AGE (2009) 31:59–66
DOI 10.1007/s11357-008-9081-0

Probiotics modulate the *Bifidobacterium* microbiota of elderly nursing home residents

Sampo J. Lahtinen · Liisa Tammela · Jaakko Korpela · Riikka Parhiala · Henri Ahokoski · Hannu Mykkänen · Seppo J. Salminen

Received: 12 June 2008 / Accepted: 23 October 2008 / Published online: 28 November 2008

Abstract Gut *Bifidobacterium* microbiota of the elderly has been suggested to differ from that of adults, possibly promoting the risk of infections and gut barrier dysfunction. Specific probiotics may improve the gut barrier. In this randomized, placebo-controlled intervention study, 66 elders consumed a fermented oat drink containing probiotic *Bifidobacterium longum* 46 and *B. longum* 2C or a non-fermented placebo oat drink for 6 months. Faecal samples were collected before, during and after the intervention. Levels of faecal bifidobacteria were determined using species-specific quantitative PCR and plate counting. The *Bifidobacterium* levels in the elderly were high and the species composition diverse. Probiotic intervention increased the levels bifidobacteria significantly. Specifically, the levels of *B. catenulatum*, *B. bifidum* and *B. breve* were enhanced. Consumption of the fermented oat drink itself was also associated with certain changes in microbiota. In conclusion, *Bifidobacterium* microbiota of elderly subjects may be modulated by probiotic administration. In some healthy elderly populations, *Bifidobacterium* microbiota may be more abundant and diverse than previously suggested.

Keywords *Bifidobacterium* · Probiotics · Elderly · Microbiota · Oat

S. J. Lahtinen · J. Korpela · R. Parhiala · H. Ahokoski · S. J. Salminen (✉)
Functional Foods Forum, University of Turku,
20014 Turku, Finland
e-mail: seppo.salminen@utu.fi

L. Tammela · H. Mykkänen
Department of Clinical Nutrition, University of Kuopio,
Kuopio, Finland

Present address:
S. J. Lahtinen
Danisco Health & Nutrition,
Kantvik, Finland

Introduction

Gut microbiota plays a critical role in maintaining human health throughout life. A healthy, diverse microbiota contributes to pathogen resistance and interacts with the host immune system. The impact of commensal microbes to colonization resistance against pathogens and healthy diversity are important to host health. Intestinal bifidobacteria in particular have received attention, due to their association with 'typical healthy intestinal microbiota' and their proposed health benefits (Leahy et al. 2005). Age-related changes in the gut microbiota composition include decreased species diversity and decreased levels of beneficial bacteria, and this may be associated with increased susceptibility to pathogen action and infection (Hopkins et al. 2001). Gut microbiota changes among the elderly may derive from dietary changes, changes in immune response, hospitalization, increased intestinal transit times and

lack of physical activity, recurrent infections, and frequent use of antibiotics (Bartosch et al. 2004). Studies relying on traditional culturing methods have suggested that compared with healthy adults, the numbers of genus *Bifidobacterium* are decreased in the elderly (Gavini et al. 2001; Mitsuoka and Hayakawa 1972; Mutai and Tanaka 1987; Woodmansey et al. 2004). However, in studies based on culture-independent methods, decrease in the numbers of bifidobacteria has not been observed (Bartosch et al. 2004; He et al. 2003).

Constipation and diarrhoea are common among the elderly, in particular in institutionalized subjects. Therefore, modulation and balancing of the gut function and microbiota composition of the elderly is desirable. Probiotics have been proposed as potential modulators of gut microbiota and function (Ouwehand et al. 2002). Despite the potential benefits of probiotics in this age group, few studies have been conducted in the elderly population. We hypothesized that specific probiotics chosen for this target group would improve gut *Bifidobacterium* microbiota composition and diversity, thereby improving the gut barrier. Using a species-specific quantitative PCR (qPCR) method and a traditional culturing method, we assessed the efficacy of an oat product fermented with two probiotic *Bifidobacterium* strains, specifically designed for the elderly, in the improvement of the gastrointestinal *Bifidobacterium* composition of elderly nursing-home residents.

