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ORIGINAL ARTICLE

Effects of yogurt and bifidobacteria supplementation on the colonic microbiota in lactose-intolerant subjects

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Abstract

Aims: Colonic metabolism of lactose may play a role in lactose intolerance. We investigated whether a 2-week supplementation of *Bifidobacterium longum* (in capsules) and a yogurt enriched with *Bifidobacterium animalis* could modify the composition and metabolic activities of the colonic microbiota in 11 Chinese lactose-intolerant subjects.

Methods and Results: The numbers of total cells, total bacteria and the *Eubacterium rectale/Clostridium coccoides* group in faeces as measured with fluorescent *in situ* hybridization and the faecal β-galactosidase activity increased significantly during supplementation. The number of *Bifidobacterium* showed a tendency to increase during and after supplementation. With PCR-denaturing gradient gel electrophoresis, in subjects in which *B. animalis* and *B. longum* were not detected before supplementation, both strains were present in faeces during supplementation, but disappeared after supplementation. The degree of lactose digestion in the small intestine and the oro-caecal transit time were not different before and after supplementation, whereas symptom scores after lactose challenge decreased after supplementation.

Conclusions: The results suggest that supplementation modifies the amount and metabolic activities of the colonic microbiota and alleviates symptoms in lactose-intolerant subjects. The changes in the colonic microbiota might be among the factors modified by the supplementation which lead to the alleviation of lactose intolerance.

Significance and Impact of the Study: This study provides evidence for the possibility of managing lactose intolerance with dietary lactose (yogurt) and probiotics via modulating the colonic microbiota.

Introduction

The occurrence of the symptoms of lactose intolerance can be influenced by the amount of lactose ingested, the small-intestinal lactase activity and transit time, and colonic processing of lactose (Vesa *et al.* 2000; Vonk *et al.* 2003), e.g. the fermentation of lactose by the colonic microbiota (He *et al.* 2006). Therefore, it can be hypothesized that modulating the composition and/or metabolism of the colonic microbiota may influence lactose intolerance. Modulation of the colonic microbiota may be achieved through the targeted use of dietary supplementation, i.e. probiotics, prebiotics and synbiotics (Collins and Gibson 1999).

Several reviews (Roberfroid 2000; Rolfe 2000; Kopp-Hoolihan 2001; de Vrese *et al.* 2001) have described that some probiotics could improve lactose digestion and eliminate the symptoms of intolerance. The mechanisms

by which these probiotics exert their effects are not fully understood yet, but may involve modifying gut pH, expressing β-galactosidase, exerting positive effects on intestinal functions and colonic microbiota. In contrast, in a systematic review by Levri *et al.* (2005), it was concluded that probiotic supplementation in general did not alleviate the symptoms of lactose intolerance in adults. *Bifidobacterium* spp., together with *Lactobacillus* spp., are the bacteria most applied as probiotics because of their potential health benefits (Goldin 1998; Arunachalam 2004).

Yogurt is defined by the *Codex Alimentarius* of 1992 as a coagulated milk product that results from the fermentation of lactose in milk by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Adolfsson *et al.* 2004). Some lactic acid bacteria can be combined with yogurt starters for their probiotic properties. In addition to the effects of yogurt-derived microbial β-galactosidase, yogurts improve lactose digestion and tolerance by delaying gastric emptying, oro-caecal transit time (OCTT) or both (de Vrese *et al.* 2001). The lactose contained in yogurts can be considered as a prebiotic for people with lactose maldigestion (Szilagyi 2002, 2004). Regular consumption of lactose influences their colonic microbiota (Ito and Kimura 1993; Kleessen *et al.* 1997) and reduces lactose intolerance (Hertzler and Savaiano 1996).

Supplementation of yogurt and/or probiotics modified the composition and metabolism of the colonic microbiota in healthy adults (Bartram *et al.* 1994; Chen *et al.* 1999), healthy infants (Guerin-Danan *et al.* 1998) and patients with functional bowel disorders (Brigidi *et al.* 2001). In those studies, enumeration of faecal bacteria was often achieved with culture-dependent methods which cannot provide a very accurate picture of the composition of the colonic microbiota as not all bacteria, especially anaraeobes, can be cultured and media are not always specific (Welling *et al.* 1997).

