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In the present study, polyphasic analysis [cultivation, combined with the fingerprinting of individual isolates, and denaturing gradient gel electrophoresis (DGGE)] was applied to study whether similar features concerning the diversity and temporal stability of selected bacterial groups could be detected intra-individually in two different niches – the oral cavity and the colon – from ten adult volunteers consuming probiotics. The predominant bacterial microbiota, *Clostridium coccoides–Eubacterium rectale* group and bifidobacterial populations, were generally stable in salivary and faecal samples, with the greater diversity seen in faeces. Furthermore, different species predominated at the two different sites. *Lactobacillus* group DGGE profiles were unstable, yet the intra-individual profiles from faecal and salivary samples collected at the same time resembled each other. The ingested probiotic product did not affect the stability of the bacterial groups studied. The culture-based analysis showed that most subjects harboured identical indigenous *Lactobacillus* genotypes in saliva and faeces (*Lactobacillus rhamnosus*, *Lactobacillus gasseri*, *Lactobacillus paracasei* and *Lactobacillus plantarum* group). Thus, identical indigenous lactobacilli were able to inhabit both ends of the orogastrointestinal tract, whereas the composition of the other bacterial groups studied varied between the two sites.

INTRODUCTION

Both ends of the orogastrointestinal tract of humans have an abundant microbiota dominated by anaerobically growing bacteria (Berg, 1996). The number of bacteria in the oral cavity is about 10^{11} (g wet weight dental plaque)$^{-1}$ and 10^8–10^9 (ml saliva)$^{-1}$ (culturable bacteria; Nisengard & Newman, 1994; Li *et al.*, 2005), whereas in faeces the corresponding figure is 6–9×10^{10} (g wet weight)$^{-1}$ (detected with molecular techniques; Thiel & Blaut, 2005). Although the same bacterial genera can be found in oral and colonic samples, to the best of our knowledge only one intra-individual comparative study of bacterial populations (in this case lactobacilli) of these two sampling sites has been performed previously in humans (Dal Bello & Hertel, 2006).

The faecal microbiota is dominated by the *Clostridium coccoides–Eubacterium rectale* (Erec) group, *Clostridium leptum* group and *Bacteroidetes* group (Franks *et al.*, 1998; Suau *et al.*, 1999; Sghir *et al.*, 2000; Eckburg *et al.*, 2005), which account for over 70 % of faecal bacteria (Sghir *et al.*, 2000). The bacteria belonging to the Erec group (clostridial

cluster XIVa) (Collins *et al.*, 1994) comprise 10–59 % of the total faecal bacteria depending on the detection method used (Franks *et al.*, 1998; Suau *et al.*, 1999; Sghir *et al.*, 2000; Eckburg *et al.*, 2005; Maukonen *et al.*, 2006a). *Bifidobacterium* and *Lactobacillus*, although found in most subjects, comprise smaller populations among faecal bacteria in adults (bifidobacteria, 1–5 %; lactobacilli, <1–2 %; Franks *et al.*, 1998; Sghir *et al.*, 2000). The oral cavity contains different micro-environments (cheeks, palate, tongue, tooth surfaces, gingival areas and saliva), each with their own microbiota (Aas *et al.*, 2005). The salivary microbiota reflects a mixture of bacteria washed off from the various surfaces, especially from the tongue (Nisengard & Newman, 1994). A large diversity of bacteria has been detected in the oral cavity, including low-mol% G + C-content Gram positive organisms (e.g. streptococci and *Clostridia* group), fusobacteria, actinobacteria, different proteobacteria, *Prevotella*, *Porphyromonas*, *Bacteroides* and spirochaetes. Members of the clostridial cluster XIVa have occasionally been detected in oral samples (Paster *et al.*, 2001). In the oral cavity, both bifidobacteria and lactobacilli can be detected, but their occurrence is reversed compared with faeces: lactobacilli are a common finding in the oral cavity, whereas bifidobacteria are detected less frequently.

The aim of this study was to investigate whether similar features concerning the diversity and temporal stability of the predominant microbiota and selected bacterial groups

– namely the clostridial cluster XIVa, bifidobacteria and lactobacilli – could be detected intra-individually in two different niches: the oral cavity and the colon. Special focus was also put on the species distribution of the genera *Lactobacillus* and *Bifidobacterium* at these two sites.