Materials and methods

Study subjects and products

The study population consisted of 66 elderly nursing-home residents participating in placebo-controlled clinical intervention trial, which was approved by the Kuopio University and Kuopio University Hospital Ethical Committee. The subjects were randomized into two groups. The probiotic group (n=33, aged 84± 8 years) was administered daily with fermented and flavoured oat drink containing 10^9 cfu ml^{-1} *Bifidobacterium longum* 46 (DSM 14583) and *B. longum* 2C (DSM 14579) for six months, while the placebo group (n=33; aged 83±7 years) consumed a non-fermented artificially acidified and flavoured oat drink, which did not contain probiotic or starter cultures. Stool samples were collected by the nursing home staff at the baseline, and after 2, 4 and 6 months of intervention, as well as after a 2-month wash-out period. Complete or nearly complete sets of stool samples were obtained from 31 and 28 subjects in the probiotic and placebo groups, respectively. Of the subjects lacking a complete set of samples, one subject in the probiotic group and four subjects in the placebo group were missing the 8-month wash-out sample. Due to the distance between the location of the sample collection (Kuopio, Finland) and the location of the sample analysis (Turku, Finland), stool samples were shipped and stored at −20°C until the analysis.

Quantification of culturable bifidobacteria by plate counts

Stool samples were homogenized and serially diluted with phosphate buffered saline (pH 7.2) and spread on blood-liver agar plates (Nissui, Tokyo, Japan). Plates were covered from light and incubated anaerobically in an anaerobic hood (80% N_2, 10% CO_2, 10% H_2) at 37°C for 3 days. *Bifidobacterium* colonies were identified by colony morphology and the identification was confirmed by PCR using *Bifidobacterium* genus-specific primers (see below). Colonies were enumerated and the results were expressed as colony-forming units per gram of stool.

Quantification of total and species-specific bifidobacteria PCR

Bacterial DNA was isolated from 1:10 diluted homogenized stool samples using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, and stored at −20°C until use. Total bifidobacteria and nine *Bifidobacterium* species were quantified using quantitative real-time PCR. Apart from the genus *Bifidobacterium* and the species assumed to be the most commonly detected in the samples (*B. longum* and *B. adolescentis*), the presence of specific *Bifidobacterium* species was first determined by qualitative PCR as described by Rinne et al. (2005). Samples which were found positive in the qualitative assay were subsequently subjected to quantitative PCR. The species-specific primers and probes used in this study have been described and validated earlier by Langendijk et al. (1995) (total *Bifidobacterium*), Gueimonde et al. (2007) (*B. angulatum, B.*

Table 1 The mean levels (SD) of culturable (measured by plating) and total (measured by qPCR) fecal *Bifidobacterium* microbiota of elderly subjects at the baseline, and the differences observed during the study between the baseline and the subsequent time points. Results presented as log cells g^{-1} (*n.s.* not significant)

Genus *Bifidobacterium*	Probiotic group (n=31)		Placebo group (n=28)	
	Plating	qPCR	Plating	qPCR
Baseline	8.45 (0.97)	9.87 (0.71)	8.33 (1.18)	10.03 (0.65)
2 months	+0.37 P=0.014 [a]	+0.21 P=0.040	n.s.	n.s.
4 months	+0.39 P=0.041	+0.22 P=0.098	+0.54 P=0.015	n.s.
6 months	+0.32 P=0.074	n.s.	n.s.	n.s.
Wash-out	+0.49 P=0.013	n.s.	n.s.	n.s.

[a] Significant difference between the baseline and the respective time point

adolescentis, B. bifidum, B. breve, B. catenulatum group, *B. dentium*), Matsuki et al. (1999) (*B. longum* and *B. infantis*) and Lahtinen et al. (2006) (*B. animalis*). Specificity of the primers was further investigated in silico by comparing the oligonucleotide sequences with BLAST database search program (Altschul et al. 1997). In the case of the *B. animalis* primers, a mismatch with certain strains of *B. infantis* was detected. The composition of reaction mixture was: 10.5 μl of H_2O, 0.5 μl of 3′ primer, 0.5 μl of 5′ primer, 12.5 μl of SYBR Green, and 1 μl of template DNA. Thermal cycling (Applied Biosystems) consisted of the following time and temperature profile: 95°C for 10 min, and 40×95°C for 15 s, annealing temperature for 1 min and 35°C for 15 s.