The subjects for this study were selected from a group of Chinese lactose maldigesters based on their high symptom scores (SSC) in lactose challenge tests. Those Chinese maldigestors harboured considerably less *Bifidobacterium* spp. (Zhong *et al.* 2004) in their colon than European subjects (Harmsen *et al.* 2002b). The objective of this study was to investigate the effects of supplementation of a yogurt enriched with *Bifidobacterium animalis* and capsules with *Bifidobacterium longum* on the colonic microbiota. Molecular techniques were applied to detect and quantify bacteria in faeces. The endogenous lactase activity in the brush border of the small intestine, the OCTT and symptom response in the subjects were also monitored. The design of the study does not focus on the analysis of the instant effect of yogurt on hydrolysis of lactose in the small intestine.

Subjects and methods

Subjects

Eleven healthy Chinese subjects (five males and six females, age range 23–54 years) were recruited for this study. The subjects were selected from a group of lactose maldigesters based on their high 6-h SSC (>10) in oral lactose challenge (25 g lactose in water) tests in the year previous to the study. The subjects had not taken antibiotics or laxatives during the 3 months prior to the study. The subjects were asked to keep their habitual diet throughout the study period. All subjects gave a verbal informed consent. The study was approved by the Medical Ethical Committee of the Groningen University Hospital and Faculty of Medical Sciences, Groningen, the Netherlands, and of the West China University of Medical Sciences, Chengdu, China.

Yogurt and bifidobacteria supplementation

The yogurt used in this study was a fermented milk with the traditional yogurt strains (*L. bulgaricus* and *S. thermophilus*) and a specific probiotic strain, *B. animalis* DN-173010 [approximately 10^8 colony forming units (CFU) per g of product] (Danone, Shanghai, China). The other supplement administered was encapsulated *B. longum* (Bifina®; Morishita Jintan Co. Ltd., Osaka, Japan), each capsule containing 2×10^8 CFU freeze-dried *B. longum*.

Study design

The study was divided into three periods (Fig. 1): a 1-week baseline period; a 2-week supplementation period; and a 1-week follow-up period. During the supplementation period, each subject consumed per day three intervals with three Bifina® capsules (daily *B. longum* intake: $1{\cdot}8 \times 10^9$ CFU) and 125 g of yogurt per interval (daily *B. animalis* intake: $3{\cdot}8 \times 10^{10}$ CFU).

Faecal samples were collected for analysis of the composition of the faecal microbiota and β-galactosidase activity. In total, five faecal samples were collected: two in the baseline period, two in the supplement period, and one in the follow-up period. Faeces was collected in a sterile bag kept at 4°C after arrival in the laboratory, and processed within 12 h after collection. For each sample, a portion of 0·5 g was stored at −20°C till the analysis of β-galactosidase activity.

Two oral lactose challenge tests (25 g of lactose in water) were carried out 1 day before and 1 day after the supplement period. A 6-h SSC was recorded as described earlier (Vonk *et al.* 2003); blood samples were collected for the measurement of the lactose digestion index (LDI)

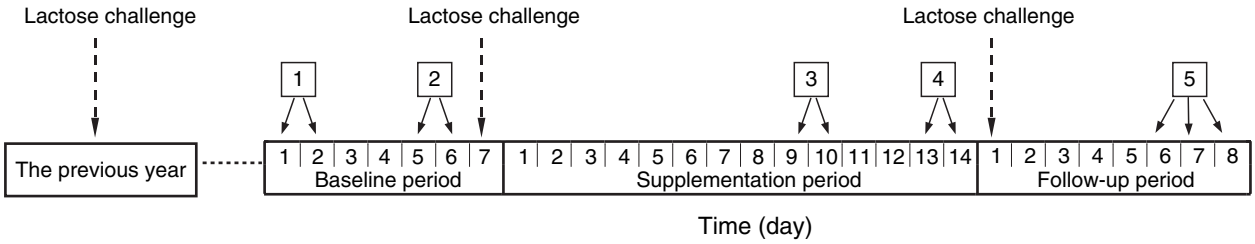

Figure 1 The study design. 1 ~ 5: collection of faecal samples.

and breath samples for the measurement of hydrogen concentration for the determination of the OCTT as described earlier (Vonk *et al.* 2003). LDI was determined with the $^{13}C/^{2}H$-glucose test as described previously (Vonk *et al.* 2001) with a slight modification: blood samples were collected before ingestion of lactose and at 45 and 60 min after ingestion of lactose. LDI was calculated as the mean value of the two samples collected at 45 and 60 min.