METHODS

Sample handling. Faecal and salivary (5 ml saliva collected with paraffin stimulation) samples were collected from ten adult volunteers (nine females, one male, aged 34–57 years) at three sampling time points (at baseline before probiotic consumption and after 1 and 2 weeks of probiotic consumption). The subjects consumed a commercial probiotic capsule preparation (Trevis, 54 capsules; Ipex Medical) according to the manufacturer's instructions (three capsules each day) for 2 weeks. Trevis capsules contain a mixture of *Lactobacillus acidophilus* LaCH-5, *Bifidobacterium animalis* subsp. *lactis* Bb-12 and yoghurt starter bacteria, totalling 10^9–10^{10} organisms per capsule. The main recruiting criterion was a normal intestinal balance (absence of repeating and/or persisting gastrointestinal symptoms). The exclusion criteria were regular gastrointestinal tract symptoms, lactose intolerance, coeliac disease and antimicrobial therapy during the 2 months prior to the study. The study was approved by the ethical committee of the VTT Technical Research Centre of Finland. All subjects gave written informed consent for participation in the study.

Part of the faecal sample was transferred to Cary–Blair transport medium (Atlas, 1997) and part of the salivary sample to VMGA III medium (Rams *et al.*, 1990), and these were analysed by culture within 1–2 days. The stability of the studied bacterial groups in the transport medium was confirmed by culturing four samples immediately after sampling and after storage for 2 days in the appropriate transport medium. The rest of the samples were frozen at −70 °C for DNA-based analyses.

Culture-based analysis

The samples were serially diluted in pre-reduced peptone saline containing 0.5 g L-cysteine/HCl l^{-1} (pH 6.3; Merck) and plated on culture medium in an anaerobic workstation (Don Whitley Scientific). The following culture media and incubation conditions were used (incubation at 37 °C): supplemented Brucella blood agar (Tammer-Tutkan Maljat) for anaerobes (anaerobic incubation for 7 days), sheep blood agar for aerobes (Tammer-Tutkan Maljat) (aerobic incubation for 4 days) and Beerens agar for bifidobacteria (Beerens, 1991) (anaerobic incubation for 4 days). For the detection of *B. animalis* subsp. *lactis* Bb-12, the samples were also acid pre-treated (Alander *et al.*, 2001). Rogosa agar was used for the detection of lactobacilli (anaerobic incubation for 3 days). For the detection of *L. acidophilus* LaCH-5, the Rogosa plates were incubated micro-aerophilically. Beerens and Rogosa agars were also used with tetracycline supplementation (8 µg ml^{-1}), as *B. animalis* subsp. *lactis* Bb-12 is intermediately resistant to tetracycline.

Isolates representing all of the different potential *Lactobacillus* and *Bifidobacterium* colony morphologies, or, in the case of uniform colony morphology, random isolates, were collected from Beerens- and Rogosa-based media (five isolates from each medium where possible) for comparison of the species distribution in saliva and faeces.

Fingerprinting of bifidobacteria and lactobacilli by randomly amplified polymorphic DNA (RAPD). A total of 587 isolates from Beerens medium were analysed by RAPD using primer OPA-2 (5′-TGCCGAGCTG-3′) and 574 isolates from Rogosa were analysed by RAPD using primer OPA-3 (5′-AGTCAGCCAC-3′) as described previously (Alander *et al.*, 2001). The RAPD fingerprints of faecal and

salivary isolates were compared with those of the *B. animalis* subsp. *lactis* Bb-12 and *L. acidophilus* LaCH-5 strains by visual inspection. All types that looked different to the ingested probiotic strains were selected for further characterization.

Partial 16S rRNA gene sequencing. Isolates representing different RAPD types were identified by partial 16S rRNA gene sequencing. Beerens isolates were amplified with Bif164-f (5′-GGGTGGTAATG-CCGGATG-3′) and Bif662-GC-r (5′-CGCCCGCCGCGCGCGGCG-GGCGGGGCGGGGGCACGGGGGGGCCACCGTTACACCGGGAA-3′) as described by Satokari *et al.* (2001a). Rogosa isolates were amplified with BSF8/20 (5′-AGAGTTTGATCCTGGCTCAG-3′) and BSR1541/20 (5′-AAGGAGGTGATCCAGCCGCA-3′) (Wilmotte *et al.*, 1993). The lactobacilli PCR mixture contained 0.2 mM each dNTP, 0.2 µM primer and 3 U Dynazyme II DNA polymerase in 1× Dynazyme buffer. PCR amplification was carried out in a thermocycler (UnoII, Biometra; 35 cycles with an annealing temperature of 56 °C). The amplification products were checked, purified and further sequenced with primers Bif164-f or BSF8/20 as described previously (Maukonen *et al.*, 2006b).