Statistics

Following logarithmic conversion, Student's *t*-test was used to determine differences between the treatment groups in the baseline levels of specific species, and in the number of different species harboured by the subjects. Paired *t*-test was used to test whether the levels of bifidobacteria or the number of different species harboured in both groups differed between the baseline and the subsequent time points.

Results

Genus *Bifidobacterium* was detected in all subjects by both culturing and qPCR. The baseline level of total bifidobacteria in the study population was log 9.94 cells g^{-1} (SD 0.68) assessed culture-independently by qPCR (no difference between the groups). Probiotic intervention resulted in elevated levels of total bifidobacteria as measured by qPCR, but this increase was significant only at 2 months, while at 4 months there was a trend for increased *Bifidobacterium* levels (Table 1). In the placebo group, the levels of total bifidobacteria remained unchanged throughout the study. The average level of culturable bifidobacteria at the baseline was log 8.39 cfu g^{-1} (SD 1.07; no difference between the groups). In the probiotic group, the level of culturable bifidobacteria was higher throughout the intervention compared with the baseline level (Table 1), and remained elevated 2 months after the intervention had ceased. In the placebo group the level of culturable bifidobacteria was elevated at 4 months of the study but not at other time points. The *Bifidobacterium* species diversity, as assessed by the average number of species harboured by the elderly subjects at the baseline, was 3.2 (SD 1.64) *Bifidobacterium* species per subject. There was no difference between the probiotic group (3.1 species; SD 1.59) and the placebo group (3.3 species; SD 1.73) at the baseline. No significant changes in the average number of different species were observed in either group during the study.

At the baseline, *B. longum* was the most prevalent *Bifidobacterium* species detected by qPCR, being present in nearly all subjects generally at high levels (Table 2). *B. adolescentis* was detected in 33.9% of the subjects and, when present, the levels of this

Table 2 The prevalence (%) and the mean baseline levels[a] (SD) of nine species of *Bifidobacterium* in the fecal samples (*n.d.* not detected)

Species	Probiotic group (n=31)		Placebo group (n=28)		All subjects (n=59)		Difference[b]
	Prevalence	log cells g^{-1}	Prevalence	log cells g^{-1}	Prevalence	log cells g^{-1}	
B. adolescentis	35.5	8.48 (1.41)	32.1	8.71 (1.59)	33.9	8.58 (1.47)	n.s.
B. angulatum	0	n.d.	0	n.d.	0	n.d.	n.s.
B. animalis/lactis	54.8	6.82 (0.64)	82.9	7.24 (0.74)	67.8	7.04 (0.72)	P=0.042
B. bifidum	29.0	7.20 (1.35)	57.1	8.90 (1.27)	42.4	8.16 (1.54)	P=0.001
B. breve	48.4	7.29 (1.26)	39.3	7.70 (1.10)	44.1	7.45 (1.19)	n.s.
B. catenulatum group[c]	19.4	7.43 (1.64)	21.4	7.61 (1.55)	20.3	7.51 (1.56)	n.s.
B. dentium	9.7	6.34 (0.49)	17.9	6.65 (0.69)	13.6	6.53 (0.61)	n.s.
B. infantis	16.1	7.52 (0.57)	9.8	8.02 (0.84)	13.6	7.72 (0.69)	n.s.
B. longum	100	8.69 (1.60)	89.3	8.49 (1.61)	94.9	8.60 (1.60)	n.s.