Symptom scores were also recorded in 10 of these 11 subjects after a lactose challenge test (25 g of lactose in water) following the same protocol as mentioned earlier in the year previous to the study.

Quantification of bacteria in faeces with fluorescent *in situ* hybridization (FISH)

16S rRNA oligonucleotide probes were used to detect the numbers of total bacteria and predominant bacterial groups in the faecal samples (Table 1). The hybridization and visualization of fluorescent cells were carried out according to the methods described previously (Jansen *et al.* 1999; Harmsen *et al.* 2002b). The detection limit of bacteria in faeces with our FISH technique is about 10^6–10^7 cells per g faeces (0·001–0·01% of the total faecal bacteria). In addition to FISH, 4′,6-diamidino-2-phenylindole (DAPI) staining was used to enumerate the total amount of cells in faeces (Jansen *et al.* 1999).

Analysis of the bifidobacterial population in faeces with polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE)

DNA extraction and PCR

0·5 g of each stool was suspended in 4·5 ml filtered PBS (phosphate-buffered saline) and homogenized on a vortex for 3 min together with a few glass beads (diameter, 4 mm). The suspension was centrifuged at 700 ***g*** for 1 min; subsequently 1 ml of the supernatant was centrifuged again at 14 000 ***g*** for 5 min. The pellet was stored at −20°C until DNA extraction. Total DNA was extracted as described previously (Harmsen *et al.* 2002a).

Table 1 Numbers of total cells, total bacteria and predominant bacterial groups in the faeces of lactose-intolerant subjects detected with fluorescent *in situ* hybridization (FISH) and 4′,6-diamidino-2-phenylindole (DAPI) staining before, during and after supplementation of yogurt enriched with *Bifidobacteria animalis* and Bifina® capsules with *Bifidobacterium longum**

		Baseline period		Supplementation period		Follow-up period	
Stain or probes	Targeted groups	Cells (10^{10})†	% Total bacteria‡	Cells (10^{10})†	% Total bacteria‡	Cells (10^{10})†	% Total bacteria‡
DAPI	Total cells	14·5 ± 6·3		20·6 ± 4·9§		17·4 ± 8·5	
Eub338	Bacteria	12·9 ± 4·9		19·5 ± 5·5¶		17·6 ± 7·9	
Bac303	*Bacteroides/Prevotella*	3·4 ± 1·7	27·7 ± 12·3	5·3 ± 2·9	26·5 ± 12·0	4·6 ± 3·3	27·1 ± 18·2
Erec482	*Eubacterium rectale/Clostridium coccoides* group	2·5 ± 1·4	19·6 ± 7·1	4·2 ± 1·3**	22·2 ± 6·9	3·3 ± 1·8	20·2 ± 9·0
Elgc01	*Eubacterium* low G+C2	0·8 ± 0·5	6·4 ± 3·1	1·2 ± 0·6	6·1 ± 2·7	1·5 ± 0·8	8·9 ± 4·4
Rbro729/Rfla730	*Ruminococcus* group	1·1 ± 1·1	7·7 ± 6·5	0·6 ± 0·5	3·6 ± 3·2	1·3 ± 0·7	8·6 ± 7·5
Bif164y	*Bifidobacterium*	0·1 ± 0·1	0·8 ± 1·0	0·2 ± 0·3	1·2 ± 1·4	0·3 ± 0·5	2·2 ± 3·6

*Values are means ± SD or %, n = 11 (baseline period and during) or 10 (after).
†Per g faeces, dry weight.
‡Percentage of bacteria (Eub338).
§P = 0·05 compared with baseline period.
¶P = 0·03 compared with baseline period.
**P = 0·04 compared with baseline period.