PCR-denaturing gradient gel electrophoresis (DGGE) analysis. DNA was extracted as described by Maukonen *et al.* (2006b). Part of the 16S rRNA gene (V_6–V_8 hypervariable region) was PCR amplified for the detection of predominant bacterial microbiota using primers U968-f+GC (5′-CGCCCGGGGCGCGCCCCGGGCGGGGCGGGG-GCACGGGGGGGAACGCGAAGAACCTTA-3′) and U1401-r (5′-CGGTGTGTACAAGACCC-3′) (Nübel *et al.*, 1996), as described by Mättö *et al.* (2005). Primers Bif164-f and Bif662-GC-r were used to evaluate the diversity and temporal stability of bifidobacteria according to Satokari *et al.* (2001a). Primers Bif164mod-f (5′-GGGTGGTAATACCGGATG-3′) and Bif662-GC-r (Satokari *et al.*, 2001b) were used for the detection of *B. animalis* subsp. *lactis* Bb-12. The *Lactobacillus* group, which comprises the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Weissella*, was amplified using primers Lac1 (5′-AGCAGTAGGGAATCTTCCA-3′ and Lac2GC (5′-CGCCC-GCCGCGCGCCCCGCGCCCGGCCCGCCG CCCCCGCCCCATTYCA-CCGCTACACATG-3′) (Walter *et al.*, 2001), as described by Vanhoutte *et al.* (2004). The Erec group was PCR amplified using primers Ccoc-f (5′-AAATGACGGTACCTGACTAA-3′; Matsuki *et al.*, 2002) and Ccoc-r+GC (5′-CGCCCGGGGCGCGCCCCGGGCGG-GGCGGGGGCACGGGGGGCTTTGAGTTTCATTCTTGCGAA-3′) according to Maukonen *et al.* (2006b).

PCR products were separated by polyacrylamide gels with a denaturing gradient of 38–60 % (predominant bacterial microbiota and Erec group), 45–55 % (bifidobacteria) or 30–60 % (*Lactobacillus* group) [where 100 % is 7 M urea and 40 % (v/v) deionized formamide] as described by Mättö *et al.* (2005). *L. acidophilus* LaCH-5 was added as a probiotic control lane to each *Lactobacillus* group-specific DGGE. Comparison of the PCR-DGGE profiles was performed as described previously (Maukonen *et al.*, 2006b).

Statistical analysis. Means ± SD were calculated for each experiment. Results with microbial numbers below the detection limit (log 4 for all microbial groups) were excluded from statistical analysis using Student's *t*-test (two-sample test assuming unequal variances).

RESULTS AND DISCUSSION

In the present study, both the culture-based approach (combined with fingerprinting of individual isolates) and PCR-DGGE were applied to study the intra-individual diversity and temporal stability of microbiota at two different sites, the oral cavity and the colon. To the best of our knowledge, there is only one previous study where the

oral and faecal bacterial populations have been compared intra-individually using molecular techniques (Dal Bello & Hertel, 2006). The probiotic product used in our study did not notably affect the stability of the predominant microbiota profile, Erec group profile or bifidobacterial profile (using non-modified primers that targeted only the intestinal bifidobacteria and not the ingested probiotic bifidobacteria), as the baseline DGGE profiles (before probiotic ingestion) were highly similar to those of the samples after 1 and 2 weeks of probiotic ingestion. All of these bacterial groups remained relatively stable in most of the subjects. Our study showed that, in the cases of predominant microbiota, bifidobacteria and Erec group bacteria, the faecal microbiota was more diverse than that in saliva. In addition, salivary and faecal DGGE profiles of these groups appeared to be substantially different. Interestingly, most subjects' faecal and salivary samples contained identical indigenous *Lactobacillus* species and genotypes.

Compliance was evaluated with culture-based techniques and DGGE analysis in this study. Probiotic strains were not found in any of the baseline samples, whereas RAPD types identical to *B. animalis* subsp. *lactis* Bb-12 were found from all faecal samples during probiotic ingestion (after 1 and 2 weeks). Ingested probiotic strains were not found in any of our salivary samples.