[a] Average faecal level of cells in subjects positive for the species, as determined by qPCR

[b] Significant difference between the groups in the average level of the species

[c] Includes *B. catenulatum* and *B. pseudocatenulatum*

species were as high as those of *B. longum*. *B. animalis* was surprisingly frequent in both groups, being present in 67.8% of the subjects at the baseline, albeit at low levels. *B. bifidum* and *B. breve* were relatively frequent species at the baseline, while *B. catenulatum* was detected in 20.3% of the subjects. Both *B. dentium* and *B. infantis* were detected in 13.6% of the subjects, both at low levels. *B. angulatum* was not detected in any of the stool samples during the study. The baseline levels of the *Bifidobacterium* species did not differ between the groups, apart from *B. animalis* and *B. bifidum*, which were detected by qPCR more frequently and at higher levels in the placebo group at the baseline compared with the probiotic intervention group (Table 2).

The intervention resulted in several species-specific changes in the faecal *Bifidobacterium* microbiota of the elderly subjects, as detected by qPCR (Table 3). The probiotic administration but not the placebo increased the levels of *B. bifidum* and *B. breve* significantly throughout the intervention. The level of *B. catenulatum* was significantly increased in the probiotic group from the baseline after 2 months, 4 months, and 6 months of intervention, while in the placebo group the increase was significant only at 2 months. In the case of *B. adolescentis*, no changes were detected in the probiotic group during the administration, but in the placebo group elevated levels were observed at 4 and 6 months into the study. The levels of *B. animalis* were significantly lower at 2 months compared to the baseline in both probiotic and placebo groups. Following the initial reduction, the levels of *B. animalis* tended to increase during the reminder of study, but this was significant only in the placebo group at 4 months and after the wash-out. No changes were observed in the levels of *B. infantis*, *B. dentium* and *B. longum* during the study.

Discussion

Bifidobacterium longum 46 and *B. longum* 2C are two probiotic strains isolated from healthy elderly subjects (Salminen et al. 2002). The strains are safe for human consumption (Mäkeläinen et al. 2003) and capable of antimicrobial activity (Hütt et al. 2006; Lahtinen et al. 2007) and toxin binding (Halttunen et al. 2007; Nybom et al. 2007). The strains are capable of adhesion to intestinal mucus and displacement of adhered pathogens from mucus (Collado et al. 2007, 2008). Moreover, the consumption of these probiotics by the elderly results in beneficial effects on health. In a randomized controlled study conducted in Helsinki, Finland, the administration of *B. longum* 46 and *B. longum* 2C resulted in normalized bowel movements in institutionalized elderly subjects (Pitkälä et al. 2007). Moreover, the probiotic administration was associated with enhanced immune function in the elderly subjects, and the changes observed in the immune parameters correlated with changes in the intestinal microbiota composition (Ouwehand et al. 2008a). In the current clinical trial, we confirm the

Table 3 Differences in the mean faecal levels of *Bifidobacterium* species between the baseline levels and subsequent time points observed during the study. Results expressed as ±log cells g^{-1}

Species[a]	Probiotic group (n=31)				Placebo group (n=28)			
	2 months	4 months	6 months	8 months	2 months	4 months	6 months	8 months
B. adolescentis	n.s.	n.s.	n.s.	n.s.	n.s.	+0.72 P=0.010	+0.47 P=0.025	n.s.
B. animalis	−0.42 P=0.015 [b]	n.s.	n.s.	+0.40 P=0.089	−0.69 P<0.001	+0.46 P=0.003	n.s.	+0.52 P=0.018
B. bifidum	+0.90 P=0.015	+1.14 P=0.005	+1.38 P=0.004	+0.65 P=0.062	n.s.	n.s.	n.s.	n.s.
B. breve	+0.67 P=0.011	+0.85 P=0.005	+0.97 P=0.006	+0.44 P=0.058	n.s	n.s.	n.s.	n.s
B. catenulatum group[c]	+1.09 P=0.005	+0.86 P=0.015	+0.94 P=0.025	n.s.	+1.20 P=0.049	+1.04 P=0.066	n.s.	n.s.