The DNA was diluted 100-fold for PCR amplification. The forward primer U515 (5′-GTGCCAGCAGCCGCGGT-3′) and *Bifidobacterium* genus-specific reverse primer 1412 (lm3, 5′-CGGGTGCTICCCACTTTCATG-3′) (Kaufmann *et al.* 1997) were used for the amplification of the 16S rRNA gene of *Bifidobacterium*. The reaction mixture (50 μl) consisted of reaction buffer (final concentrations, 15 mmol l^{-1} Tris-HCl (pH 8·0), 50 mmol l^{-1} KCl, 0·01% (v/v) Tween 20, 2·5 mmol l^{-1} $MgCl_2$, 1·6 mmol l^{-1} of each deoxynucleoside triphosphate, 200 nmol l^{-1} of each primer, 2·5 U Hot Goldstar *Taq* polymerase (Eurogentec, Seraing, Belgium) and 1 μl 100-fold diluted template DNA. The PCR was performed in a T-gradient thermocycler (Biometra, Göttingen, Germany) using the following conditions: 95°C for 10 min; 34 cycles of 95°C for 45 s, 50°C for 2 min and 72°C for 2 min; finally 72°C for 5 min. The resulted amplicons were diluted 1000-fold and used as template for a second PCR. Forward U968-GC (5′-CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGGAACGCGAAGAACCTTAC-3′) and reverse U1406 (5′-ACGGGCGGTGTGTRC-3′) primers (Nubel *et al.* 1996) were used to amplify the V6–V8 regions of bacterial 16S rRNA gene. The PCR conditions were as follows: 95°C for 10 min; 25 cycles of 95°C for 30 s, 56°C for 1 min and 72°C for 1 min; finally 72°C for 5 min. The PCR amplicons were checked for their size by electrophoresis on agarose (10 g l^{-1}) gel containing ethidium bromide.

Bifidobacterium markers

DNA fragments of bifidobacterial strains in the yogurt and Bifina® capsules, and mixed DNA fragments of six bifidobacterial strains were prepared as markers for DGGE. The six bifidobacterial strains included *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium dentum*, *B. longum* and *Bifidobacterium pseudo* longum. Each bacterial strain was cultured on Brucella blood agar (BBA) under anaerobic conditions at 37°C. *Bifidobacterium animalis* in the yogurt and *B. longum* in Bifina® capsules were obtained by culturing the yogurt and capsules on BBA, followed by re-culturing a colony which hybridized with the 16S rRNA-based probe Bif164y (Langendijk *et al.* 1995). Sequence analysis confirmed that the strain obtained from the yogurt was a *B. animalis* ssp. *lactis*. DNA extraction of the bacterial colonies and PCR amplification were performed as mentioned earlier. PCR amplicons of the six strains were mixed together to make a combined marker.

DGGE analysis

Polyacrylamide gels [9% (w/v) acrylamide-bisacrylamide (37·5 : 1)] in 20 mmol l^{-1} Tris-acetate-EDTA buffer (pH 7·4) with a denaturing gradient were prepared with a gradient mixer. The gel contained a 45–70% gradient of urea and formamide increasing in the direction of electrophoresis. A 100% denaturing solution contained 40% (v/v) formamide and 7·0 mol l^{-1} urea. The PCR amplicons were loaded on the gel and separated by electrophoresis in a PhorU system apparatus (Ingeny, Goes, the Netherlands) at a constant voltage of 140 V and temperature of 60°C for 16 h. After electrophoresis, the gel was silver stained as described previously (Sanguinetti *et al.* 1994).

Quantification of β-galactosidase activity in faeces

An aliquot of 0·5 g of each stool was kept at −20°C untill the measurement of β-galactosidase activity. The 0·5 g of stool was diluted with 4·5 ml buffer (0·02 mol l^{-1} Na_2HPO_4, 0·01 mol l^{-1} $MgSO_4$, 0·001 mol l^{-1} dithiothreitol, pH 7·0) and 0·5 ml of this suspension was sonicated on ice (4 × 1 min with 15 s intervals) with a Soniprep 150 (Beun de Ronde BV, Abcoude, the Netherlands) followed by centrifugation (16 100 ***g***, 10 min). The β-galactosidase activity in the supernatant was measured by determining the hydrolysis of *p*-nitrophenyl-β-D-galactopyranoside (PNPG) at 37°C (van Laere *et al.* 2000).