Diversity and temporal stability of the predominant bacterial microbiota, Erec group, *Lactobacillus* group and bifidobacteria as detected with DGGE

Predominant bacterial microbiota

DGGE analysis targeted to the predominant microbiota showed intra-individual diversity as well as uniqueness of both faecal and salivary microbiota (Fig. 1a). After comparison of all faecal- and saliva-based profiles, faecal profiles and salivary profiles formed two distinct clusters, with individual-based subclusters (data not shown). Indeed, the DGGE profiles of faecal and salivary samples differed substantially (mean similarity 23.7 ± 7.1 %; Table 1). The predominant faecal microbiota was relatively stable temporally (mean similarity 83.3 ± 5.3 %) and the salivary microbiota was mostly stable (mean similarity 92.2 ± 2.5 %) during the study period (Table 2). This is consistent with previous work, which showed that the faecal microbiota is host specific and relatively stable temporally (Zoetendal *et al.*, 1998; Vanhoutte *et al.*, 2004; Maukonen *et al.*, 2006b). Likewise, in a recent study by Rasiah *et al.* (2005), it was shown that the predominant salivary microbiota remained stable in one subject for 7 years. We found a significant difference between the number of amplicons detected in the faecal and salivary profiles (faeces>saliva; $P<0.05$) (Table 2). Aas *et al.* (2005) showed that 20–30 different predominant species were found from most oral sites, utilizing 16S rRNA gene clone libraries. Our universal DGGE results are in accordance with this previous work.

Erec group

DGGE analysis targeted to the Erec group showed intra-individual diversity as well as uniqueness of both faecal and salivary microbiota (Fig. 1b). Erec profiles of faecal samples clustered together according to subject, whereas salivary samples clustered according to subject in eight cases (data not shown). The similarity between faecal samples and salivary samples from the same subject at a given time point was very low or non-existent (mean similarity
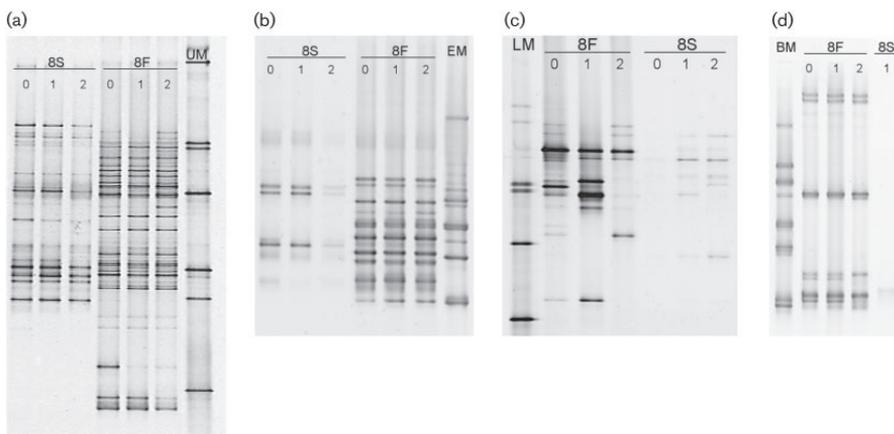
Fig. 1. Faecal and salivary DGGE profiles of a healthy subject. (a) Universal primers; (b) Erec group primers; (c) *Lactobacillus* group primers; (d) bifidobacteria primers. DNA was obtained from faecal samples (8F) and salivary samples (8S) at baseline (before probiotic consumption; lane 0), and after 1 and 2 weeks of probiotic consumption (lanes 1 and 2, respectively). BM, Bifidobacterial marker; EM, Erec group marker; LM, lactobacillus marker; UM, universal marker.

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Table 1. Intra-individual similarity values between a faecal sample and a salivary sample at a given time point for a given subject from ten healthy subjects before and during probiotic consumption

Analysis	Similarity (%)*			
	Baseline†	1 week	2 weeks	Mean
Predominant bacteria‡				
Faeces vs saliva (mean±SD)§	25.7±10.9	22.5±7.7	22.9±8.7	23.7±7.1
Range§	10.8–43.8	4.8–32.9	9.5–37.7	16.1–38.1
Erec group of clostridia‖				
Faeces vs saliva (mean±SD)§	17.4±10.0	15.7±10.4	20.7±17.8	17.9±11.1
Range§	0.0–31.3	0.0–32.2	4.9–67.3	1.6–39.0
***Lactobacillus* group¶**				
Faeces vs saliva (mean±SD)§	58.9±18.3	60.2±8.3	58.7±17.0	58.6±11.2
Range§	26.7–85.7	54.5–80.0	26.7–80.0	40.3–74.3
Bifidobacteria				
Faeces vs saliva (mean±SD)§#	19.8±13.2	23.9±28.2	26.6±32.7	26.4±27.9
Range§	0.0–33.3	0.0–77.5	0.0–81.7	0.0–81.7

*Similarity values were analysed using BioNumerics 4.50 software. Amplicons with the total surface area of at least 1 % were included in the similarity analysis.
†Sample before 2 weeks of probiotic consumption.
‡Partial 16S rRNA gene (V_6–V_8 hypervariable region).
§The salivary and faecal samples were compared only intra-individually, that is, for example, a faecal sample of subject A taken at baseline was compared only with the salivary sample of subject A taken at baseline.
‖Clostridial phylogenetic cluster XIVa (Collins *et al.*, 1994).
¶*Lactobacillus* group was comprised the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Weissella*.
#Only half of the saliva samples gave a positive result after bifidobacteria-specific PCR.