[a] *B. dentium*, *B. infantis* and *B. longum* were excluded from the table as no differences in the levels of these species were observed during the study

[b] Significant difference between the baseline and the respective time point

[c] Includes *B. catenulatum* and *B. pseudocatenulatum*

capability of these strains to beneficially modulate the composition of intestinal *Bifidobacterium* microbiota in institutionalized elderly subjects, in particular the culturable proportion of the *Bifidobacterium* microbiota. Moreover, we demonstrate that in contrast with many earlier reports, elderly subjects in this study may harbour an abundant and diverse *Bifidobacterium* microbiota.

Ageing is thought to be associated with changes in the intestinal microbiota components, including decreased levels of the genus *Bifidobacterium*. In this study, the faecal level of *Bifidobacterium* and the species diversity were high in the elderly nursing home subjects at the baseline. The levels of *Bifidobacterium* observed in this study were within the same range as commonly found in healthy adults, and also comparable to what was recently reported for healthy institutionalized elders from Helsinki, Finland (Ouwehand et al. 2008a) and healthy free-living elders from Kuopio, Finland (Ouwehand et al. 2008b). The observed high levels of bifidobacteria may relate to the high food intake and the lack of constipation among the study subjects, and possibly to the fact that the intake of dietary fibre is typically high in the Kuopio region. All subjects were consuming the food served at the institute and made to correspond with the nutrition recommendations (Nordic Council of Ministers 2004). The high levels of *Bifidobacterium* are somewhat contradictory to earlier culture-based studies, which have suggested that the levels of bifidobacteria are decreased at old age (Gavini et al. 2001; Mitsuoka et al. 1974; Mutai and Tanaka 1987; Woodmansey et al. 2004). One possible explanation for the differences between this study and the earlier culture-based studies is that rather than lowering the absolute levels of bifidobacteria, ageing may change the species composition and thereby the culturability of bacteria. Age-related differences in the *Bifidobacterium* species composition have been reported previously (Gavini et al. 2001; Woodmansey et al. 2004). Shifts in the species composition may affect the plating results because some species grow better in laboratory conditions than others.

Despite the high initial levels observed, the levels of culturable and total bifidobacteria were positively affected by the probiotic treatment. Based on plate counting, the levels of culturable bifidobacteria in the probiotic group were significantly higher than the baseline levels throughout the intervention, and remained elevated after the 2-month wash-out period. In the placebo group, increased levels of culturable bifidobacteria were observed only at 4 months of the study. Assessed culture-independently, the administration of the probiotic product resulted in increased levels of total *Bifidobacterium*, while the placebo product had no effect. These results suggest that the changes in the *Bifidobacterium* microbiota induced by

probiotic intervention may to be more pronounced in the culturable proportion of *Bifidobacterium* microbiota than in the total numbers of this genus.

The *Bifidobacterium* species composition of the elderly subjects was found to be diverse. Probiotic intervention resulted in changes in the composition of the *Bifidobacterium* microbiota composition—a result which is inline with earlier reports from studies with probiotics (Ouwehand et al. 2008a) and synbiotics (Bartosch et al. 2005; Ouwehand et al. 2008b). The most common species was *B. longum*, which was present at high levels in almost all of the subjects at the baseline. The frequency of this species in our study (89.3%) was higher than in many previous culture-dependent studies (Gavini et al. 2001; Mutai and Tanaka 1987; Silvi et al. 2003), but in line with the recent results obtained by culture-independent methods (Ouwehand et al. 2008a, 2008b). The administration of the oat drink containing two strains of *B. longum* did not affect faecal levels of this species, possibly because the initial levels of this species were already high. However, the probiotic administration resulted in significant changes in the levels of many other *Bifidobacterium* species. Consumption of the probiotic product increased the levels of *B. catenulatum* throughout the intervention, whereas in the placebo group, increase in *B. catenulatum* was observed only at 2 months into the study. This suggests that oat drink itself has a beneficial effect on *B. catenulatum*, possibly due to the proposed prebiotic effects of oat components (Mälkki and Virtanen 2001), but this effect may be further enhanced by the use of probiotics. Administration of the probiotic product but not placebo resulted in steady and significant increase in the levels of *B. bifidum* and *B. breve*. The frequency of *B. breve* was somewhat surprising, as this species is thought to be more typical of infant gut microbiota (Gavini et al. 2001; Mitsuoka et al. 1974). When present, *B. bifidum* and *B. breve* seldom represented the dominant *Bifidobacterium* species in the samples, but were nevertheless present at notable levels. *B. angulatum* was not detected from any of the subjects, although Woodmansey et al. (2004) identified *B. angulatum* as the predominant *Bifidobacterium* species in British elderly subjects, as analysed by culturing and cellular fatty acid profiles. This difference may be related to dietary or genetic differences among the study populations, or the different methodologies applied.