Data analysis

Data are expressed as mean ± SD. For statistical evaluation of FISH and β-galactosidase data, the results for baseline and supplementation periods were the average of the two samples taken in each period. Logarithmic or square root transformation of the data was performed when necessary to obtain normally distributed data, and when the data stayed skewed after transformation, non-parametric tests were applied. The repeated measures followed by the Bonferroni method for pair-wise comparison was applied to assess differences among baseline, supplementation and follow-up periods in the total number of cells and bacteria, numbers and percentages of *Bacteroides/Prevotella*, *Eubacterium rectale/Clostridium coccoides* group, *Eubacterium* low G+C2 and *Ruminococcus* group, and in β-galactosidase activity and SSC. The Friedman test followed by a Wilcoxon test for pair-wise comparison was applied to assess differences among baseline, supplementation and follow-up periods in numbers and percentages of *Bifidobacterium*. The Student *t*-test (paired, two-tailed) was applied to assess the differences in LDI and OCTT before and after supplementation. Correlations were assessed by calculating the Pearson or Spearman correlation coefficients as appropriate. $P < 0·05$ was regarded as significant. All analyses were performed using SPSS 12.0 for Windows software (SPSS Inc., Chicago, IL, USA).

Results

Effects of yogurt and bifidobacteria supplementation on the amount and composition of the colonic microbiota (Table 1)

The numbers of total cells ($P = 0{\cdot}05$), total bacteria ($P = 0{\cdot}03$) and the *E. rectale/C. coccoides* group ($P = 0{\cdot}04$) increased significantly during supplementation, but returned to the level of that in the baseline period after supplementation had stopped ($P > 0{\cdot}1$). The number of *Bifidobacterium* showed a tendency of increase in supplementation and follow-up periods, but this was not significant ($P = 0{\cdot}07$). The percentages of *Bacteroides/Prevotella*, *E. rectale/C. coccoides* group, *Eubacterium* low G+C2, *Ruminococcus* group or *Bifidobacterium* (with total bacteria as 100%) were not different among baseline, supplementation or follow-up periods ($P > 0{\cdot}1$).

Effects of yogurt and bifidobacteria supplementation on bifidobacterial population in faeces

Dynamics in faecal bifidobacterial population were monitored with PCR-DGGE. Figure 2 shows the DGGE profiles of four subjects that were representative of the 11 subjects. In the baseline period, no bands were present at the same level as the band from *B. animalis* in the yogurt in all faecal samples. In 10 of the 11 subjects, a band at the same level as the band from *B. animalis* appeared during supplementation, but disappeared again after supplementation had stopped. In six of the 11 subjects, a band at the same level as the band from *B. longum* in Bifina® capsules was already present in the baseline period and this did not change during or after supplementation. In others which did not have the *B. longum* band in the baseline period, this band was not present after supplementation, although in three of these samples the band appeared during supplementation.

The DGGE profiles of *Bifidobacterium* showed host-specific patterns. Differences were found among subjects in the positions of specific bands and the number of bands. The profile of each subject in the follow-up period was similar to that in the baseline period except for some slight changes, e.g. in three subjects, new bands appeared during and after supplementation.

In two of the 11 subjects, no band at all was present in the baseline period. In one of them, two bands at the same levels as bands from the consumed *B. animalis* and *B. longum* appeared during supplementation but disappeared after supplementation. In another subject, besides two bands as *B. animalis* and *B. longum*, another band appeared during supplementation, and while these three