17.9±11.1 %; Table 1). Faecal samples of all subjects clustered together as one distinct cluster and salivary samples of all subjects clustered as another.

Maukonen *et al.* (2006b) showed that each band position in DGGE gels contained only one phylotype. We may therefore assume that the phylotypes present in the faeces and saliva are mostly different. Erec group bacteria were temporally stable in most cases (mean similarity: faeces 94±2.9 %; saliva 90.5±7.3 %) during our study period (Table 2), as has also been described previously (Maukonen *et al.*, 2006b). In this study, the Erec group diversity in salivary samples was significantly lower than in the faecal samples ($P < 0.05$). Nonetheless, we found between 3 and 12 amplicons from each sample (Table 2), indicating that the diversity of the Erec group in saliva in some subjects may be slightly greater that that shown previously with other techniques (Downes *et al.*, 2001; Paster *et al.*, 2001).

Lactobacillus group
In this study, the DGGE profiles of the faeces- and saliva-derived *Lactobacillus* group were more similar to each other (mean similarity 58.6±11.2 %; Table 1) than the faeces- and saliva-derived profiles of the other bacterial groups studied. In addition, faecal *Lactobacillus* group profiles of nearly all subjects were fairly unstable (mean similarity 69.1±7.3 %; Table 2, Fig. 1c), whereas salivary *Lactobacillus* group profiles remained rather stable (mean

similarity 89.8±7.5 %; Table 2). Based on the comparisons made between different sampling points (baseline without probiotic consumption vs samples during probiotic consumption), the ingested *L. acidophilus* LaCH-5 strain, which was seen in the faecal lactobacilli DGGE profile during probiotic ingestion, does not alone explain the instability.

As the LaCH-5 strain produced only a single band, it did not contribute to a considerable change in the similarity values between the samples. In the salivary samples, there were no amplicons that were identical to those of *L. acidophilus* LaCH-5. In BioNumerics analysis, the lack of a stable and host-specific *Lactobacillus* group population resulted in a complete lack of subject grouping in faecal samples and sample type grouping (faeces vs saliva). This lack of subject grouping has also been reported for faecal samples by Vanhoutte *et al.* (2004). The number of amplicons detected in our study was higher in faecal samples compared with salivary samples (Table 2).

Bifidobacteria
In this study, DGGE profiles of faecal samples of a given subject clustered together in nine out of ten subjects (data not shown). In these cases, the faecal bifidobacterial population remained fairly stable temporally (one subject was unstable) (Table 2, Fig. 1d), as has also been found by, for example, Satokari *et al.* (2001a). Only about half of the

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Table 2. Intra-individual similarity values and diversity of DGGE profiles of human faecal and salivary samples obtained at three different time points from ten healthy subjects