B. adolescentis (*sensu stricto*) was detected less frequently than previously reported (Gavini et al. 2001; Mutai and Tanaka 1987; Silvi et al. 2003), but when present, *B. adolescentis* was a dominant part of the *Bifidobacterium* microbiota in the elderly. Administration of placebo oat drink increased the levels of *B. adolescentis*, possibly due to the effects of oat components such as the oat fibre including ß-glucan. It is not known why such an effect only occurred in the placebo group. It is possible that the administration of probiotics favoured *Bifidobacterium* species other than *B. adolescentis*, increasing the competition between the species. Several culture-based studies have suggested that *B. infantis* is found exclusively in infants (Mitsuoka et al. 1974; Mutai and Tanaka 1987; Woodmansey et al. 2004), but in this study *B. infantis* was detected in 13.6% of the elderly subjects. It is possible that due to the close relatedness to *B. longum* (Sakata et al. 2002), *B. infantis* has earlier been overlooked as a component of adult and elderly gut microbiota. *B. animalis* was found to be commonly present in the elderly subjects at low levels. Traditionally, *B. animalis* has not been considered a component of normal human gut microbiota, but *B. animalis* subsp. *lactis* has been reported the most common *Bifidobacterium* species in commercial probiotic products in Europe (Gueimonde et al. 2004). Although the subjects consumed a controlled diet during the study, the dietary origin of this species cannot be completely ruled out. It is possible that the presence of *B. animalis* has been overlooked in the earlier culture-based studies, as *B. animalis* may grow slowly on nutrient media used normally for the culturing of *Bifidobacterium*, and it may be difficult to detect minor components of complex microbiota due to overgrowth of dominant species. In addition, as *B. animalis* has not been thought to be a component of normal human microbiota, it often has been excluded from culture-independent analyses of human gut microbiota. According to the current results, *B. animalis* was usually present at low levels, representing less than 1% of the total genus *Bifidobacterium*, and this may also partly explain why *B. animalis* has not been detected frequently in many earlier reports. Taken together, the results of the current study are in agreement with the results reported recently for institutionalized elderly subjects from Helsinki, Finland. Nevertheless, differences in the baseline prevalence of certain species, in

particular *B. adolescentis* and *B. animalis*, can be observed between the two elderly populations. The baseline differences in the microbiota composition as well as the possible dietary, genetic and environmental factors may partly explain why the changes in the *Bifidobacterium* microbiota associated with the probiotic intervention were somewhat different between the two study populations, with the probiotic intervention enhancing the levels of *B. adolescentis* in the elders from Helsinki area and the levels of *B. bifidum* and *B. breve* in the elders from Kuopio area. In both study populations, the levels of *B. catenulatum* increased as a result of the probiotic intervention.

In conclusion, the results of this study demonstrate that the *Bifidobacterium* microbiota of the elderly may be modified by probiotic intervention. Such intervention may affect a number of different species other than the administered species, in particular the culturable proportion of the *Bifidobacterium* microbiota. Moreover, this study suggests that the average level and the species diversity of *Bifidobacterium* microbiota of the elderly may be considerably higher in certain populations than previously thought. Certain species which previously have not been associated with *Bifidobacterium* microbiota of elderly subjects were found to be relatively frequent in faecal samples in this population. Future research in this required to further study the impact of probiotic therapies on gut metabolism, pathogen resistance and intestinal function of elderly subjects.