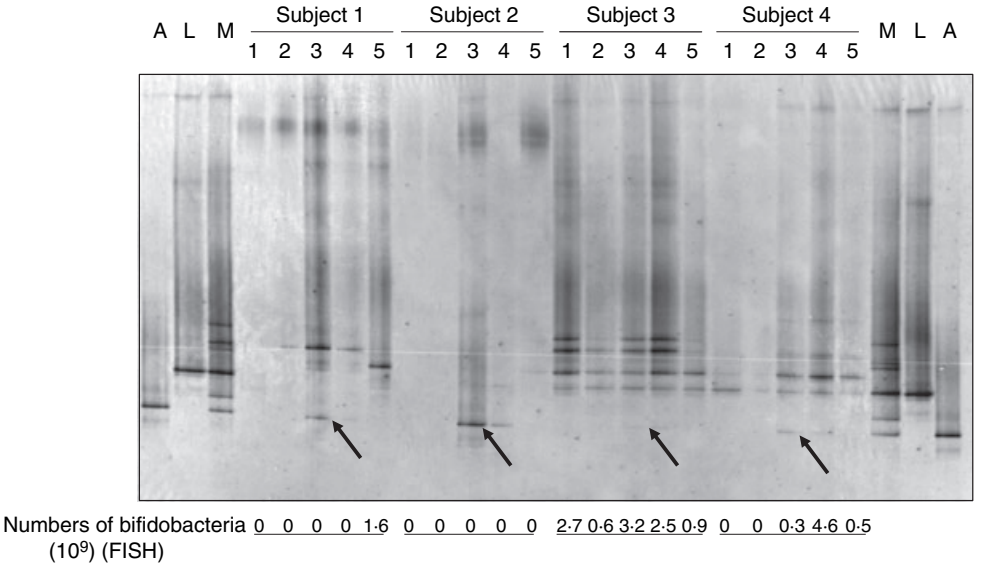

Figure 2 PCR-denaturing gradient gel electrophoresis (DGGE) analysis of bifidobacterial population in faeces of four lactose-intolerant subjects before, during and after supplementation of yogurt enriched with *Bifidobacteria animalis* and Bifina® capsules with *Bifidobacterium longum*. The profiles of the four subjects were representative of the profiles of the 11 subjects. (A) *Bifidobacterium animalis* obtained from the yogurt (L) *B. longum* obtained from Bifina® capsules; and (M) a mixture of six *Bifidobacterium* strains: (from top to bottom) *Bifidobacterium adolescentis*, *Bifidobacterium dentum*, *Bifidobacterium breve*, *B. longum* and *Bifidobacterium pseudolongum* and *Bifidobacterium bifidum*. For each subjects, samples 1–2, 3–4 and 5 were from the baseline, supplementation and follow-up periods, respectively. Arrows indicate the presence of a band at the same level as the band from *B. animalis* in the yogurt during the supplementation period. Numbers of *Bifidobacterium* (10^9 cells per g dry faeces) quantified with FISH are shown under the DGGE profile.

bands disappeared after supplementation, a band at different position appeared.

Results of *Bifidobacterium* measured with DGGE and FISH showed similar trends. Samples in which the numbers of *Bifidobacterium* were below the detection limit of FISH had no band or just a few bands on DGGE profiles, while samples with *Bifidobacterium* above the detection limit of FISH showed more bands. In some samples in which no *Bifidobacterium* were detected with FISH, there were bands present on DGGE profiles (e.g. Fig. 2, subject 1, sample 3).

Effects of yogurt and bifidobacteria supplementation on faecal β-galactosidase activity

β-Galactosidase activity in faeces increased significantly during supplementation ($P = 0{\cdot}01$, Table 2). In the follow-up period, β-galactosidase activity remained higher than that of the baseline period, but the difference was not significant ($P = 0{\cdot}37$). β-Galactosidase activity was not correlated with the total number of cells or bacteria, numbers of *Bacteroides/Prevotella*, *E. rectale/C. coccoides* group, *Eubacterium* low G+C2, *Bifidobacterium* ($P > 0{\cdot}1$) or *Ruminococcus* group ($P = 0{\cdot}09$). β-Galactosidase activity was not correlated with SSC ($P > 0{\cdot}1$).

Digestion of lactose in the small intestine and the OCTT before and after the supplementation

Lactose digestion index was measured 1 day before and 1 day after the supplementation as an indication of the degree of lactose digestion in the small intestine. Supplementation of yogurt and bifidobacteria did not change the LDI ($P = 0{\cdot}74$, Table 2), which indicates that the endogenous (brush-border) small intestinal lactase activity was not stimulated by the supplementation.

Table 2 Faecal ß-galactosidase activity, lactose digestion index (LDI), oro-caecal transit time (OCTT) and symptom score (SSC) of lactose-intolerant subjects before, during and after supplementation of yogurt enriched with *Bifidobacteria animalis* and Bifina® capsules with *Bifidobacterium longum**

	Baseline period	Supplementation period	Follow-up period
ß-Galactosidase (U mg^{-1} faeces)	6·4 ± 6·3	10·6 ± 8·3‡	8·9 ± 9·0
LDI	0·39 ± 0·14	nm†	0·38 ± 0·20
OCTT (min)	81 ± 38	nm†	68 ± 27
6 h SSC	16·1 ± 10·0	nm†	7·1 ± 5·5§

*Values are means ± SD, for OCTT, $n = 8$; for the rest, $n = 11$.
†Not measured.
§$P = 0{\cdot}01$ compared with baseline period.
‡$P = 0{\cdot}02$ compared with baseline period.

An increase in breath hydrogen was measured in eight subjects before and after the supplementation. The OCTT of these subjects was not different before and after the supplementation ($P = 0{\cdot}485$, Table 2).