Analysis	Similarity (%)*				Diversity†			
	0 vs 1	0 vs 2	1 vs 2	Mean	0 week	1 week	2 weeks	Mean
Predominant bacteria‡								
Faeces (mean ± SD)	84.3 ± 7.8	81.0 ± 7.6	84.7 ± 4.9	83.3 ± 5.3	33.7 ± 4.2	35.8 ± 4.4	35.1 ± 4.4	34.9 ± 4.0
Range	70.3–92.8	70.0–90.6	75.6–92.2	72.0–90.9	25–39	27–42	27–41	26.3–40.7
Saliva (mean ± SD)	92.1 ± 3.3	91.4 ± 2.5	93.2 ± 4.4	92.2 ± 2.5	23.9 ± 2.9	23.1 ± 2.2	23.8 ± 2.9	23.6 ± 2.4
Range	86.0–95.8	87.2–94.2	82.9–98.0	88.8–95.3	20–27	19–26	19–28	19.3–26.7
Erec group§								
Faeces (mean ± SD)	95.1 ± 2.8	92.3 ± 4.7	94.8 ± 2.0	94.0 ± 2.9	15.1 ± 2.4	15.5 ± 2.3	14.8 ± 1.9	15.1 ± 2.0
Range	89.9–98.6	81.6–97.5	90.0–97.3	87.1–96.9	11–19	13–19	12–19	12.3–18.7
Saliva (mean ± SD)	92.9 ± 7.7	86.7 ± 11.8	92.0 ± 9.2	90.5 ± 7.3	7.4 ± 2.1	7.4 ± 1.6	7.7 ± 2.0	7.5 ± 1.7
Range	73.8–98.2	66.5–98.0	70.0–99.4	77.2–98.0	3–11	5–10	5–12	4.7–9.7
Lactobacillus group‖								
Faeces (mean ± SD)	70.0 ± 8.1	68.2 ± 11.9	70.7 ± 14.0	69.1 ± 7.3	12.0 ± 5.3	10.9 ± 2.8	11.0 ± 2.4	11.5 ± 3.0
Range	60.0–85.7	50.0–85.7	50.0–88.9	61.0–85.7	7–22	7–15	7–14	8.0–16.3
Saliva (mean ± SD)	92.2 ± 13.9	87.6 ± 11.0	89.5 ± 6.5	89.8 ± 7.5	7.0 ± 2.8	7.9 ± 3.1	7.0 ± 3.4	7.3 ± 2.9
Range	66.7–100	66.7–100	80.0–100	76.9–100	3–13	5–14	4–15	3.7–14.0
Bifidobacteria								
Faeces (mean ± SD)	94.0 ± 3.5	87.1 ± 10.8	82.3 ± 16.2	87.8 ± 9.6	9.1 ± 2.8	8.8 ± 2.4	9.1 ± 3.2	9.0 ± 2.6
Range	90.9–99.6	68.2–97.8	52.5–98.1	70.7–97.1	6–15	7–15	4–14	5.3–14.7
Saliva (mean ± SD)¶	84.7 ± 17.9	97.9 ± 2.0	89.2 ± 15.7	86.4 ± 16.0	4.5 ± 1.9	5.8 ± 1.5	4.0 ± 1.7	4.4 ± 1.4
Range	62.3–98.9	95.5–99.7	65.6–98.0	62.3–98.0	2–7	4–8	2–7	3.0–6.7

*Similarity values were calculated using BioNumerics 4.50 software. Amplicons with a total surface area of at least 1 % were included in the similarity analysis. 0, baseline (before probiotic consumption); 1, 1 week of probiotic consumption; 2, 2 weeks of probiotic consumption.

†Diversity is presented as the number of bands making up >1 % of the total profile as detected by the BioNumerics 4.50 software.

‡Partial 16S rRNA gene (V_6–V_8 hypervariable region).

§Clostridial phylogenetic cluster XIVa (Collins *et al.*, 1994).

‖*Lactobacillus* group was comprised the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Weissella*.

¶Only half of the salivary samples gave a positive result after bifidobacteria-specific PCR.

salivary samples (17/30 samples) gave a positive PCR result, although several additional variations to the PCR protocol were tested (data not shown). The salivary bifidobacterial populations were temporally stable in three subjects and unstable in one subject (a different subject from the one who had an unstable faecal bifidobacterial population; data not shown). Of the remaining six subjects, only one sample from three subjects and two samples from another subject produced a PCR product, thus not allowing temporal stability to be determined. The bifidobacterial profiles did not cluster according to sample type, even though the similarity between faecal and salivary samples collected at the same time was low. The faecal samples contained significantly more amplicons than the salivary samples at all sampling time points ($P<0.05$) (Table 2). As the PCR primers used for the DGGE-based diversity assessment had one base mismatch (Satokari *et al.*, 2001b) with the sequence of the ingested *B. animalis* subsp. *lactis* Bb-12, this strain was not seen in the bifidobacterial DGGE profiles and therefore did not affect

the temporal stability of the faecal and salivary profiles. However, Bb-12 was detected in faecal samples from seven of the ten subjects using DGGE targeting *B. animalis* subsp. *lactis* Bb-12. There were no amplicons that migrated at identical positions to those of *B. animalis* subsp. *lactis* Bb-12 in any of the salivary samples.