Acknowledgements Bioferme (Kaarina, Finland) is thanked for providing the test oat products used in the study. Dr. Miguel Gueimonde is thanked for helpful discussions. Sampo Lahtinen was funded by the ABS Graduate School.

References

Altschul SF, Madden TL, Schaffer AA et al (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402. doi:10.1093/nar/25.17.3389

Bartosch S, Fite A, Macfarlane GT et al (2004) Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. Appl Environ Microbiol 70:3575–3581. doi:10.1128/AEM.70.6.3575-3581.2004

Bartosch S, Woodmansey EJ, Paterson JC et al (2005) Microbiological effects of consuming a synbiotic containing *Bifidobacterium bifidum, Bifidobacterium lactis*, and oligofructose in elderly persons, determined by real-time polymerase chain reaction and counting of viable bacteria. Clin Infect Dis 40:28–37. doi:10.1086/426027

Collado MC, Meriluoto J, Salminen S (2007) Role of commercial probiotic strains against human pathogen adhesion to intestinal mucus. Lett Appl Microbiol 45:454–460. doi:10.1111/j.1472-765X.2007.02212.x

Collado MC, Meriluoto J, Salminen S (2008) Adhesion and aggregation properties of probiotic and pathogen strains. Eur Food Res Technol 226:1065–1073. doi:10.1007/s00217-007-0632-x

Gavini F, Cayuela C, Antoine JM et al (2001) Differences in the distribution of bifidobacterial and enterococcal species in human faecal microflora of three different (children, adults, elderly) age groups. Microb Ecol Health Dis 13:40–45. doi:10.1080/089106001750071690

Gueimonde M, Delgado S, Baltasar M et al (2004) Viability and diversity of probiotic *Lactobacillus* and *Bifidobacterium* populations included in commercial fermented milks. Food Res Int 37:839–850

Gueimonde M, Debor L, Tölkkö S et al (2007) Quantitative assessment of faecal bifidobacterial populations by real-time PCR using lanthanide probes. J Appl Microbiol 102:1116–1122

Halttunen T, Salminen S, Tahvonen R (2007) Rapid removal of lead and cadmium from water by specific lactic acid bacteria. Int J Food Microbiol 114:30–35. doi:10.1016/j.ijfoodmicro.2006.10.040

He T, Harmsen HJ, Raangs GC et al (2003) Composition of faecal microbiota of elderly people. Microb Ecol Health Dis 15:153–159. doi:10.1080/08910600310020505

Hopkins MJ, Sharp R, Macfarlane GT (2001) Age and disease related changes in intestinal bacterial populations assessed by cell culture, 16S rRNA abundance, and community cellular fatty acid profiles. Gut 48:198–205. doi:10.1136/gut.48.2.198

Hütt P, Shchepetova J, Loivukene K et al (2006) Antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uropathogens. J Appl Microbiol 100:1324–1332. doi:10.1111/j.1365-2672.2006.02857.x

Lahtinen SJ, Gueimonde M, Ouwehand A et al (2006) Comparison of four methods to enumerate probiotic bifidobacteria in a fermented food product. Food Microbiol 23:571–577. doi:10.1016/j.fm.2005.09.001

Lahtinen SJ, Jalonen L, Ouwehand AC et al (2007) Specific *Bifidobacterium* strains isolated from elderly subjects inhibit growth of *Staphylococcus aureus*. Int J Food Microbiol 117:125–128. doi:10.1016/j.ijfoodmicro.2007.02.023

Langendijk PS, Schut F, Jansen GJ et al (1995) Quantitative fluorescence in situ hybridization of *Bifidobacterium* spp. with genus-specific 16S rRNA-targeted probes and its application in fecal samples. Appl Environ Microbiol 61:3069–3075

Leahy SC, Higgins DG, Fitzgerald GF et al (2005) Getting better with bifidobacteria. J Appl Microbiol 98:1303–1315. doi:10.1111/j.1365-2672.2005.02600.x