Effects of yogurt and bifidobacteria supplementation on SSC

The SSC recorded in the follow-up period was significantly lower than that in the baseline period ($P = 0{\cdot}02$, Table 2). Compared with the SSC obtained in the year previous to the study (18·3 ± 10·3, means ± SD, $n = 10$), SSC in the baseline period was not different ($P = 1{\cdot}00$). However, SSC in the follow-up period was significantly lower than that of the previous year ($P = 0{\cdot}01$). Diarrhoea is a relatively objective measure for symptom response after lactose challenge. In the baseline period, four subjects developed diarrhoea after lactose challenge, while none of them did in the follow-up period. Only one subject had diarrhoea in the follow-up period.

Discussion

This study shows that a 2-week supplementation of probiotic bacteria *Bifidobacterium longum* and a yogurt enriched with *B. animalis* modified the amount of bacteria and increased the β-galactosidase activity in faeces from lactose-intolerant subjects. The composition of the predominant bacterial groups in faeces remained unchanged. There was no evidence, which administered that bifidobacterial strains colonized in the colon. The brush-border lactase activity in the small intestine and the OCTT did not change; however, SSC after the lactose challenge decreased after the supplementation.

Yogurt and bifidobacteria supplementation increased the numbers of total cells, total bacteria and *E. rectale/C. coccoides* group in faeces in this study. The increase in bacterial numbers could be attributed to, at least partly, the lactose present in the yogurt. During the production of yogurts, only 20–30% of the lactose is hydrolysed in the fermentation process (Adolfsson *et al.* 2004). The supplemented yogurt per day contained about 11·5 g lactose. Only ∼40% of the ingested lactose (in water) could be digested in the small intestine of the subjects (as indicated by LDI). Lactose in yogurt is better digested, but not all lactose in yogurt can be digested in lactase-deficient subjects (Kolars *et al.* 1984; Wytock and DiPalma 1988). Thus, during the supplementation period, up to 7 g of lactose would enter the colon per day and serve as a substrate for fermentation by the colonic microbiota. The presence of lactose is expected to stimulate the bacterial β-galactosidase activity. The *E. rectale/C. coccoides* group has been shown to possess β-galactosidase activity (He

et al. 2005). In two other studies, yogurt supplementation did not result in an increase in the number of total anaerobes in healthy infants (Guerin-Danan *et al.* 1998) or healthy German adults (Bartram *et al.* 1994). This may be attributed to the fact that healthy infants and most German adults can digest lactose well. Supplementation of yogurt in this case, would not bring a considerable amount of lactose to their colon. In contrast to this, a study carried out in Chinese subjects in Taiwan (Chen *et al.* 1999) showed increased counts of total anaerobes after yogurt ingestion. Most Chinese adults have genetically determined low lactase activity (Sahi 1994). The aforementioned studies lend support to our assumption that maldigested lactose may serve as a substrate for the colonic microbiota and thus, will stimulate the growth of bacteria. Furthermore, the supplemented bifidobacteria, which were found to be present in the colon during the supplementation, may influence the colonic microbiota, considering their positive effects on intestinal ecology (Bartram *et al.* 1994; Picard *et al.* 2005).

Despite the change in the numbers of bacteria, the composition of the faecal microbiota did not change after the supplementation. The stability of the colonic microbiota in healthy subjects (Zoetendal *et al.* 1998) and during similar dietary supplementation as in the present study (Bornside 1978; Harmsen *et al.* 2002a) have been reported earlier.