Culture

Mean numbers of culturable bacteria on media without tetracycline at different sampling occasions are shown in Fig. 2. The number of culturable bacteria remained stable during probiotic consumption in both faecal and salivary samples. The number of culturable anaerobic bacteria was significantly higher in faeces (mean log 10.5 c.f.u. g^{-1}) than in saliva (mean log 8.3 c.f.u. g^{-1}) ($P<0.05$), whereas the number of culturable aerobic bacteria was significantly higher in saliva (mean log 7.8 c.f.u. g^{-1}) than in faeces (mean log 7.1 c.f.u. g^{-1}) ($P<0.05$). The numbers of both bifidobacteria and lactobacilli were significantly higher in
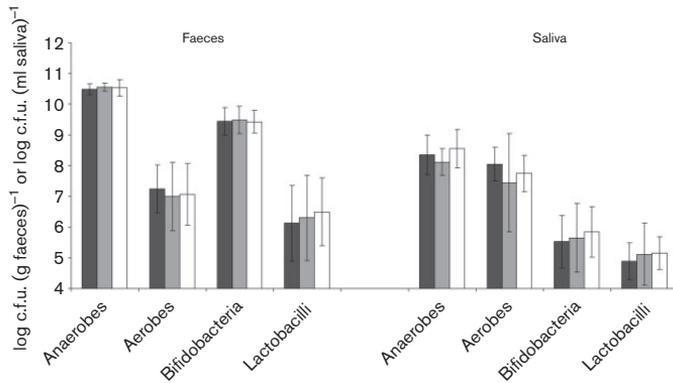
Fig. 2. Bacterial numbers (counts of bacteria growing on non-selective and bifidobacteria- or lactobacilli-selective culture media) in faecal and salivary samples of ten healthy volunteers. The samples were obtained at baseline before probiotic consumption (black bars), after 1 week of probiotic consumption (grey bars) and after 2 weeks of probiotic consumption (white bars).

faeces compared with saliva ($P<0.05$) (Fig. 2). The numbers of culturable bacteria found in salivary and faecal samples were in accordance with previous studies (Sanyal & Russel, 1978; Matsuki *et al.*, 1999; Tannock *et al.*, 2000).

Isolates with an identical RAPD type to the ingested *B. animalis* subsp. *lactis* Bb-12 strain were recovered from faecal samples but not from the salivary samples of all subjects during probiotic consumption (samples from 1 and 2 weeks), whereas isolates of *L. acidophilus* LaCH-5 strain were recovered from faecal samples of all subjects after 1 week of probiotic consumption and from nine out of ten subjects after 2 weeks.

From each subject, three to six indigenous salivary lactobacilli RAPD types and one to five indigenous faecal lactobacilli RAPD types were detected (Table 3). In addition, identical indigenous lactobacilli RAPD types were found in saliva and faeces for eight of the ten subjects (Table 3). These included *Lactobacillus rhamnosus*, *Lactobacillus gasseri*, *Lactobacillus paracasei*, *Lactobacillus plantarum* group (comprising *L. plantarum*, *Lactobacillus arizonensis* and *Lactobacillus pentosus*) and a *Lactobacillus* sp.

In a study by Dal Bello & Hertel (2006), *Lactobacillus* populations of salivary and oral samples of three subjects were compared by culture and RAPD typing in addition to DGGE. They found that *L. gasseri*, *L. paracasei*, *L. rhamnosus* and *Lactobacillus vaginalis* were most commonly detected among the predominant lactobacilli in the saliva and faeces of their three subjects. However, we only found *L. vaginalis* from one subject's saliva and none of the faecal samples of our subjects. *L. gasseri*, *L. paracasei* and *L. rhamnosus* were commonly detected from our subjects. The similarity of the faecal and salivary *Lactobacillus* populations found in this and another study (Dal Bello & Hertel, 2006) indicate that at least some *Lactobacillus* species/strains are able to live in different niches in the human orogastrointestinal tract, although the oral cavity and colon differ in several aspects including redox potential, nutrients, mucosal surfaces and co-existing members of the specific microbial community.

Between two and eight indigenous RAPD types were found per subject from faecal Beerens isolates and between two and nine per subject from salivary Beerens isolates. However, only one to seven faecal and zero to two salivary RAPD types per subject were confirmed to be bifidobacteria (after bifidobacteria-specific PCR with primers Bif164-f and Bif662-GC-r; Satokari *et al.*, 2001a) (Table 4), indicating that most salivary Beerens biotypes were not bifidobacteria. Whereas 80 % (37/46; Table 4) of the faecal Beerens biotypes were bifidobacteria. No identical indigenous RAPD types were found between saliva and faeces. After sequencing of the different bifidobacteria RAPD types, multiple indigenous bifidobacterial genotypes, including *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Bifidobacterium catenulatum/ Bifidobacterium pseudocatenulatum*, *Bifidobacterium bifidum* and *Bifidobacterium angulatum* were detected in faeces within an individual, whilst only *Bifidobacterium dentium* and *B. bifidum* were detected from saliva (Table 4). We found *B. dentium* in the saliva of five subjects. *B. dentium* was not found in faecal samples, which is consistent with a published culture-based analysis (Mättö *et al.*, 2004). However, it has been found in faeces in DNA-based studies (Matsuki *et al.*, 1999). We used culture-based identification in the present study, as we wanted to see whether the bifidobacteria detected from the faeces were alive and therefore most likely to represent autochthonous strains. The levels of *B. dentium* in our salivary samples gave a mean value of 2×10^5 c.f.u. ml^{-1}, which accounts for 10^6–10^8 cells in 1000 ml saliva; this is the mean quantity of saliva ingested daily (Nisengard & Newman, 1994). Therefore, the *B. dentium* strains found in the faecal samples by DNA-based methods may originate from saliva and may actually be allochthonous.