Mäkeläinen H, Tahvonen R, Salminen S et al (2003) In vivo safety assessment of two *Bifidobacterium longum* strains. Microbiol Immunol 47:911–914

Mälkki Y, Virtanen E (2001) Gastrointestinal effects of oat bran and oat gum. Lebensm-Wiss u-Technol 34:337–347

Matsuki T, Watanabe K, Tanaka R et al (1999) Distribution of bifidobacterial species in human intestinal microflora examined with 16S rRNA-gene-targeted species-specific primers. Appl Environ Microbiol 65:4506–4512

Mitsuoka T, Hayakawa K (1972) Die Faecalflora bei Menschen I. Mitteilung: Die Zusammensetzung der Faecalflora der verschiedenen Altersgruppen. Zbl Bakt Hyg I Abt Orig A 223:333–342

Mitsuoka T, Hayakawa K, Kimura N (1974) Die Faecalflora bei Menschen II. Mitteilung: Die Zusammensetzung der Bifidobakterienflora der verschiedenen Altersgruppen. Zbl Bakt Hyg I Abt Orig A 226:469–478

Mutai M, Tanaka R (1987) Ecology of *Bifidobacterium* in the human intestinal flora. Bifidobact Microflora 6:33–41

Nordic Council of Ministers (2004) Nordic nutrition recommendations 2004. Integrating nutrition and physical activity, 4th edn. Nordic Council of Ministers, Copenhagen

Nybom SM, Salminen SJ, Meriluoto JA (2007) Removal of microcystin-LR by strains of metabolically active probiotic bacteria. FEMS Microbiol Lett 270:27–33. doi:10.1111/j.1574-6968.2007.00644.x

Ouwehand AC, Salminen S, Isolauri E (2002) Probiotics: an overview of beneficial effects. Ant Van Leeuw 82:279–289. doi:10.1023/A:1020620607611

Ouwehand AC, Bergsma N, Parhiala R et al (2008a) *Bifidobacterium* microbiota and parameters of immune function in elderly subjects. FEMS Immunol Med Microbiol 53:18–25. doi:10.1111/j.1574-695X.2008.00392.x

Ouwehand AC, Tiihonen K, Saarinen M et al (2008b) Influence of a combination of *Lactobacillus acidophilus* NCFM and lactitol on healthy elderly: intestinal and immune parameters. Br J Nutr. doi:10.1017/S00007114508003097

Pitkälä KH, Strandberg TE, Finne-Soveri UH et al (2007) Fermented cereal with specific bifidobacteria normalizes bowel movements in elderly nursing home residents. A randomized, controlled trial. J Nutr Health Aging 11:305–311

Rinne MM, Gueimonde M, Kalliomäki M et al (2005) Similar bifidogenic effects of prebiotic-supplemented partially hydrolyzed infant formula and breastfeeding on infant gut microbiota. FEMS Immunol Med Microbiol 43:59–65. doi:10.1016/j.femsim.2004.07.005

Sakata S, Kitahara M, Sakamoto M et al (2002) Unification of *Bifidobacterium infantis* and *Bifidobacterium suis* as *Bifidobacterium longum*. Int J Syst Evol Microbiol 52:1945–1951. doi:10.1099/ijs.0.02221-0

Salminen S, Ouwehand A, Salminen E et al (2002) Method for screening probiotic strains of the genus *Bifidobacterium*. World Patent WO 02/38798

Silvi S, Verdenelli MC, Orpianesi C et al (2003) EU project Crownalife; functional foods, gut microflora and healthy aging. Isolation and identification of *Lactobacillus* and *Bifidobacterium* strains from faecal samples of elderly subjects for a possible probiotic use in functional foods. J Food Eng 56:195–200. doi:10.1016/S0260-8774(02)00249-2

Woodmansey EJ, McMurdo ME, Macfarlane GT et al (2004) Comparison of compositions and metabolic activities of fecal microbiotas in young adults and in antibiotic-treated and non-antibiotic-treated elderly subjects. Appl Environ Microbiol 70:6113–6122. doi:10.1128/AEM.70.10.6113-6122.2004