Bifidobacterium in faeces from the 11 subjects was enumerated with FISH in the year previous to the study. The number [7·3 (8·7) $\times 10^8$ cells per g dry faeces, mean (SD)] and the percentage of *Bifidobacterium* (0·7% of total bacteria) in these subjects were considerably lower than those in European subjects [6·0 (4·0) $\times 10^9$ cells per g dry faeces, 4·8% of total bacteria] (Harmsen *et al.* 2002b). *Bifidobacterium* is generally believed to possess health-beneficial properties (Leahy *et al.* 2005). Unfermented milks containing *B. longum* might be effective in reducing breath hydrogen response and symptoms from lactose malabsorption (Jiang *et al.* 1996). *Bifidobacterium animalis* DN-173 010 is shown to exhibit probiotic properties in the colon (Picard *et al.* 2005). Effects of supplementation of *B. longum* in capsules and a yogurt containing *B. animalis* on the faecal bifidobacterial population were investigated with FISH and PCR-DGGE in this study. FISH analysis shows a trend of increase in the numbers of bifidobacteria. PCR-DGGE analysis shows host-specific patterns of *Bifidobacterium* that were relatively stable. *Bifidobacterium longum* was already present in the faeces of some of the subjects before the supplementation. In other subjects, which did not have *B. longum* before the supplementation, *B. longum* could not be detected after the supplementation had ceased. The *B. animalis* strain in the supplemented yogurt was not present in any subject before the supplementation. Our data suggest that it was transiently present during the supplementation, but disappeared after consumption had stopped. It has been reported that once the adult gut microbiota is established, colonization with new strains is usually difficult and transient, and sustained oral doses are required for their middle- and long-term maintenance (Mackie *et al.* 1999). In most studies, supplemented probiotic strains did not colonize the intestine, as observed in the present study (Tannock *et al.* 2000; Massi *et al.* 2004; Olivares *et al.* 2006). Duez *et al.* detected *B. animalis* DN-173 010 1 week after its ingestion had stopped with a colony immunoblotting method (Duez *et al.* 2000). It is good to bear in mind the possibility that the study of faecal samples alone might not be sufficient in evaluating colonization by a probiotic strain. In one study, the administered probiotic strain was detected in colonic biopsies after its disappearance from faeces (Alander *et al.* 1999).

For the detection of *Bifidobacterium* in faeces, PCR-DGGE is more sensitive than FISH in the present study. The detection limit of PCR-DGGE seems to be lower than that of FISH ($\sim 10^6$–10^7 cells per g faeces).

Faecal β-galactosidase activity was increased during the supplementation period. One of our recent studies indicate that a major part of the colonic microbiota possess β-galactosidase activity (He *et al.* 2005) and the abundance of β-galactosidase is not involved in lactose intolerance (He *et al.* 2006). Therefore, the increase in the faecal β-galactosidase activity may not necessarily be related to the reduced SSC, but rather, could be an indication of altered metabolic activities of the colonic microbiota or the presence of the administered probiotic bacteria in the colon. Probiotics are found to be able to modify colonic fermentation (Jiang and Savaiano 1997a,b). Modification of colonic fermentation of lactose might affect the occurrence of lactose intolerance. An increase in faecal β-galactosidase activity could not be correlated with the changes in the amount of the colonic microbiota. The reasons for this could be: firstly, changes in metabolic activities occur without changes in the composition of bacteria; and secondly, our FISH method is not sensitive enough to detect the changes in bacterial composition responsible for the enzymatic changes. The large variation in bacterial numbers among individuals might make it difficult to clarify the relationship.

The SSC was reduced after the supplementation. Hertzler and Savaiano observed colonic adaptation to regular lactose ingestion and suggested that this adaptation reduces lactose intolerance symptoms (Hertzler and Savaiano 1996). Changes in the amount and metabolic pattern of the colonic microbiota as observed in this study can be among those adaptive changes. Furthermore, yogurt and

probiotics are generally regarded to be able to improve lactose digestion and alleviate symptoms of intolerance. In this study, SSC of the baseline period was not different from the SSC of the previous year, whereas SSC decreased significantly after supplementation compared with the baseline values. This indicates that the supplementation of yogurt and bifidobacteria alleviates the symptoms of lactose intolerance.

Yogurt can efficiently improve lactose digestion by providing active microbial β-galactosidase and slowing gastrointestinal transit (de Vrese *et al.* 2001). However, the consumption of yogurt or yogurt combined with probiotics cannot stimulate the endogenous (brush-border) lactase activity in the small intestine, as indicated by the study from Lerebours *et al.* (1989) and the present study. Moreover, the OCTT did not change after the supplementation. Therefore, the alleviation of intolerance symptoms observed in the follow-up period of this study is not caused by improved digestion of lactose in the small intestine.

Colonic metabolism of lactose has been suggested to play a role in lactose intolerance (Vesa *et al.* 2000; Vonk *et al.* 2003; He *et al.* 2006). In this study, the changes in the colonic microbiota might be among the factors modified by the supplementation which lead to the reduction of symptoms. Modulation of colonic metabolism through dietary supplementations could be a useful approach for the management of lactose intolerance.

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