Conclusions

In conclusion, the predominant bacteria, bifidobacteria and Erec group bacteria, of the oral cavity and intestines were generally stable during probiotic consumption,

Table 3. Heterogeneity of indigenous *Lactobacillus* populations

Subject	Saliva		Faeces		Comparison of saliva and faeces	
	No. of RAPD types	Species*	No. of RAPD types	Species*	Identical RAPD types	Species*
1	5	*L. rhamnosus, L. casei/L. paracasei, L. brevis*	5	*L. brevis*	0	–
2	4	*L. rhamnosus, L. gasseri, L. paracasei*	4	*L. rhamnosus, L. gasseri, L. sakei, L. parabuchneri*	2	*L. rhamnosus, L. gasseri*
3	4	*L. rhamnosus, L. gasseri, L. brevis, L. plantarum* group†	1	*L. gasseri*	1	*L. gasseri*
4	6	*L. rhamnosus, L. paracasei, L. parabuchneri, L. plantarum* group†	2	*L. rhamnosus, L. parabuchneri/L. ferintoshensis*	1	*L. rhamnosus*
5	5	*L. rhamnosus, L. paracasei, L. jensenii*	3	*L. rhamnosus, L. paracasei*	1	*L. paracasei*
6	5	*L. gasseri, L. paracasei, L. parabuchneri, L. fermentum*	4	*L. gasseri, L. paracasei, L. rhamnosus*	1	*L. gasseri*
7	5	*L. rhamnosus, L. paracasei, L. vaginalis, L. plantarum* group†	4	*L. rhamnosus, L. crispatus, L. plantarum* group†	2	*L. rhamnosus, L. plantarum* group†
8	6	*L. gasseri, L. paracasei, L. salivarius, L. fermentum, L. kitasonis/L. crispatus*	1	*L. gasseri*	0	–
9	3	*L. rhamnosus, L. brevis, Lactobacillus* sp.	4	*L. paracasei, L. plantarum* group†, *Lactobacillus* sp.	1	*Lactobacillus* sp.
10	3	*L. rhamnosus, L. gasseri, L. casei/L. paracasei*	4	*L. rhamnosus, L. gasseri, L. barabucneri*	1	*L. gasseri*

*Determined by 16S rRNA gene sequencing.
†*L. plantarum* group: *L. plantarum, L. arizonensis* and *L. pentosus.*

Table 4. Heterogeneity of indigenous *Bifidobacterium* populations

Subject	Saliva			Faeces		
	No. of initial RAPD types*	No. of confirmed RAPD types†	Species‡	No. of initial RAPD types*	No. of confirmed RAPD types†	Species‡
1	5	2	*B. dentium, B. bifidum*	4	1	*B. longum*
2	6	2	*B. dentium, B. bifidum*	8	5	*B. longum, B. adolescentis, B. ruminantium/B. adolescentis*
3	4	2	*B. dentium*	3	2	*B. longum*
4	9	1	*B. dentium*	2	2	*B. longum, B. catenulatum/B. pseudocatenulatum*
5	3	0	–	4	4	*B. longum, B. adolescentis*
6	4	0	–	5	5	*B. longum, B. adolescentis, B. bifidum, B. angulatum*
7	6	0	–	8	7	*B. longum, B. adolescentis, B. bifidum*
8	6	0	–	4	4	*B. longum, B. adolescentis, B. catenulatum/B. pseudocatenulatum*
9	5	0	–	4	4	*B. longum, B. adolescentis, B. catenulatum/B. pseudocatenulatum, B. ruminantium/B. adolescentis*
10	2	1	*B. dentium*	4	3	*B. longum, B. adolescentis*

**Bifidobacterium-like isolates that were initially RAPD typed.
†Number of RAPD types of the bifidobacterial isolates that gave a positive result with a bifidobacteria-specific PCR (Satokari *et al.*, 2001a).
‡Determined by 16S rRNA gene sequencing.

showing more diversity in faeces than saliva, and having different species compositions for the two sampling sites. In contrast, the lactobacilli, which are known to inhabit several ecological niches, showed temporal instability in both faeces and saliva. Furthermore, faecal and salivary samples contained identical indigenous *Lactobacillus* genotypes (*L. rhamnosus, L. gasseri, L. paracasei, L. plantarum* group and a *Lactobacillus* sp.) in most subjects.

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