolated shared a close genetic relationship, as their PFGE DNA fingerprints were similar. For example, the L. gasseri strain isolated from an infant in our study is possibly genetically related to the L. gasseri strain isolated from an adult. Similarly, a genetic relationship is also possible between the L. casei/paracasei strains dominating in two adults. It is possible that these strains may be undergoing an increased evolutionary rate imposed on them by the local environment of the intestine.

Comparison of the Lactobacillus isolates cultivated from the adults and infants with well-characterized type strains showed that the L. gasseri strain isolated from an infant and an adult, which also seemed to be related to each other, is also possibly genetically related to L. gasseri LMG 9203T. In addition, one of the dominant L. salivarius strains cultivated from one of the infants showed a similar genetic fingerprint to L. salivarius LMG 9477T. The Lactobacillus population in this study was also found to be relatively simple with regard to the strain composition that was characteristic of the particular human host. The adult samples further contained a more diverse Lactobacillus population, with three genetically distinct strains recovered in two cases, while the infant stool samples generally harboured one dominant Lactobacillus strain. Only one infant (aged 3 months) harboured multiple Lactobacillus strains (L. rhamnosus and L. casei/paracasei), which could be associated with its older relative age. In one case, the same strain was isolated from two nonrelated individuals, and this had indistinguishable PFGE patterns following restriction with both ApaI and XbaI. This strain was identified as representing L. casei/paracasei.

All the strains showing different macro restriction PFGE patterns were assayed for carbohydrate fermentation, and species identities obtained by 16S rRNA gene sequencing were consistent with carbohydrate fermentation patterns, using the API 50 CH system. Some strains within the same species group that exhibited different PFGE patterns...
showed the same fermentation pattern, demonstrating that carbohydrate fermentation analysis on its own is not sufficient to differentiate strains within the same species.

The isolation of lactobacilli from faeces is generally difficult, owing to the presence of competing microorganisms, such as bifidobacteria, streptococci and enterococci, which are present in higher numbers. Lactobacillus-selective agar was used in this study to achieve optimal recovery of Lactobacillus species, which prefer a low-pH habitat for growth, such as that provided by the LBS medium. As one of the most commonly chosen media for selective culturing of lactobacilli from faecal samples (Jackson et al., 2002), LBS medium is considered to be reliable. However, its use requires caution and further confirmation as some nonlactobacilli strains were isolated from the human faecal samples, including enterococci and bifidobacteria.

Discriminatory power and reproducibility are important attributes in any typing system. In several Lactobacillus studies, PFGE has been shown to be the most powerful method for strain typing (Tynkkynen et al., 1999; Burton et al., 2003) but it is laborious and expensive. RAPD PCR, by contrast, is rapid and cheap, but careful optimization is needed to ensure reproducibility. Under the conditions used in the present study, PFGE was found to discriminate more strains than RAPD PCR, as found for other genera (Bjorkroth et al., 1996; Bert et al., 1997; Tynkkynen et al., 1999). Strain discrimination was only considered when RAPD patterns were less than 70% similar, as suggested by Kurzak et al. (1998). PFGE was also found to provide greatest discrimination of Lactobacillus strains by Kimura et al. (1997) and therefore appears to be the method of choice for population studies of these bacteria obtained from human sources.

Lactobacillus has been reported to increase antimicrobial protection in the GI tract and to combat infectious and other diseases and so could be of importance for maintaining the health of the host and an important part of the human gut microbiota. To deliver these health benefits, lactobacilli must survive and persist within the GI tract, which are major criteria for selecting probiotics (Bernet et al., 1994). The data from the present study support the earlier observation that the composition and abundance of the intestinal Lactobacillus community fluctuates temporally and is variable among individuals. Moreover, the differences in dominant species between infants and adults are noteworthy and highlight the importance of species/strain selection when testing potentially probiotic cultures for differently aged groups.

Acknowledgements

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References


amplified polymorphic DNA analysis, and pulse-field gel electrophoresis in typing of *Lactobacillus rhamnosus* and *L. casei* strains. *Appl Environ Microbiol* 65: 3908–3914.


